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Research Article

Effects of management complexity on the composition, plant functional dominance relationships and physiognomy of high nature value grasslands

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Abstract

A significant proportion of Europe's species-rich grasslands are semi-natural habitats. They have a long history of traditional management. Several studies have been carried out to conserve them, resulting in the establishment of subsidised conservation management schemes. On the other hand, many of these conservation management schemes have failed to provide locally adaptive solutions to maintain the diversity and functional status of species-rich grasslands. In addition, few studies have compared the conservation effectiveness of different levels of management complexity. The levels of management complexity in our study are based on how different management types (e.g. grazing and mowing etc.) and how different herbage removal intensities (e.g. lower and higher grazing intensities) are combined within and between years. To investigate this, we compared the overall effects of management complexity, herbage removal intensity and management type on plant diversity, plant functional type dominance relationships and plant physiognomy. Our field sampling was carried out in the sandy meso-xeric grasslands of the Turján Region of the Great Hungarian Plain (Central Hungary). We sampled nine 2 m × 2 m plots per grassland site (n = 12), recorded all the rooted plant species and estimated their percentage cover in each plot. High level of management complexity had significant positive effects on plant diversity, grazing had positive effects on plant diversity and phanerophyte density, while the studied levels of herbage removal intensity had no effect on diversity, plant functional types or plant physiognomy. In parallel, mowing and/or low levels of management complexity had some negative effects on conservation value (e.g. lower Shannon and Simpson diversity). In this landscape, the dominance of grazing and the more complex management is more optimal

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than relatively homogeneous mechanical mowing. The choice of management type and intensity is an important tool in the conservation management system of this landscape, but so too is its appropriate application in space and time. Through a detailed analysis of the effects of management complexity levels compared to management types and herbage removal intensity levels, we provide a new opportunity to make grassland management practices more effective for conserving biodiversity in this region, but it would be important to investigate these in different landscapes and conditions.

Key words: Grassland conservation system, management effects, management efficiency

Introduction

A significant proportion of European landscapes are cultural landscapes that have been transformed and managed by humans (Plieninger et al. 2006; Dahlström et al. 2013). The majority of the grasslands in these landscapes are semi-natural, i.e. created and actively maintained by local communities (Maurer et al. 2006; Marini et al. 2007; Niedrist et al. 2009). Nevertheless, the number of endemic species in these grasslands is exceptionally high in Europe, underlining their high conservation value (Hobohm and Bruchmann 2009; Habel et al. 2013).

Species-rich, semi-natural grasslands have been managed for centuries by small family farms to provide summer forage in pastures and to produce winter fodder in hay meadows for livestock (Babai and Molnár 2014). Economic, socio-cultural and political factors, such as intensification and abandonment, have led to the disappearance of extensive grassland management systems across Europe since the mid-twentieth century (Bakker and Berendse 1999; MacDonald et al. 2000; Öckinger et al. 2006). As a result, the habitat mosaic of the cultural landscapes has changed, with fragmentation and disappearance (scrub encroachment, afforestation) of species-rich semi-natural grasslands having occurred, while the biodiversity of these habitats has declined (Eriksson et al. 2002; Krauss et al. 2004; Öckinger and Smith 2006; Flynn et al. 2009; Laliberte et al. 2010).

As semi-natural grasslands have been developed and maintained by human management, active and adaptive nature conservation management should be implemented to maintain the species composition and vegetation structure of these habitats. Through a long learning process, nature conservation aimed to mimic the patterns and disturbance regimes of former non-intensive, traditional grassland management (Szépligeti et al. 2018; Kun et al. 2021). These principles recognise the importance of the co-formation of the vegetation and extensive management and the adapted nature of the species pool to the former management practices (Poschlod et al. 1998).

Conservation management of grasslands should also draw on the experience of local communities still practising traditional and adaptive grassland management (cf. Niedrist et al. (2009); von Glasenapp and Thornton (2011)), as in the case of experiments in conservation biology (e.g. Vadász et al. (2016)). One of the most significant trends in conservation management research is the over-simplified ‘one-factor’ view, where ecologists study the effects of only one management factor, mostly focusing on the general effects of management type (mostly grazing and/or mowing) and management intensity (Tälle et al. 2016;

Török et al. 2018; Kun et al. 2021). The explanatory power of these specialised and generic few-factor models often has major limitations in their applicability to specific and local grassland conservation practices (Vadász et al. 2016; Kun et al. 2019). Therefore, more effective and practice-orientated nature conservation also requires more detailed and comprehensive studies to fill the knowledge gaps on the complex, locally specific effects of different management factors (e.g. locally appropriate management types, regimes, spatial and temporal applications etc.) on species-rich grasslands (Babai et al. 2015; Kun et al. 2021). One of the potentially important management factors for grassland conservation that should be investigated is how different management factors are applied spatially and/or temporally on grasslands (Allan et al. 2014). Based on some previous studies (Vadász et al. 2016; Kun et al. 2019), the spatial and temporal application of management types (e.g. grazing or mowing) or different herbage removal intensities (temporal speed of grazing or mowing, based on standard livestock unit and mowing frequency, see Table 1) can represent management complexity. Levels of management complexity are based on how different management types (e.g. grazing or mowing) and different management intensities are varied within and between years on a given grassland (see Table 1 for details). With more knowledge about the appropriate application of levels of management complexity, we would be able to more effectively adapt our conservation objectives in different grassland conservation management cases (Kun et al. 2019).

In this study, we aim to reveal the effects of management complexity, management intensity levels and management types on plant diversity, plant functional type dominance relationships and plant physiognomy in species-rich meso-xeric, sandy grasslands of central Hungary. We hypothesise that high management complexity and low herbage removal intensity will positively affect plant diversity, plant functional state and physiognomy. We also hypothesise that grazing, in particular, has a positive effect on higher plant diversity and less graminoid (Poales) cover, more forbs and shrubs (Phanerophytes) cover. Our specific question is: How do low and high levels of management complexity affect plant diversity, vegetation physiognomy and plant functional type cover in relation to management type and herbage removal intensity?

Table 1. A list and an introduction to the management factors and their categories and sub-categories.

Management factor categories	Management factor subcategories
Type of grassland management (*T*)	**Mowing (*M*):** Mechanical mowing at the end of June or the first half of July with 10–15 cm of stubble. See details of management complexity later in this Table.
	Grazing (*G*): Pastures are mainly grazed by cattle from the end of April to the beginning of October each year. Shepherds often work with them.
	Combined (*C*): Mowing and grazing are combined within the same year or between years. For more details, see management complexity later in this Table.
Herbage removal intensity (*I*)	**Low:** Grazing at < 0.5 Standard Livestock Units (SLU) per hectare or mown once a year. LUI value: 0.1 (Schneiders et al. 2011).
	High: Grazing at **>** 0.5 Standard Livestock Units (SLU) per hectare or mown once a year followed by grazing in the same year. LUI value: 0.2 (Schneiders et al. 2011).
Management complexity (*C*)	**Low:** Grazing with a standard sequence of two grazing units per year or one mowing with 10% uncut per year or one mowing per year combined with subsequent grazing.
	High: Mowing and grazing combined between years or grazing with different start times between years in a four-year rotation.

Methods

Study area

The study sites are located in the Turján Region of the Great Hungarian Plain along the Danube in central Hungary, in the northern Kiskunság area. The study sites are relatively close to each other, within a circle of about 10 km diameter around the neighbouring villages of Kunpeszér, Tatársszentgyörgy and Kunadacs (Appendix 1). The climate is mainly continental with sub-Mediterranean influences. The average annual temperature is 10.5–11 °C, while the average annual precipitation is 500–550 mm (Kocsis 2018). The potential natural vegetation is the Euro-Siberian steppic woods with *Quercus* spp. A significant part of the region consists of semi-natural *Molinia* meadows, which are mown or grazed by cattle and Pannonic sand steppes. These grasslands are mainly grazed by Hungarian Grey cattle and Charolais breeds and, to a lesser extent, by sheep.

Most of the studied sites have been modified by local people in the past and present, through woodcutting and long-term grazing (Molnár et al. 2022). Some of the grasslands studied are old fields, abandoned several decades to a few centuries ago. These areas are fully regenerated and are well developed. Their species pool, species composition and physiognomy do not differ significantly from the other grasslands studied. Constant management is essential in these grasslands to prevent reforestation and the spread of some native disturbance-tolerant or invasive alien species (Erdélyi et al. 2023). Over the past century, a network of drainage canals has been constructed throughout the area, resulting in the drying out of wet grasslands and the creation of a significant amount of drier grassland (Tölgyesi et al. 2022). All of the grasslands studied are meso-xeric habitats, representing the transitional zone between the *Molinia* meadows (*Molinion caeruleae*) and the dry Pannonic sand steppes (*Festucion vaginatae*), with a similar vegetation composition and state of development. This species-rich grassland covers a large area in the study area; but it is threatened in a regional and wider context. The meso-xeric grassland habitats are important for the whole Eurasian forest-steppe zone and can be considered as its species-rich grassland component (Mathar et al. 2016; Willner et al. 2019). The dominant and characteristic graminoid (*Poales*) species of the studied grasslands include *Chrysopogon gryllus*, *Brachypodium pinnatum* and *Molinia caerulea* and some forb species, such as *Serratula tinctoria*, *Sanguisorba officinalis*, *Peucedanum cervaria*, *Betonica officinalis* and *Genista tinctoria*, as well as some Hungarian protected forb species, such as *Ophrys sphegodes*, *Iris spuria*, *Centaurea scabiosa* subsp. *sadleriana* etc. All the grasslands studied are part of the Kiskunság National Park. As a result, these grasslands have been managed according to conservation principles in the last decades which means a lower management intensity and a more complex management in space and time in general. Conservation is carried out throughout the study area by the Kiskunság National Park Directorate.

Data collection

The surveys were conducted in June 2018 on 12 grassland sample sites, all of which were at least 5 ha and at most 10 ha in size (Appendix 1). Three of the sites were mown, six were pastures with varying levels of herbage removal intensity and complexity and, in three grasslands, these had combined use (both mowing and grazing). For each study site, nine plots (2 m × 2 m each) were located in the inner zone of the grassland to exclude edge effects. In each grassland, a random starting point was chosen and the plots were sampled along two parallel line transects with a maximum length of 200 m and a minimum distance of 4 m between plots (Appendix 2).

The coordinates of the plots were recorded by GPS. Data were collected from 108 plots in the 12 grassland sites mentioned above, nine plots per site (see Appendices 1 and 2). During sampling, we recorded each vascular plant species found in the sample plots and visually estimated its percentage cover. In addition, we visually estimated four vegetation physiognomic characteristics: 1) percent litter cover, 2) total plant cover, 3) the amount of bare soil surface and 4) average plant height. Average plant height was estimated using a tape measure and reported in centimetres. Due to overlapping layers of vegetation, total plant cover in plots could exceed 100%.

We defined plant functional types (PFTs) as groups of species based on three growth forms: *forbs* including non-grassy herbs, graminoids (*Poales*) including grasses, sedges and rushes and *phanerophytes* including shrubs and small trees (Raunkiær 1934; Box 1996; Király 2009). We calculated the proportions of PFTs in each plot by summing the cover values of the species assigned to them.

At each grassland site, we recorded three management factors at different levels, including intensity of herbage removal (***I***, with low and high levels), complexity of management (***C***, with low and high levels) and different types of management (***T***, including grazing, mowing and combined types) (Table 1). Prior to our field sampling, we interviewed the conservation practitioners of the later sampled grasslands of the National Park Directorate and sampled grassland sites were selected, based on low and high levels of complexity and herbage removal intensity of management, as well as management types (grazing, mowing or combined). On each of the sampled grassland sites (n = 12), all three management factor categories (***T***, ***I***, ***C***) were applied, but only one subcategory of each management factor category was applied, for example, on a grazed site (one management type), only low or only high level of herbage removal intensity and only low or only high level of management complexity were applied (see Appendix 3). These management techniques on grasslands have been stable in the last decades and were only started by the Kiskunság National Park Directorate in the Turján Region (see Vadász et al. (2016)).

Data analysis

We calculated diversity measures, namely species number, Shannon index and Simpson index, from the plant species and estimated percent cover data recorded in each plot. The use of both diversity indices was important because the Shannon diversity index is more sensitive to the higher proportion of rare (often specialist) species, while the Simpson index is more sensitive to the balance of more dominant species. We built linear and generalised linear mixed effects models (with ‘lmer’ and ‘glmer’ functions from the ‘lme4’ package) to test the effect of management factors ***T***, ***I*** and ***C*** as three fixed factors on plant diversity indices, on the abundance of PFTs and on vegetation physiognomy. Different families of distributions (Gaussian and Gamma) were used to treat each differently distributed dependent variable in the modelling (the ‘gamma_ test' function from the 'goft' package was used). In our analyses, site was a random factor in all models. To assess model fit, marginal R^2_{LR} was applied (Ives 2019) from the 'MuMIn' package in R 3.5.1. The beta R^2_{LR} statistic (Edwards et al. 2008) was applied using the 'r.squaredLR' function to assess the best-fitting model amongst those run with each factor (*T*, *I* and *C*) separately as a predictor. The levels of the fixed factors ***T***, ***I*** and ***C*** were compared using the LMER Tukey post hoc test with the Bonferroni adjustment method (Hothorn et al. 2009) from the 'multcomp' package and with the 'glht' function. PERMANOVA analysis (with the 'adonis' function from the 'vegan' package) was used to investigate general patterns in species composition via possible effects of management factors. Principal component analysis (PCA) (using the 'pca' function from the 'vegan' package) was used to investigate the relationships between plant diversity, plant functional types and physiognomic factors in relation to different management factors. Our analyses were performed in the R 3.5.1 (R Core Team 2018) software environment (R Core Team 2018).

Results

Management type, levels of herbage removal intensity and management complexity had similarly strong effects on species number based on model fits ($R^2 > 0.320$, Table 2). There were no differences between low and high levels of herbage removal intensity for diversity, plant functional types and physiognomic factors. High levels of management complexity resulted in significantly higher Shannon and Simpson diversity (Fig. 1). In the case of ***T***, grazing and combined management resulted in significantly higher Shannon and Simpson diversity than mowing and grazing had significantly higher phanerophyte cover than mowing, but no significant difference in species number was observed (Appendix 4).

With PFT categories as dependent variables, management type showed a strong relationship with graminoid and forb cover (Table 3). Grazed sites had a significantly higher proportion of phanerophyte cover than mown sites and combined sites were between the two (Appendix 4). Herbage removal intensity showed a strong relationship with forb and graminoid cover, but a weaker

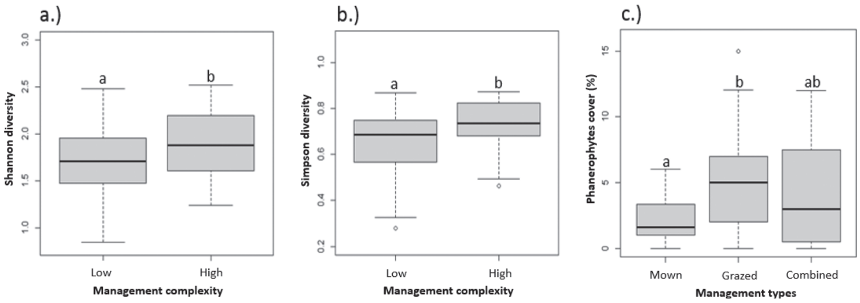

Figure 1. Significant differences in diversity and cover of phanerophytes in grasslands with low and high management complexity and different management types. Only models with minimum $R^2_{LR} \geq 0.100$ fit (see Tables 2–4) and significant differences (Appendices 4–6) were selected for inclusion. Significance of differences between groups is based on the LMER Tukey post hoc tests. Significant differences ($p < 0.05$) between factor levels are indicated by letters ('a' and 'b') above the boxplots. Non-significant differences are indicated by the letters 'ab'.

relationship with phanerophyte cover (Table 3). There were no significant differences between the levels of herbage removal intensity (Appendix 5). ***C*** had a stronger relationship with the forbs and graminoid groups, but a weaker relationship with the phanerophyte group (Table 3). Apart from these relationships, no significant differences were found between ***C*** levels for PFTs (Appendix 6). ***T***, ***I*** and ***C*** strongly influenced average plant height, litter cover and total plant cover, in general (Table 4). On the other hand, no significant differences in average plant height, litter cover and total plant cover were observed between grasslands exposed to different levels of ***T***, ***I*** and ***C*** (Appendices 4–6).

The two main components were presented in relation to forbs and graminoid (Poales) cover, based on principal component analysis. Higher graminoid cover was associated with mowing and higher forbs cover was mostly associated with grazing and combined management was intermediate between mowing and grazing (Fig. 2). High herbage-removal intensity was associated with higher graminoid cover and low herbage-removal intensity was associated with higher forbs cover (Fig. 3). A high level of management complexity was associated with higher forbs cover and a low level of management complexity was asso-

Table 2. Effects of different management factors, namely ***T*:** management type; ***I*:** herbage removal intensity of management; ***C*:** management complexity, on diversity measures in terms of model fit. Goodness-of-fit is expressed as R^2_{LR} values.

Management factors	Species number	Shannon diversity	Simpson diversity
	R^2	R^2	R^2
T	0.324	0.096	0.057
I	0.325	0.023	0.011
C	0.324	0.072	0.053

Table 3. Effects of different management factors, namely ***T*:** management type; ***I*:** herbage removal intensity of management; ***C*:** management complexity in relation to forbs, graminoid and Phanerophyte cover. Goodness-of-fit is also presented in R^2_{LR} values.

Management factors	Forb species cover (%)	Graminoid species cover (%)	Phanerophyte species cover (%)
	R^2	R^2	R^2
T	0.368	0.430	0.121
I	0.365	0.420	0.075
C	0.368	0.415	0.097

Table 4. Effects of different management factors, namely ***T*:** management type; ***I*:** herbage removal intensity of management; ***C*:** management complexity in relation to physiognomic factors in relation to grasslands. Goodness-of-fit is also presented in R^2_{LR} values.

Management factors	Litter cover (%)	Total plant cover (%)	Bare soil surface (%)	Average plant height in plots (cm)
	R^2	R^2	R^2	R^2
T	0.579	0.709	0.120	0.355
I	0.572	0.705	0.090	0.298
C	0.559	0.703	0.115	0.318

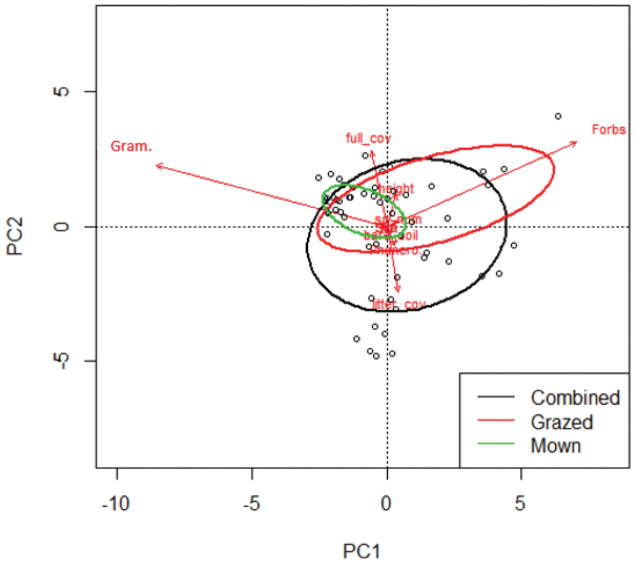

Figure 2. Principal Component Analysis of diversity indices, plant functional type cover and physiognomic factors across management types. The diversity indices examined are species number (sp_num), Shannon (Sha) and Simpson (Sim) diversity. Plant functional type cover includes graminoids (Gram.), forbs and phanerophytes (Phanero.). Plant physiognomic factors are average plant height (height), total plant cover (full_cov), bare soil surface (bare_soil) and litter cover (litter_cov). Management types: mown, grazed and combined management. The direction, width and different colours of the ellipses in the figure show us the relationship between the samples of different management types. The length and direction of the arrows show the explanatory power and relationship of each variable studied with management types and other variables.

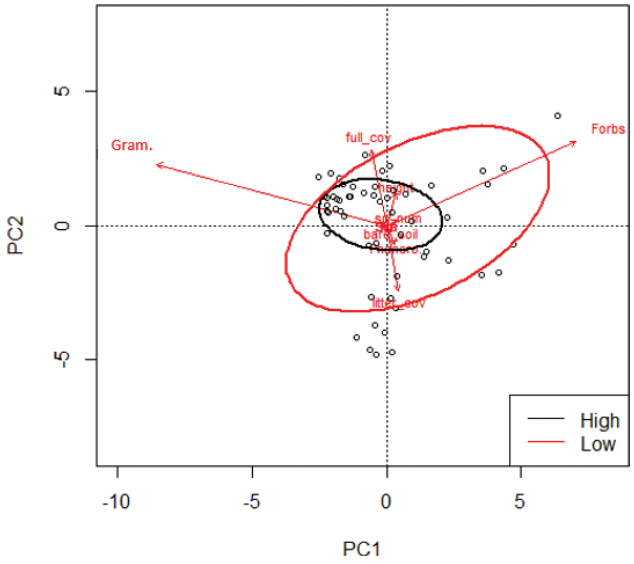

Figure 3. Principal Component Analysis of diversity indices, plant functional type cover and physiognomic factors across herbage removal intensity levels. The meaning of the abbreviations used in this Figure is given in the legend to Fig. 2.

ciated with higher graminoid cover (Fig. 4). Further details on the importance of the principal components, based on the proportion of variance explained by them, can be found in Appendix 8.

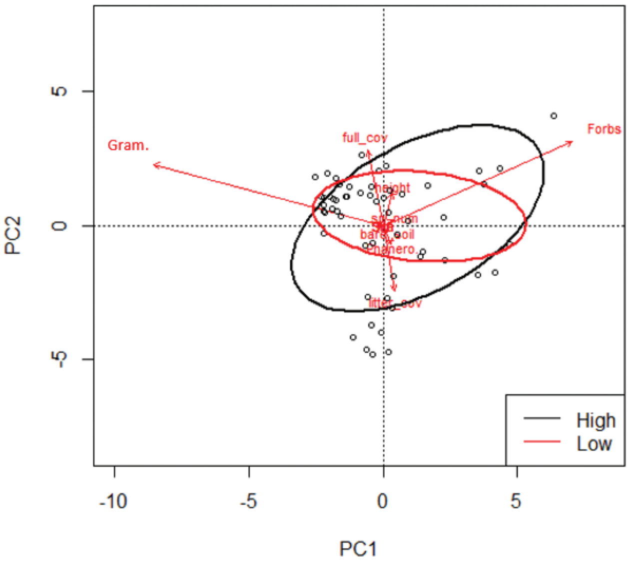

Figure 4. Principal Component Analysis of diversity indices, plant functional type cover and physiognomic factors across management complexity levels. The meaning of the abbreviations used in this Figure is given in the legend to Fig. 2.

Discussion

Effects of different management, plant functional type cover and physiognomic factors on grassland diversity

Different management types, mainly mowing and low and high levels of herbage removal intensity and management complexity, significantly affected the species composition and dissimilarity ratios of the grasslands studied (Fig. 1, Appendix 7). In addition, we found a strong positive effect of high management complexity (***C***) on species number and, to a lesser extent, on Shannon and Simpson diversity and forbs and a negative effect on predominantly perennial and clonal graminoids (Figs 1, 4). The ***C*** increases when different management types (***T***) and herbage removal intensities (***I***) are varied in space and time (see Table 1; Vadász et al. (2016)). Certain species or groups of species are likely to prefer certain combinations of ***T*** and ***I***, while they may become locally extinct if other combinations are practised for a long time. When ***C*** is high, many combinations of ***T*** and ***I*** occur at least once within a time-frame of a few years, providing opportunities for most species to experience a favourable year, preventing extinction (Catorci et al. 2014; Kun et al. 2021). For physiognomic factors (e.g. litter cover and average plant height), ***C*** levels did not play a significant role and these variables are better determined by the type of management.

Although different ***T*** choices played a less important role in influencing compositional diversity, the choice of the appropriate management type was also significant: grazing had a more positive effect on phanerophytes than mowing (Appendix 4). This difference can be explained by the most extensive, professional cattle grazing on the studied grasslands and by the selective and structuring grazing behaviour of cattle (i.e. cattle avoid shrubs etc.) and/or other grazers (Dumont et al. 2012; Molnár et al. 2020). The presence of phanerophyte species and their adequate control by grazing can lead to greater structural or physiognomic heterogeneity of grasslands. The effect of grazing is in contrast to that of mowing machines, which cut all plants uniformly in mid-summer with a low stubble height. As a result, mown sites could become more homogeneous in vegetation structure. By creating microhabitats and increasing structural variability by allowing a greater cover of phanerophyte species (mostly native shrubs, such as *Crataegus monogyna*, *Prunus spinosa* etc.), extensive grazing can contribute to the generative reproduction of herbaceous plants in grasslands (Kelemen et al. 2017). Furthermore, the application of grazing, mowing or a combination of both also resulted in slight differences in Jaccard-based species composition (but not low or high ***I*** and ***C*** levels) (Appendix 7). We argue that these phenomena may positively influence species richness. The nurturing effect of shrub species may help the generative and vegetative reproduction of grassland species in the actively managed natural and semi-natural grassland communities in the forest-steppe zone (Kelemen et al. 2017). On the other hand, it is fundamental to keep the phanerophyte cover within an optimal range (~ 1–10%), which prevents reforestation. An extensive grazing regime can be an efficient way to optimally control the number of shrubs on grasslands. Like shrubs, many forbs can be considered important microhabitat and structure-providing species, based on our field observations (e.g. *Serratula tinctoria*, *Sanguisorba officinalis* and *Genista tinctoria*). Due to several rare and specialist members (e.g. *Iris spuria*, *Centaurea scabiosa* subsp. *sadleriana* and *Ophrys* spp., etc.), native, annual and characteristic forbs are also important conservation targets. The occurrence and diversity of forbs in European steppe or forest-steppe grasslands have a long evolutionary history (Bråthen et al. 2020).

The increase of clonal, often highly competitive graminoid species with higher biomass production can reduce plant diversity (Deák et al. 2011; Házi et al. 2011; Szentes et al. 2012) and suppress conservation target species in grasslands (Kőrösi et al. 2014; Szépligeti et al. 2018), for example, several native forb species and their proportions (Figs 2–4). Therefore, the optimal and continuous control of clonal, competitive graminoids and the maintenance of optimal proportions of native and often specialist forbs is important in conservation practices for high nature value grasslands (Kun et al. 2021). This is most likely to be facilitated by high levels of management complexity and low levels of herbage removal intensity grazing (Figs 2–4). On the other hand, there was no significant difference between low and high levels of spatio-temporal complexity, herbage removal intensity or management type on graminoid cover, based on linear mixed model post hoc tests and, therefore, further studies are needed to analyse these relationships.

Importance and challenges of studying the management complexity and other management factors in grassland conservation locally and across regions

Based on our results, special attention should be paid to the multiplicity of management factors (e.g. different management types or herbage removal intensity levels), including their spatio-temporal variability (Kun et al. 2021). We argue that taking these aspects into account can provide practitioners and stakeholders with more straightforward guidelines for conserving and restoring grassland diversity in the Turján Region. Local and regional scale case studies, as well as large-scale, comprehensive and comparative analyses of the effectiveness of different grassland conservation management techniques on different high nature value grassland communities in different regions, should be carried out in the future to gain more detailed and broader knowledge (see, for example, Fischer and Wipf (2002); Socher et al. (2012); Vadász et al. (2016); Kun et al. (2019); Rac et al. (2020)). This should provide a more complex view of the relationship between management practices and conservation objectives at the regional level, which could help to adapt grassland management to local conditions and challenges. Due to the often poor explanatory power of one-factor models, controversial management practices may arise in several cases (Babai et al. 2015; Kun et al. 2019), which may lead to locally ineffective conservation management (Vadász et al. 2016).

On the other hand, although we found that high management complexity is beneficial for grassland conservation, it may be difficult to apply such management complexity and the same methods in practice in other regions, for example, for several individual farmers. Our conclusions are most relevant in terms of the exact management complexity which we have investigated in our study. Each region is different in terms of management possibilities and environmental factors. It can be difficult to graze a site one year and mow it the next or to vary the intensity of management. It is also important to note that spatially and temporally complex management can be achieved in more ways than we have explored in our study. There are other and/or simpler ways, for example, mowing only every other year, mowing at the beginning of summer one year and at the end of summer the next. The use of different grazing animals and the leaving of uncut lines in different places on a grassland between years can also be effective tools for more complex management, depending on local conservation objectives and opportunities.

However, there are often practical difficulties in applying multiple aspects of management to the modelling of community diversity. Including more explanatory variables in a model requires larger sample sizes and a more balanced sample distribution (Harrison et al. 2018). Ideally, all possible factor combinations should be present in sufficient replicates without spatial autocorrelation across the study area. However, ongoing management plans are typically designed to meet different, often non-scientific, objectives and the actual management design rarely satisfies statistical assumptions. One can sample what is out there and if certain combinations of factors simply do not exist in reality, they will not be present in the statistical model. This increases multicollinearity in the models and makes it more difficult to distinguish the effects of different management factors (Graham 2003). This is a likely explanation for why the explanatory power of management type, intensity and complexity was similar in our models. Balanced sampling designs are relatively easier to achieve in experiments where factor levels and spatial structure can be varied to meet statistical requirements. On the other hand, more detailed assessments, based on multiple management factors in different parts and regions of Europe, would allow us to identify more comprehensively and accurately what should be included in conservation systems at larger scales, as well as in local practices.

Implications

Our aim was to collect, organise and compare the elements of the hard-to-compare, mosaic-like landscape of use according to various parameters, using systematic sampling and to quantify and generalise the treatment results obtained mainly through experience. We must emphasise as an important message to legislators and developers of support schemes that because each site is different, generalisation is limited.

High levels of management complexity and grazing as a management type are more positive and have a greater significance for grassland conservation (i.e. result in higher plant diversity, higher proportion of forbs etc.) than the intensity of herbage removal in our study area. At the same time, mowing and/or low levels of management complexity may have some negative effects on conservation value. These analyses can be used to identify what are the strong or direct and less strong or indirect effects in the conservation of high nature value grasslands. Further research is needed to verify these relationships across a wider range of different study systems in order to provide generalisable guidelines for conservation.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Appendix 1

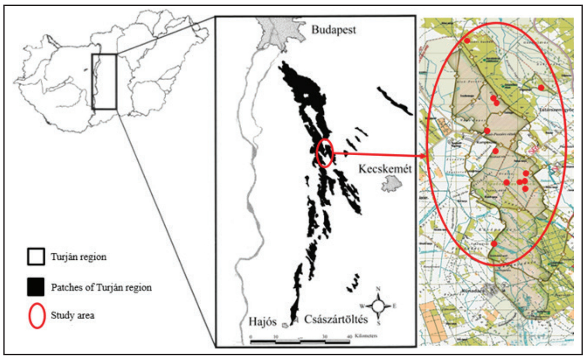

Figure A1. Study area and location of the twelve sampled meso-xeric grassland sites (Knipl and Sümegi 2012).

Appendix 2

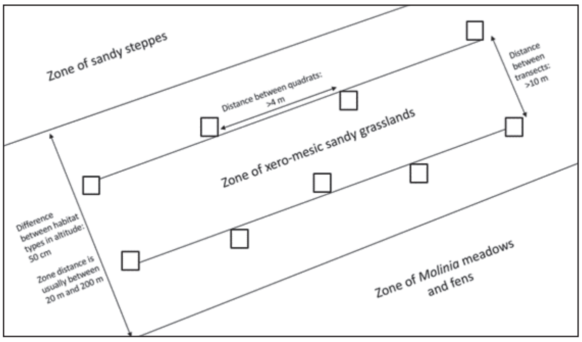

Figure A2. Distances between an elevation of Molinia meadows and fens and a sandy steppe zone. For the field sampling, nine plots were established along two transects in each grassland site. The distance between two transects was at least 10 m and the distance between the quadrats was at least 4 m.

Appendix 3

Table A1. Sampling design in the study area with factor combinations at each site and number of replicates.

Number of sites	Management type	Herbage removal intensity	Management complexity	Number of management factor combinations	Number of plots per site
1	Mown	Low	Low	1	9
2	Mown	Low	Low	1	9
3	Mown	Low	Low	1	9
4	Grazed	Low	High	2	9
5	Grazed	Low	High	2	9
6	Grazed	High	Low	3	9
7	Grazed	High	Low	3	9
8	Grazed	High	Low	3	9
9	Grazed	High	High	4	9
10	Combined	Low	Low	5	9
11	Combined	Low	High	6	9
12	Combined	High	High	7	9

Appendix 4

Table A2. Differences in PFT cover and diversity indices between different management types (mowing: *M*, grazing: *G* and combined: *C*) of semi-natural grasslands. The Table shows means and standard deviations of PFT groups and diversity indices. Significant differences in LMER Tukey post hoc tests between different management types are indicated by the letters 'a', 'b' and 'c'.

	MOWN	***GRAZED***	***COMBINED***
Species number	34.1±3.2a	34.9±5.8a	35.4±3.5a
Shannon diversity	1.6±0.4a	1.9±0.3b	1.8±0.3b
Simpson diversity	0.6±0.2a	0.7±0.1b	0.7±0.1b
Forbs cover (%)	19.0±11.9a	24.4±15.2a	31.1±20.2a
Graminoid cover (%)	82.2±11.3a	74.3±18.2a	57.8±22.2a
Phanerophytes cover (%)	2.2±1.7a	5.1±3.8b	4.0±4.0ab
Mean plant height (cm)	31.4±12.8a	26.7±8.4a	21.0±8.4a
Total plant cover (%)	94.0±3.3a	95.6±3.1a	87.6±8.7a
Bare soil surface (%)	0.6±0.4a	1.6±1.6a	1.8±1.9a
Litter cover (%)	5.7±3.3a	3.8±2.7a	10.9±7.7a

Appendix 5

Table A3. Effects of herbage removal intensity of management on plant diversity and cover of PFTs. Table shows means and standard deviations of PFT cover and diversity indices. Results are based on LMER Tukey post hoc tests. Significant differences between different intensity levels are indicated by the letters 'a' and 'b'.

	LOW	***HIGH***
Species number	34.3±4.7a	35.6±4.6a
Shannon diversity	1.7±0.4a	1.8±0.4a
Simpson diversity	0.7±0.1a	0.7±0.1a
Forbs cover (%)	26.4±19.1a	22.5±11.3a
Graminoid cover (%)	66.9±21.3a	79.5±15.1a
Phanerophytes cover (%)	4.4±3.9a	3.7±3.3a
Mean plant height (cm)	25.9±10.8a	26.5±9.2a
Total plant cover (%)	91.3±6.8a	96.0±3.3a
Bare soil surface (%)	1.3±1.7a	1.6±1.6a
Litter cover (%)	8.0±5.9a	3.2±2.4a

Appendix 6

Table A4. Differences between two levels of management complexity (low and high) on plant diversity and plant functional types. Table shows means and standard deviations of PFT cover and diversity indices. Results are based on LMER Tukey post hoc tests. Significant differences between different levels of management complexity are indicated by the letters 'a' and 'b'.

	LOW	***HIGH***
Species number	35.0±3.8a	34.6±5.8a
Shannon diversity	1.7±0.4a	1.9±0.4b
Simpson diversity	0.6±0.1a	0.7±0.1b
Forbs cover (%)	24.2±17.2a	25.6±15.3a
Graminoid cover (%)	76.3±21.2a	66.3±16.2a
Phanerophytes cover (%)	3.4±2.9a	5.2±4.3a
Mean plant height (cm)	29.7±10.2a	22.2±8.6a
Total plant cover (%)	94.4±4.5a	91.5±7.5a
Bare soil surface (%)	1.0±0.9a	1.8±2.0a
Litter cover (%)	5.2±3.9a	7.3±6.8a

Appendix 7

Table A5. Differences in species composition dissimilarity between management types and levels of herbage removal intensity and management complexity, based on PERMANOVA analyses and Jaccard dissimilarity index.

	Df	**Sums of Sqs**	**Mean Sqs**	**F-test**	**R^2**	**Pr(> F)**
T	2	3.126	1.563	5.792	0.099	0.001 ***
Residuals	105	28.332	0.270		0.901	
Total	107	31.458			1.000	
I	1	0.980	0.980	3.409	0.031	0.001 ***
Residuals	106	30.478	0.288		0.969	
Total	107	31.458			1.000	
C	1	1.783	1.783	6.368	0.057	0.001 ***
Residuals	106	29.675	0.280		0.943	
Total	107	31.458			1.000	

Appendix 8

Table A6. Proportion of principal components expressed by eigenvalues, explained and cumulative proportions and their contribution to the variance.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**
Eigenvalue	724.490	179.844	50.093	14.880	7.097	2.952	0.420
Explained share	0.739	0.184	0.051	0.015	0.007	0.003	0.000
Cumulative share	0.739	0.923	0.974	0.989	0.997	0.100	1.000

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Research Article

Environmental characteristics, including soil and vegetation composition, in relation to the occurrence patterns of an endangered lizard, *Eremias argus*, in a fluvial island, South Korea

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Abstract

Eremias argus, known as the Mongolian racerunner, is a reptile that has been designated as a level II endangered species in South Korea since 2005 despite being listed as "Least Concern" by the International Union for Conservation of Nature. Particular vegetation and soil characteristics are critical components of the habitat of *E. argus*, which is an ectotherm. However, research on the environmental characteristics of *E. argus* living on a fluvial island is lacking. This study sought to characterize the soil environmental factors and vegetation composition of *E. argus* habitats on Doriseom Island, South Korea by dividing the island into an area in which *E. argus* occurred frequently (F zone) and an area in which *E. argus* occurred rarely (R zone). Both soil hardness and cobble cover were significantly higher in the R zone (soil hardness: 1.6 ± 0.2 kg·cm^{-2}, mean ± standard error; cobble cover: 40 ± 5%) than in the F zone (soil hardness: 0.9 ± 0.1 kg·cm^{-2}; cobble cover: 18 ± 3%). Plant litter cover did not differ significantly based on *E. argus* occurrence. The vegetation composition within F and R zones appeared distinct, though *Coreopsis lanceolata* dominated both zones. A sand dune sedge, *Carex pumila*, thrived in F zone sites, where soil hardness was low, while the endemic *Aster danyangensis*, which prefers cobble areas, was found largely in the R zone. In conclusion, *E. argus* was most commonly found in areas with low soil hardness dominated by dune vegetation. Understanding endangered species' habitat requirements can provide important clues for establishing conservation plans and restoration measures.

Key words: Cobble cover, conservation, Mongolian racerunner, reptile, soil hardness

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Introduction

The Intergovernmental Panel on Climate Change (IPCC) has stated that endangered species are at risk under projected climate change during and beyond the 21st century, especially as climate change interacts with other factors, such as habitat modification (Doherty et al. 2020), over-exploitation (Auliya et al. 2016), environmental pollution (Gibbons et al. 2000), and invasive species (Dueñas et al. 2021). Specifically, a considerably larger percentage of amphibians and reptiles are at risk compared to birds or mammals (Cordier et al. 2021; IUCN 2023). Although reptiles are important members of food chains and play pivotal roles in maintaining ecosystem dynamics (Ali et al. 2018), reptile populations have experienced serious declines worldwide (Chang et al. 2022), and approximately 20% of reptile species are considered threatened (IUCN 2023).

Eremias argus, known commonly as the Mongolian racerunner, is a small lacertid lizard in the family Lacertidae. The species has a body length of 150–200 mm, including the tail, and has a limited distribution in the Korean Peninsula, Mongolia, China, and Russia (Park et al. 2014). It is known as a specialist that generally occupies grassy sand dunes and riverbanks with little vegetation (Park et al. 2014). *Eremias argus* has been designated as a level II endangered species by the Ministry of Environment in South Korea since 2005 (Song et al. 2013), although it is listed as Least Concern by the International Union for Conservation of Nature (IUCN) (Orlova et al. 2019). In South Korea, *E. argus* populations are suffering a sharp decline because of habitat loss, degradation, and fragmentation resulting from coastal sand dune development (Kim et al. 2012b). In addition, *E. argus* populations in South Korea exhibit genetic fragmentation, indicating limited movement of individuals between subpopulations even when subpopulations are geographically close to one another (Park et al. 2014). In light of the *E. argus* populations' restricted range within Korea, Mongolia, China, and Russia, it is evident that the long-term viability of the species hinges on the conservation and restoration of its native habitats in these countries.

Globally, several studies on *E. argus* have been conducted, including research into the species' population genetics (Zhao et al. 2011), metabolism (Chen et al. 2016), herbicide sensitivity (Wang et al. 2021), and reproductive strategy (Sun et al. 2013). Within South Korea, studies considering population genetics (Park et al. 2014), mating behavior (Kim et al. 2012a), population size (Song et al. 2013; Chang et al. 2021), habitat distribution (Do et al. 2022), and food resources (Jeong and Song 2010) have been conducted. While extensive studies have been conducted on the coastal sand dune and mountain habitats of *E. argus* (Kim et al. 2011; Kim et al. 2012b), research into the species' habitat environment, such as the vegetation structure and soil characteristics, on a fluvial island remains notably limited.

The effectiveness of conservation programs targeting endangered species relies heavily on a comprehensive understanding of these species' specific habitat needs (Souter et al. 2007). Environmental characteristics are the result of interactions between biotic and abiotic factors, such as those relating to soil, vegetation, and microorganisms; these factors are highly interrelated and each is important to habitat formation (Reynolds et al. 2003). Soil properties are significantly influenced by certain environmental factors such as climate, topography, parent materials, vegetation, and disturbance due to human activity (Chen et al. 1997; Bakhshandeh et al. 2014; Cazzuffi et al. 2014). In particular, plant roots influence soil slope stability and erosion processes by impacting both hydrological processes and the geomechanical structure of the soil (Cazzuffi et al. 2014). In addition, plant roots alter their local environment in a number of ways, from modifying the soil's biophysical, chemical, and mechanical properties, to stimulating microbial abundance and diversity, ultimately reinforcing soil mechanically (Stokes et al. 2014). The physical characteristics of soil, such as its hardness and porosity, are largely influenced by vegetation (Huang et al. 2007) and can impact the burrowing choices of different lizard species (Zaady and Bouskila 2002; Zeng et al. 2016). In addition, the growth and regenerative capacity of vegetation can be significantly affected by soil compaction (Amrein et al. 2005). Furthermore, external disturbances can exacerbate soil compaction in a positive feedback loop when direct compaction destroys vegetation cover (Garden et al. 2007). For reptiles in particular, vegetation and soil are crucial components of species' habitat requirements, underscoring the significance of understanding these factors.

The goal of this study was to characterize the vegetation composition and soil environmental factors of *E. argus* living on the fluvial island, Doriseom Island, in South Korea. Because many elements constitute a habitat, this study was conducted by measuring not only abiotic factors such as soil hardness, cobble cover, and plant litter cover, but also biotic factors such as vegetation composition. We then compared how these environmental components differed between areas where *E. argus* individuals are common and areas where they are rare.

Methods

Study site

This study was conducted from May to November 2022 on Doriseom Island (37°13'34.6"N, 127°43'17.9"E), which is located in Yeoju-si, Gyeonggi-do, South Korea. Doriseom Island is a fluvial island at the confluence of the Namhangang River and the Cheongmicheon Stream (Fig. 1a, b). The island is approximately 906 m in length at its longest point, approximately 520 m wide, and has an area of approximately 292,370 m^2. During the nearly 30 years from 1991 to 2020, the average annual temperature on Doriseom Island was 11.7 °C, and the average annual precipitation was 1,315.9 mm (KMA 2023). Doriseom Island has an entirely flat terrain formed by the accumulation of alluvial soil approximately 5 m above the surface of the Namhangang River and the Cheongmicheon Stream (National Geographic Information Institute 2021). There is no river running through the interior of the island, and during major floods in the summer, the entire island can be affected by flooding. There are no inhabitants and houses, although some researchers visit the island to study the plants and animals.

Doriseom Island is a valuable and unique habitat, particularly for the resident reptile *Eremias argus* and flowering plant *Aster danyangensis*, both of which are classified as endangered wildlife class II by the Ministry of Environment (Macias et al. 2021). However, anthropogenic activities such as a river maintenance project have led to habitat reduction, which has been identified as a major threat to *E. argus* (Macias et al. 2021).

We first recorded the distribution of *E. argus*, which largely occurs in the southwest of Doriseom Island (86 sites) and is rarer in the northeast (10 sites; Fig. 1c). We then used the frequency of *E. argus* occurrences to divide Doriseom Island into two zones: a zone in which *E. argus* frequently occurs in the southwest (F zone) and a zone in which *E. argus* rarely occurs in the northeast (R zone; Fig. 1d).

Vegetation survey

The vegetation survey to identify plant community structure was conducted by randomly installing 1 m × 1 m quadrats (n = 138; 78 in the F zone and 60 in the R zone) in Doriseom Island from May to November 2022. In each quadrat, the plant cover (%) was surveyed and recorded in 5% increments, and plant species

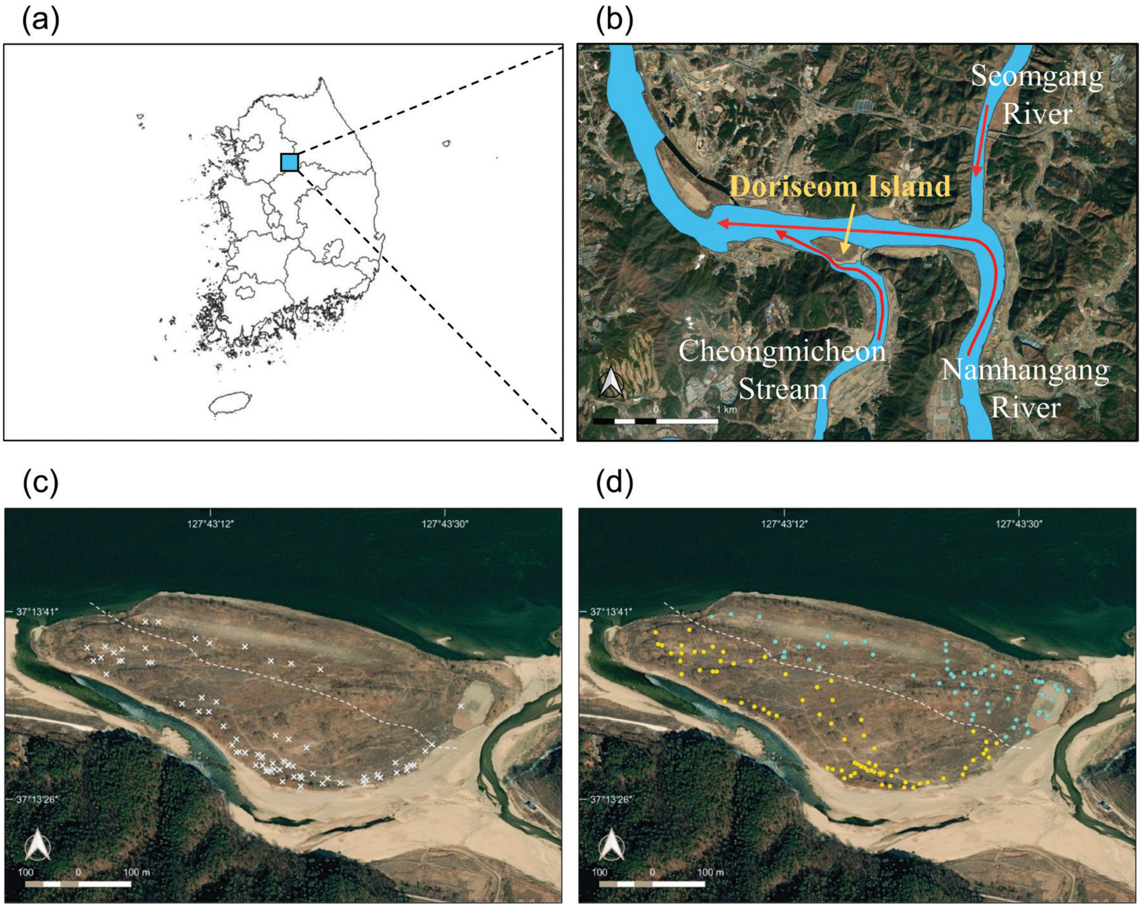

Figure 1. Location of *Eremias argus* study sites on Doriseom Island **a** location of the survey site on a map of South Korea **b** Doriseom Island is at the confluence of the Namhangang River and the Cheongmicheon Stream **c** the 96 sites where *E. argus* was found are indicated with white cross symbols (Bing Maps, working with QGIS 3.22.16) **d** the zones where *E. argus* occurred frequently (frequent zone, hereafter F zone) and rarely (rare zone, hereafter R zone) were determined. F zone sites are marked with yellow points (n = 78), while R zone sites are marked with mint-colored points (n = 60). At each point, the vegetation composition and soil environmental factors were surveyed.

were identified using various references (Lee 2003; Park 2009). In addition, we classified the observed plants into exotic and native plants. The nomenclature followed the "World Flora Online" (https://wfoplantlist.org/plant-list), the "Korea Biodiversity Information System Database" (http://www.nature.go.kr/main/Main.do) and Korea National Arboretum (2022).

Measurement of environmental factors

Three environmental factors were measured: soil hardness (kg·cm^{-2}), cobble cover (%), and plant litter cover (%). Soil hardness was measured with five randomly selected replicates in each quadrat using a pocket penetrometer (Forestry Suppliers, Inc., 77114, Jackson, United States), and an average value of soil hardness was calculated from the five replicates. If the soil was covered with cobbles and it was impossible to measure soil hardness, soil hardness was recorded as 4.5 kg·cm^{-2}, which was the maximum value of the soil hardness gauge (measurement range 0.01–4.5 kg·cm^{-2}). Like the plant cover, the cobble and plant litter covers (%) were recorded in 5% increments within each quadrat.

Data analysis

All statistical analyses were performed using R statistical software ver. 4.2.1 (R Core Team 2022) and the vegan (Oksanen et al. 2022), lawstat (Gastwirth et al. 2020), and ggplot2 (Wickham 2016) packages.

First, to identify significant differences in vegetation composition between the F zone and the R zone, we performed a permutational multivariate analysis of variance (PMAV) with the "adonis" function from the vegan package using Bray–Curtis distance (Oksanen et al. 2022). Standard deviational ellipses ("ordiellipse" function) were plotted in the NMDS ordination to describe zone site compositional differences. Species cover values were log-transformed (log(cover + 1)) to minimize the influence of rare plant species (Májeková et al. 2016). To examine how the dominant plant species differed between the two zones, we performed a rank-sum analysis. The percent cover of plant species in each quadrat was summed and each species' total sum of cover was sorted in descending order and visualized in a rank sum plot. In addition, native and exotic species richness and the percent cover of *Coreopsis lanceolata*, which was the dominant plant species in Doriseom Island, were compared using a *t*-test. Prior to the *t*-test, homogeneity of variance was tested through the "levene.test" function in the lawstat package (Gastwirth et al. 2020). In addition, the environmental factors (soil hardness, cobble cover, and plant litter cover) were compared between F and R zones using a *t*-test analysis for equal variances and Welch's test for heteroscedasticity (Zimmerman and Zumbo 1993; Burns 2006).

Second, non-metric multidimensional scaling (NMDS) was performed to elucidate the relationship between vegetation composition and the environmental factors (soil hardness, cobble cover, and plant litter cover) using the "metaMDS" and "envfit" functions from the vegan package (Oksanen et al. 2022). In addition, vegan's "ordisurf" function was used to visualize the gradient of environmental factors (soil hardness, cobble cover, and plant litter cover) in the NMDS ordinations.

Results

Vegetation composition

In a vegetation survey, 69 species belonging to 47 genera within 18 families were observed: Poaceae (25 species, 36%), Asteraceae (9 species, 13%), Cyperaceae (6 species, 8%), and other families (Appendix 1). Exotic plant species, including ecosystem-disturbing species designated by the Korean Ministry of Environment, accounted for approximately 42% of the total flora and were represented by 29 species. There were 27 species of plants common to both F and R zones, as well as 23 species found only in the F zone and 19 species found only in the R zone. For example, the dune sedge *Carex pumila* was found only in the F zone, while *Aster danyangensis*, which thrives in cobble environments, was found only in the R zone.

Coreopsis lanceolata showed an overwhelmingly high total cover in both zones (F zone: 2,771%, R zone: 1,571%; Fig. 2). Of the 20 plant species with the highest total percent cover, *C. lanceolata*, *Carex pumila*, and *Artemisia indica* had the highest percent cover in the F zone, while *C. lanceolata*, *Themeda triandra*, and *A. indica* had the highest percent cover in the R zone.

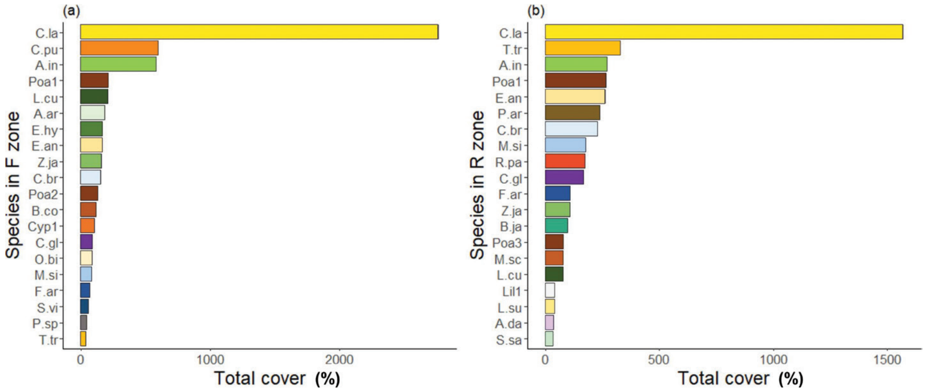

Figure 2. Rank-sum plot showing the sum of the total cover of plant species within the surveyed quadrats **a** F zone (*n* = 78) and **b** R zone (*n* = 60). Only the top 20 cover values for each zone are shown. Species are ordered from most abundant to least abundant. Abbreviations indicate plant species names, and the full species name is provided in Appendix 1.

Table 1. Species richness and average cover of *Coreopsis lanceolata* according to the frequency of *Eremias argus* occurrence in F and R zones. SE, standard error. *, significant difference ($p < 0.05$).

Index	F zone (*n* = 78)		*R zone* (*n* = *60*)	
	Mean	SE	Mean	SE
Total plant richness	4.7*	0.2	4.0	0.2
Native species richness	2.5*	0.2	2.1	0.2
Exotic species richness	2.2	0.1	2.0	0.2
Cover of *Coreopsis lanceolata* (%)	36*	3	26	3

Native plant richness in the F zone (2.5 ± 0.2, mean ± standard error) was significantly higher than that in the R zone (2.1 ± 0.2; Table 1). On the other hand, exotic plant richness was 2.2 ± 0.1 in the F zone and 2.0 ± 0.2 in the R zone, representing no significant difference. *C. lanceolata* average cover was 36 ± 3% in the F zone and 26 ± 3% in the R zone. *Coreopsis lanceolata* was the dominant species in the two zones, but its percent cover was significantly higher in the F zone ($p < 0.05$).

Relationship between vegetation composition and soil environmental factors

In the comparison of communities using Bray–Curtis distance, the vegetation composition in F and R zones differed significantly according to the frequency of *E. argus* occurrence (PMAV; $F = 4.28$; $p = 0.001$; $r^2 = 0.03$), although the r^2 value is very low. An NMDS was performed to elucidate the relationships between vegetation and soil environmental factors in Doriseom Island (stress value = 0.186). As a result, all three environmental factors (soil hardness, cobble cover, and plant litter cover) had a significant effect on the vegetation composition ($p < 0.05$; Fig. 3). In addition, as soil hardness increased, the amount of plant litter (such as dead plant leaves and twigs) on the surface tended to decrease.

Soil hardness showed a positive relationship with *C. lanceolata* (C.la) and *Metaplexis japonica* (M.ja), but was negatively correlated with *A. indica* (A.in) and *Fallopia dumetorum* (F.du), which were related with low plant litter cover.

Cobble cover was positively correlated with *Galium verum* subsp. *asiaticum* (G.ve) and *Populus nigra* (P.de), but showed a negative correlation with *Ambrosia artemisiifolia* (A.ar) and *Miscanthus sinensis* var. *purpurascens* (M.si).

Comparison of soil environmental factors

We compared soil environmental factors according to the frequency of *E. argus* occurrence and found significant differences in soil hardness and cobble cover. Soil hardness was 0.9 ± 0.1 kg·cm^{-2} in the F zone and significantly higher at 1.6 ± 0.2 kg·cm^{-2} in the R zone ($p < 0.05$; Fig. 4a). Particularly, a significant difference was observed in cobble cover, which was 18 ± 3% in the F zone and 40 ± 5% in the R zone ($p < 0.01$; Fig. 4b). On the other hand, plant litter cover was 59 ± 3% in the F zone and 49 ± 5% in the R zone, being statistically similar regardless of the frequency of *E. argus* occurrence ($p > 0.05$; Fig. 4c).

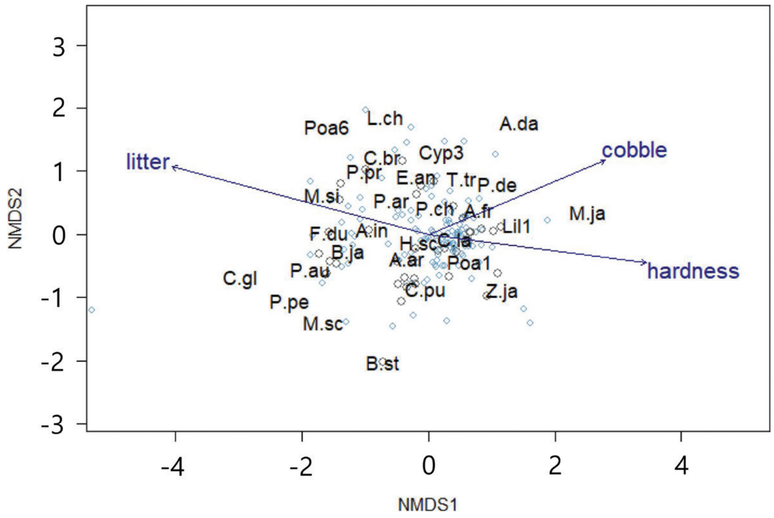

Figure 3. Non-metric multidimensional scaling (NMDS) plot representing the relationships between vegetation composition and soil environmental factors (stress value = 0.18). Plant cover data were log-transformed (log(cover + 1)), and raw, untransformed environmental factor data were used. Abbreviations indicate plant species names, and the full species name is provided in Appendix 1. Blue points indicate the surveyed quadrats, and grey points indicate rare plant species. The vectors with blue arrows show significant soil environmental factors ($p < 0.05$).

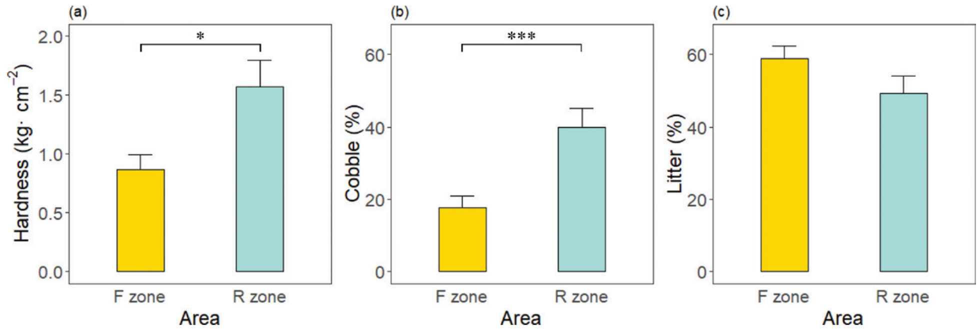

Figure 4. Differences in soil environmental factors (soil hardness, cobble cover, and plant litter cover) according to the occurrence patterns of *Eremias argus*. The yellow bar represents the zone with a high frequency of *E. argus* (F zone, $n = 78$), and the mint-colored bar represents the zone where *E. argus* is rare (R zone, $n = 60$). Differences in **a** soil hardness **b** cobble cover, and **c** plant litter cover between these two zones were considered. Standard errors are shown as error bars, and signs above the bars indicate whether the values differ from two zones (* $p < 0.05$, *** $p < 0.001$).

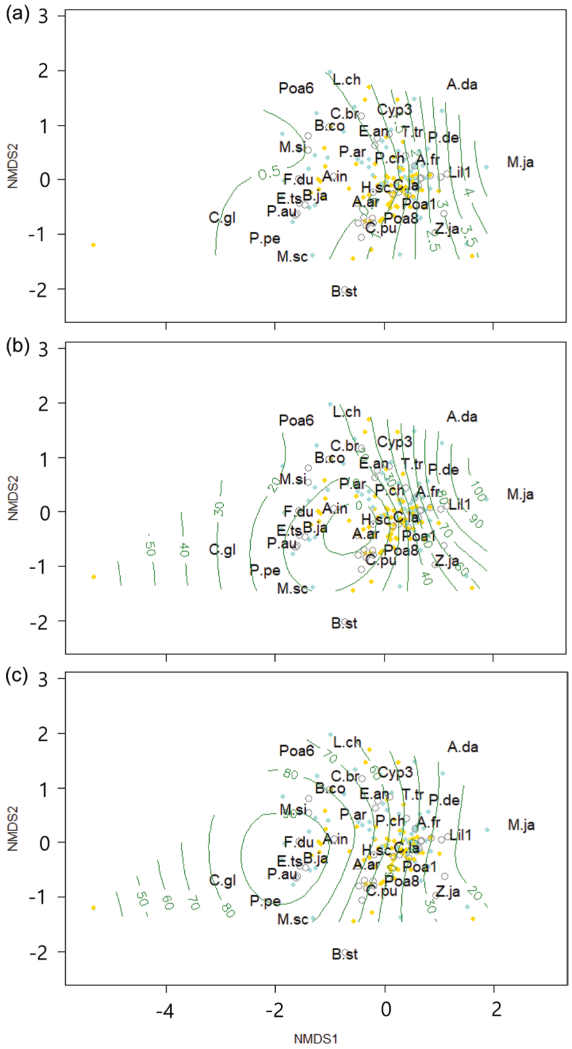

Figure 5. NMDS representation of the relationship between soil environmental factors and surveyed areas based on *Eremias argus* occurrence patterns. The yellow points indicate the zone with a high frequency of *E. argus* (F zone), and the mint-colored points indicate the zone where *E. argus* was rare (R zone). Differences in **a** soil hardness **b** cobble cover, and **c** plant litter cover between these two zones were considered. Additionally, the "ordisurf" function from the vegan package was used to separate the range of environmental factors with green lines.

Soil hardness ranged between 0.5–2 kg·cm^{-2} in most quadrats regardless of the frequency of *E. argus* occurrence, but high hardness values (2 kg·cm^{-2} or more) were more common in the R zone ($p < 0.05$; Fig. 5a). Cobble cover ranged from 0 to 40% in most areas, but values above 70% appeared only in the R zone ($p < 0.001$; Fig. 5b). Plant litter cover was similar in both zones ($p > 0.05$; Fig. 5c).

Discussion

Given the wide variation in *E. argus* occurrence within Doriseom Island, we divided the island into two zones based on the frequency of *E. argus* occurrence and examined how the two areas differed in terms of vegetation composition and soil physical environment. *Eremias argus* frequently appeared in areas with lower soil hardness and less cobble cover.

Vegetation composition

According to the vegetation survey on Doriseom Island, *Coreopsis lanceolata*, *Artemisia indica*, and *Erigeron annuus* were dominant in both F and R zones, but there were slightly significant differences in species composition. In the F zone, the percent cover of *Carex pumila* and *Ambrosia artemisiifolia* was high, while in the R zone, grasses such as *Themeda triandra*, *Phalaris arundinacea*, and *Miscanthus sinensis* var. *purpurascens*, as well as a Korean endemic plant, *Aster danyangensis*, showed high percent cover (Fig. 2).

The most dominant herbaceous species on Doriseom Island, *C. lanceolata*, was positively correlated with soil hardness (Fig. 3). However, *C. lanceolata* was more abundant in the F zone, where the soil hardness was lower than that in the R zone, indicating that this species was widely distributed throughout the island. Compared to the R zone, which was mostly covered with cobbles and had high soil hardness, the F zone, in which *E. argus* was more common, had some sandy soil with low soil hardness. In addition, plant richness and native plant richness was higher in the F zone than in the R zone, where the soil hardness was especially high. High soil hardness can obstruct plant growth for several reasons (Unger and Kaspar 1994), including hindrance of root penetration (Singh et al. 2015), low soil water retention (Ngo-Cong et al. 2021), and reduced soil aeration (Watson and Kelsey 2006).

Generally, *C. lanceolata* readily adapts to diverse ecosystems such as cut slopes, roads, and riverbanks (KIE 2018). It is also known to thrive in a wide range of climates, from temperate to subtropical (Batianoff and Halford 2002). *Coreopsis lanceolata* is native to the eastern United States and is considered a potentially serious weed in temperate to subtropical regions of Australia (Batianoff and Halford 2002). In addition, it is listed as one of Japan's 100 most noxious invasive plants (Arifin and Okamoto 2023). This plant species limits the light available to native vegetation (Arifin and Okamoto 2023) and spreads from the approximately 12,000 seeds produced each flowering season (Zeng et al. 2014) or by vegetative growth (Batianoff and Halford 2002). In the future, it will become necessary to monitor how quickly *C. lanceolata* will spread in Doriseom Island.

Other species that demonstrated high cover in both the F and R zones were *A. indica* and *E. annuus*, which were generally distributed across Doriseom Island. *Artemisia indica* and *E. annuus* are typical pioneer species, dominating areas shortly after a disturbance (Pacanoski 2017). This pattern suggests that Doriseom Island frequently experiences disturbances such as periodic flooding and human visits.

Unlike *C. lanceolata*, *A. indica*, and *E. annuus*, a few species, like the sedge *C. pumila*, exhibited a particularly high percent cover in areas with relatively low soil hardness in the range of 1.0–1.5 kg·cm^{-2} (Fig. 5a). *Carex pumila* is a typical dune plant that thrives in sandy soil with low hardness (Burgess 1984). This plant can be seen in the coastal sand dunes of Sinduri in South Korea, which is the habitat for the Mongolian racerunner, *E. argus*, (Lee et al. 2020), mirroring our findings. *Carex pumila* is also found in the coastal sand dunes of southern South Korea and Jeju Island (Oh and Kim 2008). Therefore, *E. argus* and *C. pumila* appear to inhabit similar environments.

On the other hand, certain species were rarely found in the F zone but were common in the R zone. This pattern was especially true of *A. danyangensis*. This plant is endemic to South Korea, growing in limited areas of the Namhangang River basin, and is listed as a class II endangered species by the Ministry of Environment (Kim et al. 2022). *A. danyangensis* was especially abundant in the R zone and exhibited a strong positive correlation with cobble cover. More specifically, *A. danyangensis* only thrived in areas where cobble cover exceeded 80% (Fig. 5b). *A. danyangensis* typically inhabits riverside gravel sandbars, creating a shaded environment when the gravel and cobble remain unembedded and exposed on the surface (Lee et al. 2017). This microenvironment is known to be an important factor in the germination and establishment of *A. danyangensis* seedlings (Kagaya et al. 2008). In fact, an alternative habitat was created to restore the population of *A. danyangensis* in the eastern part of the R zone. Since the surface of the alternative habitat was covered with cobbles with a diameter of about 7 cm or more, the frequency and cover of *A. danyangensis* is judged to have a large positive relationship with cobble.

In general, herbaceous species dominate Doriseom Island, but woody and shrub species such as black poplar (*Populus nigra*), black locust (*Robinia pseudoacacia*), and indigo bush (*Amorpha fruticosa*) are becoming more common. Menke (2003) reported that a number of lizard species that occupy grassland habitats are threatened by the invasion of woody bushes. Therefore, the environmental condition of *E. argus* living on Doriseom Island may also be threatened by the expanding tree population. In addition to trees, river flooding and human visits represent disturbances on Doriseom Island. Garden et al. (2007) found a positive correlation between the presence of native reptiles and a moderate level of weed cover, ranging from 25% to 50%, indicating that the structure offered by vegetation (e.g., refuge) may be more important than vegetation composition for lizard habitat. These findings support those of Pinto et al. (2018) who noted that on a micro-habitat level, the likelihood of reptile presence, as well as their abundance and species diversity, grew as plant diversity increased. On the other hand, Jellinek et al. (2004) reported that overall lizard species richness was negatively associated with increased plant cover. The relationship between reptiles and vegetation can vary greatly depending on the species (Scott et al. 2006; Mizsei et al. 2020). Some reptiles prefer habitats with dense vegetation for a variety of reasons, including the vegetation's role in offering shelter and protection from adverse conditions (Attum et al. 2006), refuge from predators (Sato et al. 2014), and reproduction sites (Martin and Murray 2011). Moreover, unlike in our study, *E. argus* have been found to be more abundant in areas with dense vegetation, preferring the warm thermal environment of denser habitats (Zeng et al. 2016). Consequently, generalizing about the significance of plant cover for all reptiles is challenging.

Soil environmental characteristics for *E. argus*

Our result establishing that the areas where *E. argus* frequently appeared had a low cobble cover and low soil hardness is consistent with the findings of previous studies, which have shown that reptiles are absent from environments with high soil compaction (Garden et al. 2007). Individuals in one coastal dune region consistently utilized the grass dune habitat throughout the year, using it even for hibernation (Kim et al. 2012b). *Eremias argus* is most commonly found in areas with low soil hardness that are dominated by dune vegetation, which is similar to *E. argus* habitat in South Korea (Song et al. 2013; Lee et al. 2020). Since sand dunes are typically arid environments known for their very low solidity (Zaady and

Bouskila 2002), they are preferred by *E. argus*, which lay eggs on the ground. Female *E. argus* lay around six eggs in a minimum of two batches, typically from late May to early July in South Korea (Kim et al. 2012a). Soil surface resistance, measured in this study as soil hardness, has been understood as important in burrow site selection for some burrowing lizards (Souter et al. 2007). Although lizards' preferred habitats may vary slightly, generally, habitats must provide essential areas for foraging, hiding, hibernation, egg-laying, and mating (Kim et al. 2012b).

Eremias argus is known as a specialist in dune areas (Park et al. 2014). However, habitat specialists are most likely to become locally extinct because they are rarely able to adapt to drastic habitat changes (Cordier et al. 2021). Furthermore, Doriseom Island is likely to be affected by habitat changes due to human activities and riverside development, resulting in changes in animal movement patterns (Doherty et al. 2021).

Environmental management strategies for *E. argus*

Vegetation composition and soil environment were found to differ according to the frequency of *E. argus* occurrence. However, several predominant plant species were ubiquitously distributed throughout Doriseom Island, and soil environment, especially soil hardness and cobble cover, may affect vegetation structure. In previous research conducted by Souter et al. (2007), no specific vegetation structure was found to be correlated with lizard occupancy within grassland habitats, but potential for soil type to influence the plant community was found. Carpio et al. (2017) also indicated that a more diverse herbaceous ground cover, containing a greater variety of species and resembling natural vegetation, would offer a better solution for promoting diversity in reptile assemblages within agricultural landscapes. Because specific plant species are less important for reptile habitats than the physical conditions that they create, a broader array of habitats, such as a combination of open spaces in close proximity to vegetation cover, can fulfill the requirements of a habitat with diverse structure (Edgar et al. 2010).

Coreopsis lanceolata, an exotic plant that is expanding and forming uniform patches across Doriseom Island, poses a potential threat to the habitat of *E. argus*. As the climate crisis intensifies, it facilitates the spread of invasive exotic species, which in turn changes the composition of native communities (Vilà et al. 2011). Some have also reported that when the average annual temperature rises by 1 °C, invasive exotic plants become better competitors; in addition, it is known that when invasive exotic species dominate a specific ecosystem, species diversity decreases and a highly homogenized community is created (Kortz and Magurran 2019; Mathakutha et al. 2019). Moreover, negative relationships have been observed between exotic plant cover and lizard species abundance and diversity (Jellinek et al. 2004). Hence, it is crucial to persistently monitor and conduct follow-up research regarding the proliferation of *C. lanceolata* on Doriseom Island.

Ecotones—the transitional zones between habitats such as sunny woodland edges, grassland-scrub interfaces, and interfaces within grasslands of varying vegetation heights—are especially important for reptiles (Urbina-Cardona et al. 2006; Edgar et al. 2010). These zones are characterized by having a diverse range of plant species and habitat structures, providing various microhabitats and microclimates that are particularly favored by reptiles (Edgar et al. 2010). Pinto et al. (2018) found that a Mediterranean lizard, *Psammodromus algirus*, showed a positive reaction to habitat diversity, with its population growing as habitat complexity increased. Furthermore, to ensure the viability of *E. argus* populations, it is crucial to preserve habitat connectivity and natural dispersal dynamics (Park et al. 2014).

The population size and distribution of *E. argus* may be related to factors shaped by surrounding vegetation, such as food availability, preferences for specific microhabitats, or interspecific interactions. Some preys of *E. argus* are known (grasshoppers, beetles, ants, leafhoppers, moths, bees, and spiders), which belong to Orthoptera, Lepidoptera, Araneae, Dermaptera, and Amphipoda, among others (Jeong and Song 2010; Huang et al. 2016). In our study, Poaceae family plants accounted for the highest proportion, comprising 25 species among 69 total plant species, providing *E. argus* populations protection from predators and allowing light to enter between the plants and help *E. argus* populations to bask properly (Kim et al. 2011; Chang et al. 2021). Although this study did not unveil the intricate connections among vegetation, soil, and food sources, future studies investigating habitat characteristics (vegetation structure and soil) and prey sources should yield additional knowledge to help inform a conservation strategy for *E. argus*.

Conclusions

This study investigated the soil environmental factors and vegetation composition of Doriseom Island, South Korea, where *E. argus* has been found. The island was divided into two zones: the frequently occurring *E. argus* area (F zone) and the rarely occurring *E. argus* area (R zone). The results revealed significant differences in soil hardness and cobble cover between the F and R zones. The R zone had higher soil hardness (1.6 ± 0.2 $kg{\cdot}cm^{-2}$) and greater cobble cover (40 ± 5%) compared to the F zone (soil hardness: 0.9 ± 0.1 $kg{\cdot}cm^{-2}$; cobble cover: 18 ± 3%). However, plant litter cover did not show significant variation based on *E. argus* occurrence. Distinct differences were observed in vegetation composition between the F and R zones, although *Coreopsis lanceolata* was dominant in both areas. *Carex pumila*, a sand dune sedge, thrived in the F zone where soil hardness was low, while the endemic *Aster danyangensis*, which prefers cobble areas, was predominantly found in the R zone. In conclusion, *E. argus* preferred environments with low soil hardness that were dominated by dune vegetation. Understanding the environmental requirements of endangered species like *E. argus* is crucial for developing effective conservation and restoration strategies. To provide inhabitable environments for the endangered lizard *E. argus* and endemic plant *A. danyangensis* within Doriseom Island, it is crucial to create a diverse soil environment (including sand and cobble). Additionally, it is important to prevent the spread of invasive plants and protect against excessive riverside development.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Eunhee Cho, fieldwork, plants identification, data analysis and document writing; Deokjoo Son, fieldwork, plants identification, document writing and completed document review.

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Data availability

All of the data that support the findings of this study are available in the main text

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Appendix 1

Table A1. Plant species code and scientific name.

Species code	Scientific name	Family	Common name	Zone
A.ar	*Ambrosia artemisiifolia* L.	Asteraceae	Common ragweed (돼지풀)	F, R
A.ca	*Artemisia capillaris* Thunb.	Asteraceae	Capillary wormwood (사철쑥)	F, R
A.da	*Aster danyangensis* J.Y.Kim & G.Y.Chung	Asteraceae	Danyang aster (단양쑥부쟁이)	R
A.fr	*Amorpha fruticosa* L.	Fabaceae	False indigo-bush (족제비싸리)	R
A.in	*Artemisia indica* Willd.	Asteraceae	Korean wormwood (쑥)	F, R
A.se	*Arenaria serpyllifolia* L.	Caryophyllaceae	Thyme-leaf sandwort (벼룩이자리)	F, R
B.co	*Bromus commutatus* Schrad.	Poaceae	Hairy chess (털큰참새귀리)	F
B.ja	*Bromus japonicus* Thunb.	Poaceae	Common brome (참새귀리)	F, R
B.st	*Bromus sterilis* L.	Poaceae	Barren brome (까락빕새귀리)	F
C.bi	*Cosmos bipinnatus* Cav.	Asteraceae	Garden cosmos (코스모스)	F
C.br	*Carex breviculmis* R.Br.	Cyperaceae	Short-stem sedge (청사초)	F, R
C.gl	*Carex glabrescens* (Kük.) Ohwi	Cyperaceae	Glabrate sedge (곱슬사초)	F, R
C.la	*Coreopsis lanceolata* L.	Asteraceae	Lanceleaf coreopsis (큰금계국)	F, R
C.pu	*Carex pumila* Thunb.	Cyperaceae	Dwarf sand sedge (좀보리사초)	F
Cyp1	N/A (Cyperaceae species 1)	Cyperaceae	Cyperaceae species 1 (사초과 1)	F
Cyp2	N/A (Cyperaceae species 2)	Cyperaceae	Cyperaceae species 2 (사초과 2)	F
Cyp3	N/A (Cyperaceae species 3)	Cyperaceae	Cyperaceae species 3 (사초과 3)	R
D.ch	*Dianthus chinensis* L.	Caryophyllaceae	Rainbow pink (패랭이꽃)	F, R

Species code	Scientific name	Family	Common name	Zone
D.in	*Duchesnea indica* (Andrews) Teschem.	Rosaceae	Wrinkled mock strawberry (뱀딸기)	R
D.ne	*Draba nemorosa* L.	Brassicaceae	Woodland whitlow-grass (꽃다지)	F
E.an	*Erigeron annuus* (L.) Pers.	Asteraceae	Annual fleabane (개망초)	F, R
E.ar	*Equisetum arvense* L.	Equisetaceae	Field horsetail (쇠뜨기)	F, R
E.hy	*Equisetum hyemale* L.	Equisetaceae	Scouringrush horsetail (속새)	F, R
E.ra	*Equisetum ramosissimum* Desf.	Equisetaceae	Branched horsetail (개속새)	F, R
E.ts	*Elymus tsukushiensis* Honda var. *transiens* (Hack.) K.Osada	Poaceae	Wheatgrass (개밀)	F
F.ar	*Festuca arundinacea* Schreb.	Poaceae	Tall fescue (큰김의털)	F, R
F.du	*Fallopia dumetorum* (L.) Holub	Polygonaceae	Copse buckwheat (닭의덩굴)	F
G.ve	*Galium verum* L. subsp. *asiaticum* (Nakai) T.Yamaz.	Rubiaceae	Asian yellow spring bedstraw (솔나물)	F
H.ly	*Hemisteptia lyrata* (Bunge) Fisch. & C.A.Mey	Asteraceae	Lyre-shape hemistepta (지칭개)	F
H.sc	*Humulus scandens* (Lour.) Merr.	Cannabaceae	Wild hop (환삼덩굴)	F, R
I.cy	*Imperata cylindrica* (L.) Raeusch.	Poaceae	Blady grass (띠)	F
L.ch	*Leymus chinensis* (Trin.) Tzvelev	Poaceae	False wheatgrass (개밀아재비)	R
L.cu	*Lespedeza cuneata* (Dum.Cours.) G.Don	Fabaceae	Sericea lespedeza (비수리)	F, R
L.su	*Leucanthemum × superbum* (Bergmans ex J.W.Ingram) D.H.Kent	Asteraceae	Shasta daisy (샤스타데이지)	R
L.vi	*Lepidium virginicum* L.	Brassicaceae	Virginia peppergrass (콩다닥냉이)	F, R
Lil1	N/A (Liliaceae species 1)	Liliaceae	Liliaceae species 1 (백합과 1)	F, R
M.ja	*Metaplexis japonica* (Thunb.) Makino	Apocynaceae	Rough potato (박주가리)	R
M.sa	*Miscanthus sacchariflorus* (Maxim.) Benth. & Hool.f. ex Franch.	Poaceae	Amur silvergrass (물억새)	F
M.sc	*Melica scabrosa* Trin.	Poaceae	Rough melic (참쌀새)	R
M.si	*Miscanthus sinensis* Andersson var. *purpurascens* (Andersson) Matsum.	Poaceae	Purple maiden silvergrass (억새)	F, R
O.bi	*Oenothera biennis* L.	Onagraceae	Evening primrose (달맞이꽃)	F, R
P.ar	*Phalaris arundinacea* L.	Poaceae	Reed canary grass (갈풀)	R
P.ar2	*Plantago aristata* Michx.	Plantaginaceae	Bracted plantain (긴포꽃질경이)	R
P.au	*Phragmites australis* (Cav.) Trin. ex Steud.	Poaceae	Common reed (갈대)	R
P.ch	*Potentilla chinensis* Ser.	Rosaceae	East Asian cinquefoil (딱지꽃)	F, R
P.de	*Populus nigra* L.	Salicaceae	Black poplar (양버들)	R
P.hy	*Persicaria hydropiper* (L.) Delarbre	Polygonaceae	Water pepper (여뀌)	F
P.pe	*Persicaria perfoliata* (L.) H.Gross	Polygonaceae	Asian tearthumb (며느리배꼽)	R
P.pr	*Poa pratensis* L.	Poaceae	Kentucky bluegrass (왕포아풀)	F, R
P.sp	*Poa sphondylodes* Trin.	Poaceae	Hard bluegrass (포아풀)	F
Poa1	N/A (Poaceae species 1)	Poaceae	Poaceae species 1 (벼과 1)	F, R
Poa2	N/A (Poaceae species 2)	Poaceae	Poaceae species 2 (벼과 2)	F
Poa3	N/A (Poaceae species 3)	Poaceae	Poaceae species 3 (벼과 3)	R
Poa4	N/A (Poaceae species 4)	Poaceae	Poaceae species 4 (벼과 4)	F
Poa5	N/A (Poaceae species 5)	Poaceae	Poaceae species 5 (벼과 5)	F, R
Poa6	N/A (Poaceae species 6)	Poaceae	Poaceae species 6 (벼과 6)	R
Poa7	N/A (Poaceae species 7)	Poaceae	Poaceae species 7 (벼과 7)	R
Poa8	N/A (Poaceae species 8)	Poaceae	Poaceae species 8 (벼과 8)	F
R.cr	*Rumex crispus* L.	Polygonaceae	Curly dock (소리쟁이)	R
R.pa	*Rubus parvifolius* L.	Rosaceae	Trailing raspberry (멍석딸기)	R
R.ps	*Robinia pseudoacacia* L.	Fabaceae	Black locust (아까시나무)	F, R
S.ar	*Silene armeria* L.	Caryophyllaceae	Sweet william catchfly (끈끈이대나물)	F
S.sa	*Sedum sarmentosum* Bunge	Crassulaceae	Ster sedum (돌나물)	R
S.vi	*Setaria viridis* (L.) P.Beauv.	Poaceae	Bristlegrass (강아지풀)	F
T.gl	*Turritis glabra* L.	Brassicaceae	Tower rockcress (장대나물)	F, R
T.tr	*Themeda triandra* Forssk.	Poaceae	Brush grass (솔새)	F, R
V.am	*Vicia amurensis* Oett.	Fabaceae	Amur vetch (벌완두)	F
V.ma	*Viola mandshurica* W.Becker	Violaceae	Manchurian violet (제비꽃)	F, R
Z.ja	*Zoysia japonica* Steud.	Poaceae	Korean lawngrass (잔디)	F, R

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Research Article

A detailed assessment of the land cover development in a territory with dispersed settlement area (case study Hriňová – Snohy, Slovakia)

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Abstract

This study focuses on the development of the landscape during a period of significant social changes in Central Europe from the middle of the 20th century, and evaluates the impact of the selected natural factors on landscape dynamics in the Snohy dispersed settlement area (cadaster of Hriňová, Slovakia). During the periods of feudalism and the democratic Czechoslovak Republic after 1918, private ownership of land prevailed on the territory of Slovakia. After 1948, in the period of the socialist establishment, the municipality was one of the few localities without collectivisation. We focused on the state and changes to the landscape structure based on remote sensing data analysis (1949, 2003, and 2018), as well as field research and archive photographs inspection. The study area is a part of the proposed Special Area of Conservation SKUEV4026 Meadows of Poľana and is situated near the border between the transition and buffer zones of the Poľana Biosphere Reserve. The state of land cover and its changes have been evaluated using a CORINE Land Cover modification at the 5th level. Results showed that the influence of the slope on agricultural extensification, deforestation, and afforestation, was the most pronounced. Since 1949, more than half of the land cover has changed across the study area. The most extensive changes were recorded in the spatial structure and composition of forest communities.

Key words: Dispersed area settlement, georelief, Hriňová, land cover, Poľana Biosphere Reserve, Slovakia, soil conditions

Introduction

Land use and land cover

Stamp (1931) conducted some of the earliest studies dealing with the inspection of land use, utilizing legends built upon a combination of several functional and physiognomic features. Other crucial works were released by Anderson et al. (1976) and Heymann et al. (1994). When identifying and interpreting different types of remote sensing images, physiognomic differences between the objects on the Earth's surface were emphasized by these authors. Similar objectives were aimed at by the CORINE Land Cover (CLC) programme, which established a technique for identifying land cover from satellite imagery with a resolution of 10–50 m, while including aspects of land use. With a scale of 1:100,000 and a minimum monitored area size of 25 ha, the CLC technique (Heymann et al. 1994; Büttner et al. 1998; Bossard et al. 2000) is appropriate for national or regional investigations.

The term land cover represents spatial objects on the Earth's surface, which are identified on the basis of morphostructural and physiognomic attributes (Feranec and Oťaheľ 2001). According to (Kopecká 2006), the term expresses the actual state of the landscape structure. By the term land cover change, Coppin et al. (2003) categorized change or gradual transition of one land cover class to another. Treitz and Rogan (2004), Rogan and Chen (2004), and Comber et al. (2005) all understand land cover as a physical formation of matter on the Earth's surface, which is represented by forests, grasslands, artificial surfaces, rocks, water, etc.

Oťaheľ and Feranec (1997, 2006) were among the first in Slovakia to focus on land cover changes identified from CORINE Land Cover data from 1970 to 2000. Analyses of landscape dynamics based on the spatial intensity of changes were published by Feranec et al. (2000), Feranec and Oťaheľ (2001), Boltižiar (2007), Oťaheľ and Feranec (1997) and Oťaheľ et al. (2011, 2017). Classifications of land cover changes were published by Čerňanský et al. (2003) and Kopecká (2006).

In recent decades, the topics of land use and land cover have been a relatively frequent part of historical, geographical, and landscape ecological research in Slovakia, as well as throughout the world. Some studies claim (Huba 1989; Petrovič 2006b; Hunziker et al. 2008; Bürgi et al. 2017), that landscape changes evaluation is largely linked to ongoing social processes. Land-use changes are increasingly being influenced by distant forces due to the globalization of many markets (Lambin and Meyfroidt 2011). Conversely, climate change can also influence land cover, resulting in a loss of forest cover from climate-related increases in disturbances (Flannigan et al. 2009), and the expansion of woody vegetation into grasslands (Kulmatiski and Beard 2013).

Dispersed settlement

In addition to Slovakia (Lauko 1985; Huba 2004; Petrovič 2006a; Petrovič and Petrikovičová 2021), dispersed settlement also occurs in many countries. As examples of studies of this type of settlement, we mention the following: Scandinavia (Norling 1960; Hansen 1972), Bulgaria (Hoffman 1964), Africa (Stone 1991; Donaldson and Boshoff 2001), Czechia (Löw and Míchal 2003; Vaishar and Šťastná 2019; Šťastná and Vaishar 2020), and the United States (Barnes and Robinson 1940). However, its occurrence in the aforementioned regions is spatially limited.

In Slovakia, dispersed settlement is a specific form of settlement, which is conditioned by specific historical and environmental factors. As a result of colonization waves in Slovakia, groups of irregularly-scattered settlements in different types of georelief were created. However, its genesis was rather diverse

(Huba 1989, 1990, 1997). The origin of dispersed settlements began in medieval times (Hoffmann 2014; Hanušin et al. 2020). Its basic function was soil cultivation in the marginal areas (Sitár 1967). The formation of dispersed settlements was linked to the three colonization waves: Wallachian (Chaloupecký 1947); Highlander and the "Kopaničiarska" (Petrovič and Petrikovičová 2021). Nowadays, in Slovakia, five different regions with scattered settlements and different land uses can be found (Verešík 1974).

Poľana Biosphere Reserve

One specific dispersed settlement was created in the Podpoľanie area. Within Poľana, as a part of the Western Carpathians Mountains, is one of the greatest European former volcanoes (original elevation at the time of its activity reached approximately 2500 m a.s.l.), and the highest volcanic mountain range in Slovakia. Elevation at the highest point is 1458 m a.s.l., while the lowest one reaches 460 m a.s.l. Poľana Biosphere Reserve (BR), established in 1990, is characterized by the common occurrence of both thermophilous and mountain plant species. Forests cover almost 85% of its area, with the moderate prevalence of coniferous forests (https://en.unesco.org/biosphere/eu-na/polana). To date, 1220 species of vascular plants have been described in the BR, out of which 80 species are in the categories of protection, threat, and rareness. With 174 species of birds, the Poľana BR has been recognized as an Important Birds Area of Slovakia. Besides biodiversity, land use dynamics is a crucial part of BRs research in Slovakia (Olah and Boltižiar 2009).

Historical evolution of Podpoľanie

The area of Podpoľanie is characterized by a specific type of homestead and colonization. In the initial phase of Wallachian colonization in the 13th–14th century, there were successive waves of settlement by inhabitants with a nomadic way of life, who were influenced by the political situation (Cojacaru 2014). Wallachian colonization was continuously followed by mining colonization (in some areas it overlapped it) from the 17th to 19th century (Petrovič and Petrikovičová 2021). Colonization affected not only sheep farming, but also culture, folklore, domestic production, and the very way of life in mountain and foothill areas. This area of Podpoľanie is characterized by high biodiversity and is connected mainly to the boundaries, which consist of the edges of field roads, original meadows, pastures, permanently grassy areas on former fields, or various waterlogged areas (Ružičková et al. 1999).

Koza (2018) evaluated the historical landscape structures with a focus on the agricultural landscape in the cadastral territory of Hriňová. Vašková (2015) analyzed the development of ecosystems in relation to forms of land use in Podpoľanie. Lapinová (2004) dealt with the development of dispersed settlements in the Detva dispersed area region, and together with Paučová (2014), they conducted research in the neighboring district of Detva. Paučová (2014) focused on preserved areas in Detva, specifically on the local part of Skliarovo. Private land ownership, however, was not encouraged after the establishment of socialism in 1948. The established regulations clearly delineated the limits of land that could have been used for farming by private farmers, and the remaining land was predisposed to collectivization. In the middle of the 20th century, through the process of collectivization, land was collectively pooled. And so, peasant families were left with only small areas up to 0.5 ha (Spišiak 2005).

The shift after 1989 resulted in the conversion of the central planned economy to a market-oriented economy. Despite the fact that farmers in the area once farmed as owners of the agricultural soil, many young people moved for employment to other nearby cities. The growth of Hriňová's population began to decline in 1998 (KPSS 2018). Since then, the city of Hriňová's demographics have taken a negative turn as fewer children are being born, the population is ageing, or the population is post-productive. Nowadays, the area of Podpoľanie is sparsely inhabited, including three settlements, one recreation center, several farms and forest houses (https://en.unesco.org/biosphere/eu-na/polana). Only a few of the 400 permanent residents work in forestry or agriculture, and the majority of them are retired. Those who are working commute to industrial facilities outside the area.

Objectives of the study

Our study provides a detailed description of the historical landscape structures development in the Hriňová – Snohy location. With the years 1949, 2002–2003 and 2018 under examination, the early period of socialism, entry to the European Union and current land use are represented. Landscape dynamics were evaluated on the basis of aerial imagery GIS-based expert classification, in accordance with the CORINE Land Cover methodology modification by Oťaheľ et al. (2017).

We introduced our approach to detailed research of land cover changes of dispersed settlement area in relation to soil and geomorphological conditions. In this paper, we also aim to emphasize any threats that could potentially endanger the future existence of dispersed settlements in Slovakia.

Materials and methods

Study area

The city of Hriňová is located in the Detva district in central Slovakia. Snohy is a local part of Hriňová, situated approximately 10 km north of the city center on the southern slopes at an elevation range 751–828 m a.s.l. The area under investigation is located in Snohy, with a size of 2.5 km^2. It belongs to a moderately cold, very humid district of a cold climate area with an average air temperature in July of 12 °C – 16 °C. The average air temperature in January is from -5 to -6 °C (https://app.sazp.sk/atlassr/).

This is a specific type of dispersed settlement (named „lazy"), where traditional farming has been preserved until today. Hriňová was one of the few municipalities where the collectivization of agriculture never took place, and the Unified agricultural cooperative was thus never established. To this day, it has preserved the traditional structure of land use, which is characterized by terraced fields (Fig. 1). While in the surrounding municipalities of Detva and Očová only a few fragments of agrarian historical structures have remained, compact historical farms have been conserved in the Hriňová dispersed area, being among the best preserved in Slovakia (Urban 2015). Historical landscape

Figure 1. Landscape structure of Hriňová with dispersed settlements and terraced fields (Photo: K. Švoňavová, 2019).

structures are represented by terraced fields and several farms. The study area is a part of the proposed Special Area of Conservation SKUEV4026 Meadows of Poľana with agricultural management (Figs 2, 3). Landscapes of the Poľana Mts. have been protected as the Poľana has been designated as a protected landscape area since 1981, as well as within the Poľana Biosphere Reserve from the UNESCO Man and Biosphere programme since 1990.

The volcanic relief of the Poľana mountain range was remodeled in the Quaternary, mainly by fluvial activity. The soil cover consists mainly of cambisols, andosols, and fluvisols. The territory is located at the upper borderline of a moderately warm climate zone. The region is covered mainly by woodlands (85% of the BR), the rest being agricultural land, including grassland and pastures, except for 50 ha of water reservoir. The forest habitats are diverse, from oak and beech forests to spruce forests growing on andesites, and are well-known for their southernmost occurrence within the Western Carpathians, covering the highest part of the mountain range. Many forests have the character of an old primeval forest. Throughout their history, the Poľana forests have been modified by various natural disturbances and human interventions (e.g., beech forests were replaced by spruce monocultures).

Data

For the purposes of our research, imagery from an aerial survey was used. For the year 1949, panchromatic aerial photographs from the archive of the Topographic Institute in Banská Bystrica with a resolution of 0.5 m (http://mapy.tuzvo.sk) were used. An orthophotomosaic of the Slovak Republic, provided by Geodis ltd. and Eurosense ltd. with a resolution of 0.5 m, was applied for 2003. As for 2018, the open-source Orthophotomosaic of the Slovak Republic was used, carried out by the National Forest Center and Cadastre Authority of the Slovak Republic (https://www.geoportal.sk/en/zbgis/orthophotomosaic).

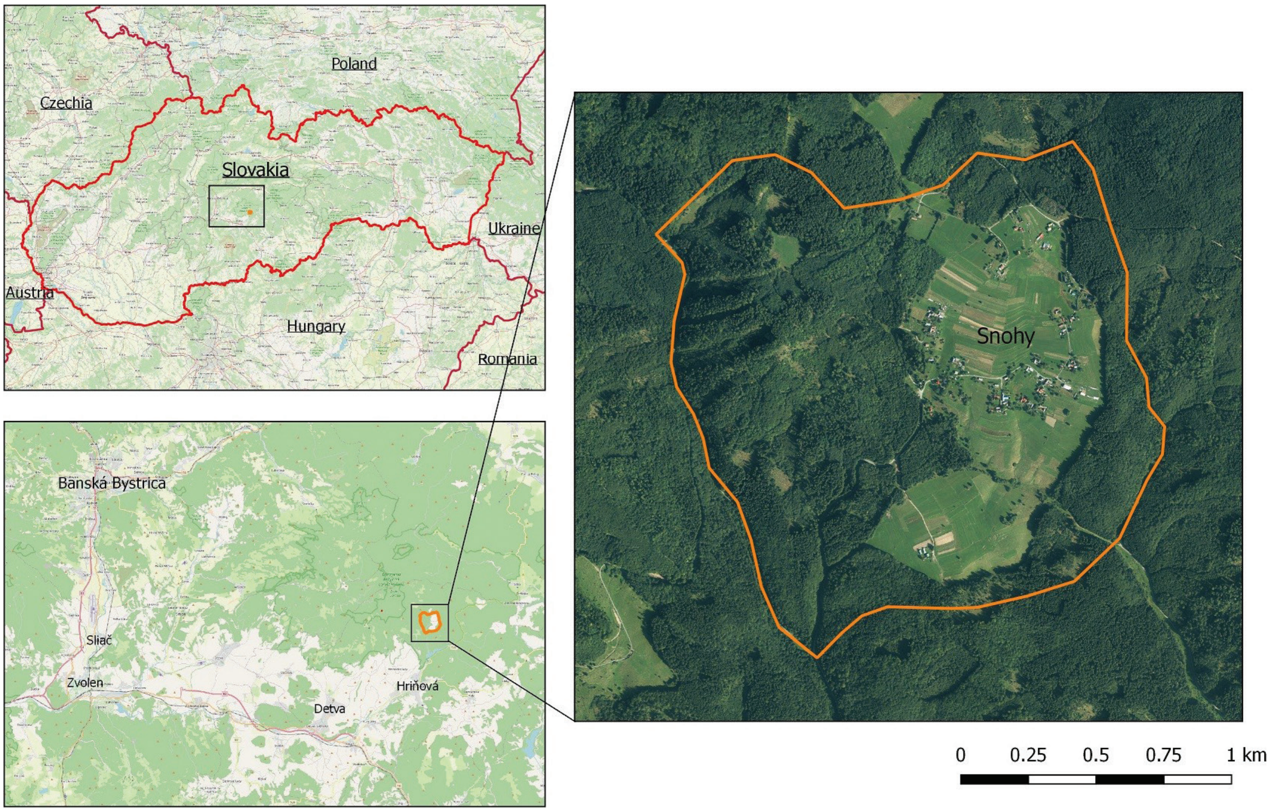


Figure 2. Location of the study area.

Figure 3. Landscape of the study area (Photo: K. Švoňavová, 2019).

Data for the soil maps creation (the map of certified soil-ecological units and forest soil maps) was provided by the Research Institute of Soil Science and Landscape Protection (http://www.podnemapy.sk/portal/verejnost/bpej/) and the National Forest Center (http://gis.nlcsk.org/ArcGIS/services/Inspire/PodneTypy/MapServer/WMSServer?).

The basemap of the Slovak Republic on a scale of 1:10,000 (https://zbgisws.skgeodesy.sk/ZMSR_wms/service.svc/get) and the digital elevation model (DEM) with a resolution of 25 m (https://land.copernicus.eu/imagery-in-situ/eu-dem/eu-dem-v1.1?tab=mapview) were used to create topographic and insolation outputs.

During the whole analysis, the coordinate system of the unified trigonometric network of the cadastral S-JTSK (5514) was used.

Analysis of land cover changes

The vectorization, as well as the following spatial analysis, were performed in QGIS 2.8.14. The creation of land cover layers was based on visual interpretation. Moreover, data for 2018 was verified by the field research. For identification of land cover classes, the modification of the CORINE Land Cover nomenclature on the 5th level at a scale of 1:10,000 by Oťaheľ et al. (2017) was used.

The method is based on the concepts of hierarchically higher classes (Heymann et al. 1994; Bossard et al. 2000). Most importantly, morpho-structural and physiognomic attributes distinguishing individual landscape objects were respected. The size of the least-identified and simultaneously-recorded area is identical with the study by Druga et al. (2015), who established it at 0.1 ha. It corresponds approximately to a square with a 31.5 m side, a circle with a 35.5 m diameter, or a 20 × 50 m rectangle (Falťan et al. 2020). The minimum area of change is set to 0.02 ha and the minimum width of a polygon to 10 m. Exclusion is made for polygons of linear elements – water courses, railways and road networks – which has a minimal width of 2 m and a minimal length of 50 m.

When mapping land cover for the years 1949 and 2003, the retrospective approach was applied (Feranec et al. 2004), meaning only areas with an interpreted change (larger than the size of the minimum area of the change) were edited. As a reference layer, the layer from the following analyzed year was used. The Change Detection Toolbox tool, which was created as a practical output in Lukáš Žubrietovský's diploma thesis (Žubrietovský 2016), was used in the analysis of the land cover. Classification of changes was carried out according to Feranec et al. (2010).

Temporal stability of each land cover class reflects the degree of its connection to the selected landscape natural components (Súľovský 2017).

The stability of the types of areas used for the land, or of the land cover, also shows the degree of their connection to the selected relevant natural components of the landscape (Súľovský 2017). Land cover stability maps were carried out in the ArcGIS 10.7.1, with land cover change data (1949–2003 and 2003–2018) being the input layers. These layers were overlaid using Intersect tool, and thus we selected unstable areas.

The Merge tool was applied when delineating neutral areas, by merging both layers of the land cover changes. Stable areas are defined as the territory outside any unstable and neutral areas, i.e., an area where no change was recorded.

Analysis of soil and georelief

Based on the underlying soil data, a map was created on a scale of 1:10,000 and the soils were classified according to the soil type and subtype. To unify soil names, a conversion table, which is freely available on the ForestPortal website about the forests of Slovakia, was used (http://www.forestportal.sk/lesne-hospodarstvo/hospodarska-uprava-lesov/Stranky/prevody-pody.aspx).

A map of morphographic-positional types of georelief according to Tremboš (Minár et al. 2001) was prepared on the basis of the Basic Map of the Slovak Republic in a scale of 1: 10,000 and DEM. When delineating the debatable areas, we used an extracted layer of slope, orientation to the cardinal points, and a layer of contours computed in ArcGIS 10.7.1. Auxiliary contours were created at intervals of 10 m and 5 m. To evaluate the relationship between relief forms and areas of stability, the aforementioned layers were overlaid, and their relationship was examined.

The slope of the soil is an important physical parameter that significantly affects the quality and method of use of the soil and the given location. Layers of slope and insolation were computed from the DEM. In the case of slope, we reclassified raster into six classes. For practical purposes and the needs of agricultural practice, it is possible to express the representation of the slope of agricultural land in the following classes: 0–1°, 1–3°, 3–7°, 7–12°, 12–17°, 17° – 25°, 25° and others. In the case of slope, we reclassified the data in degrees into these classes. Classes 12–17° and 17–25° were merged, as one of the classes was not represented.

Subsequently, raster was converted to the vector feature, while polygons with an area of less than 100 m^2 were merged with the nearest related area. A similar method was used to create the insolation layer: raster was reclassified into six classes, ranging from the minimum value up to 400,000, 400,000–430,000, 430,000–460,000, 460,000–490,000, 490,000–520,000 and 520,000–550,000 WH/m^2. Consequently, layers of slope and insolation were overlaid with the layer of land cover stability so that the underlying relationships could be analyzed.

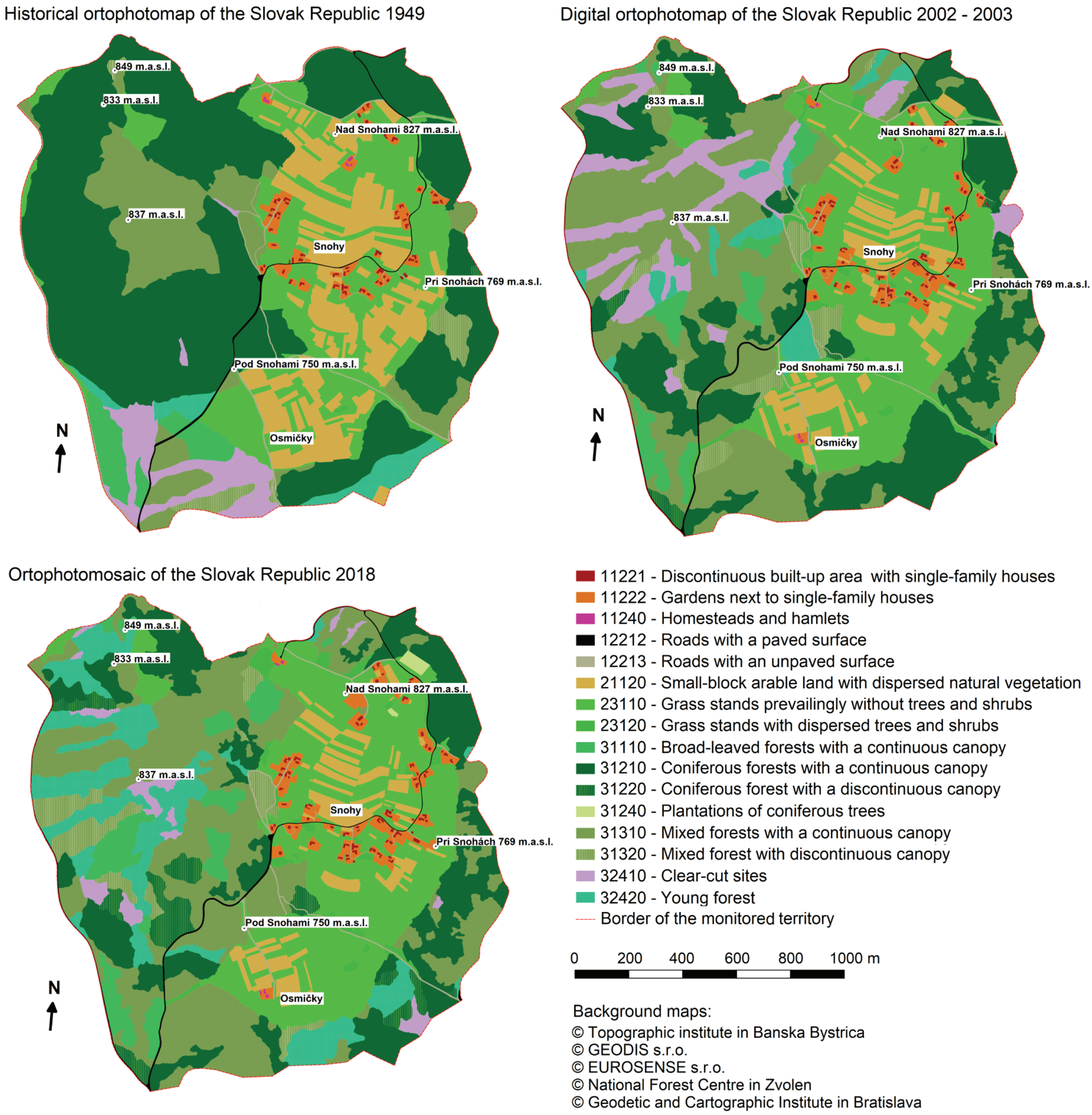
Results

Analyses of land cover development

In the territory of the Snohy area (Fig. 4), a significant change of the land cover was observed (Table 1). During the period 1949–2003, 52.9% of the total area changed, while during 2003–2018, a change of 28.3% was recorded. In terms of land cover flows during both periods, the most prominent were other changes (61.1 ha = 23.6%), for example, changes in the species composition of stands in the forests or within settlement classes, followed by deforestation (28.0 ha = 10.8%), caused by forest harvesting, and extensification of agriculture (21.8 ha = 8.4%).

In 1949–2003, the most prominent changes were recorded in the structure and composition of forest communities (Fig. 5). The changes in the period of 2003–2018 were similar, however, the other changes had already reached lower values (25.7 ha = 9.9%), yet still being the highest among the others.

This was followed by changes in deforestation (20.8 ha = 8%) and afforestation (18 ha = 6.9%). Artificial areas, fields and meadows in Snohy have changed in all the observed periods (Fig. 6). Significant changes took place in forests under conservation of the Poľana protected landscape area and the


Historical ortophotomap of the Slovak Republic 1949

Digital ortophotomap of the Slovak Republic 2002 - 2003

Ortophotomosaic of the Slovak Republic 2018

Background maps:
© Topographic institute in Banska Bystrica
© GEODIS s.r.o.
© EUROSENSE s.r.o.
© National Forest Centre in Zvolen
© Geodetic and Cartographic Institute in Bratislava

Figure 4. Land cover of the Snohy area in 1949, 2003 and 2018.

UNESCO Biosphere Reserve. It must be noted, however, that both active forest management and sustainable agriculture are not prohibited, and are thus encouraged in this zone.

The most stable land cover types are grasslands, followed by coniferous and deciduous forests with a continuous canopy.

Analysis of the influence of slope angle and insolation on the stability of the land cover areas

Unstable areas are connected predominantly to the slope angles 12–25° (19.1 ha) and 7–12° (17.0 ha), being associated with the increased values of deforestation in 1949–2003 (Fig. 8). Areas with a single recorded change are

Table 1. Land cover classes and its area (ha) in each observed time horizon.

Code	land cover type	1949	2003	2018
11221	Discontinuous built-up area with single-family houses	0.90	1.06	1.04
11222	Gardens next to single-family houses	3.78	5.75	6.59
11240	Homesteads and hamlets	0.08	0.05	0.06
12212	Roads with a paved surface	1.37	2.20	2.20
12213	Roads with an unpaved surface	1.99	1.18	0.91
21120	Small-block arable land with dispersed natural vegetation	31.86	15.84	12.05
23110	Grass stands prevailingly without trees and shrubs	46.78	64.69	65.30
23120	Grass stands with trees and shrubs	0.11	3.38	4.14
31110	Broad-leaved forests with a continuous canopy	6.74	8.02	11.51
31210	Coniferous forests with a continuous canopy	116.62	55.85	42.06
31220	Coniferous forests with a discontinuous canopy	0.23	1.51	8.43
31240	Plantations of coniferous trees	0.00	0.00	0.58
31310	Mixed forests with a continuous canopy	29.11	67.92	65.51
31320	Mixed forests with a discontinuous canopy	3.97	5.96	8.29
32410	Clear-cut sites	9.86	18.51	4.73
32420	Young forests	5.36	6.85	25.37

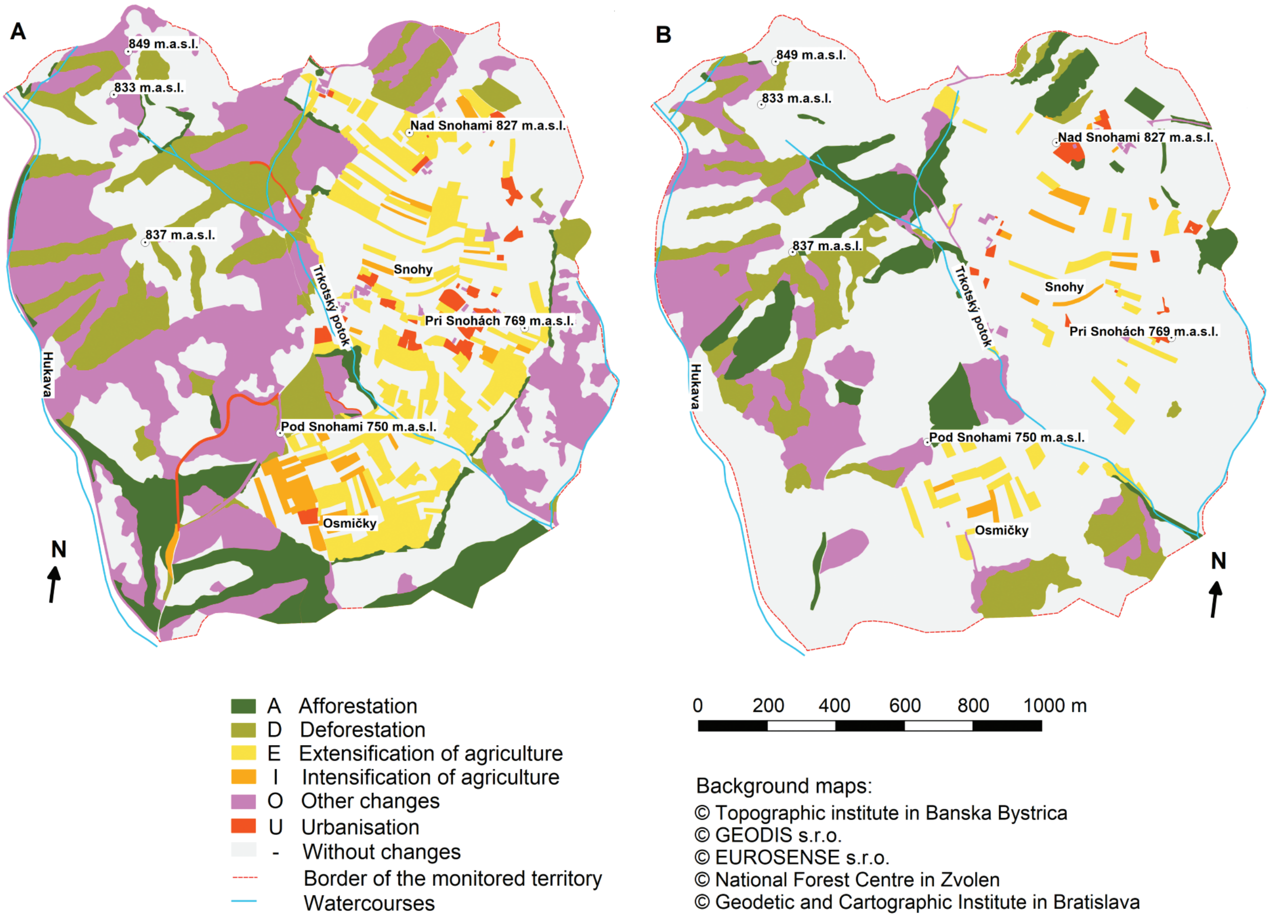


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Figure 5. Land cover changes in the Snohy area in 1949-2003 (**A**) and 2003-2018 (**B**).

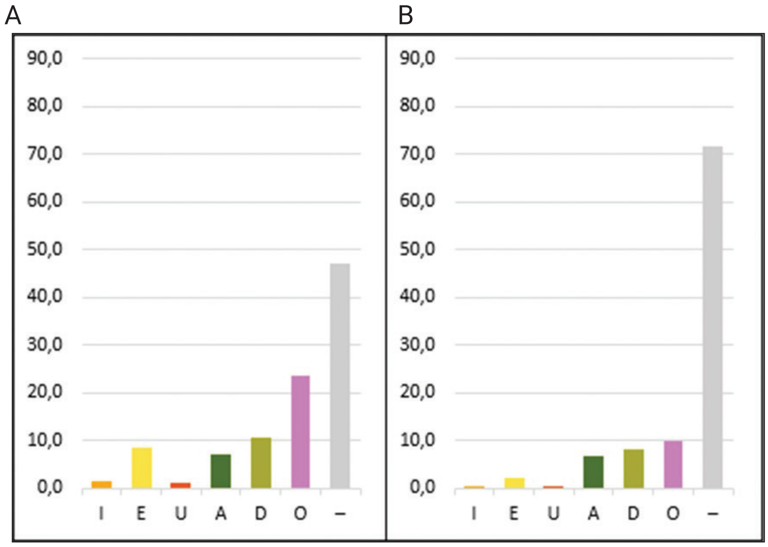

Figure 6. Proportions of individual types of land cover changes (%) in 1949–2003 (**A**) and 2003–2018 (**B**). Type of changes: I – intensification of agriculture, E- extensification of agriculture, U – urbanization, A- afforestation, D - deforestation, O – other change, - - without change.

linked to afforestation, significant extensification after 1950, and subsequent intensive deforestation between 2003–2018 (Fig. 8). Areas with steep slopes were marginally modified during 1949–2018, being covered mostly by forests.

The most significant category of the total insolation in the entire territory was 460,000–490,000 Wh/m^2 (104.5 ha = 40.4%). Moreover, the amount of insolation ranging from 490,000 to 520,000 WH/m^2 took place in the area of 69.3 ha (26.8%). The category of 520,000–536,300 Wh/m^2 was spatially detected in the area of 3.5 ha, being the highest value of insolation among dispersed settlements throughout the entire Hriňová region.

Unstable areas with higher insolation values showed the most significant processes of afforestation, deforestation, and other changes in the entire observed period of 1949–2018 (Fig. 7). Neutral areas in the sunniest parts of the territory were characterized by changes in deforestation, other changes until 2003, and increased extensification and other changes after 2003. The land cover in the areas with a higher insolation values was represented by forest communities, arable land, and grasslands.

The influence of georelief forms and soil types on the stability of land cover areas

In the study area, the predominant soil type is locally-andotermic cambisol (Fig. 7). In the floodplains, fluvisols take place, being characteristic for the areas modified by a single change type (mostly deforestation). In the areas with prevailing cambisols, the land cover types stability was influenced mainly by the forms of georelief. The most significant changes were linked to deforestation in the second half of the 20th century, as well as afforestation and extensification at the beginning of the 21st century. These changes took place on 46% of the transport slopes.

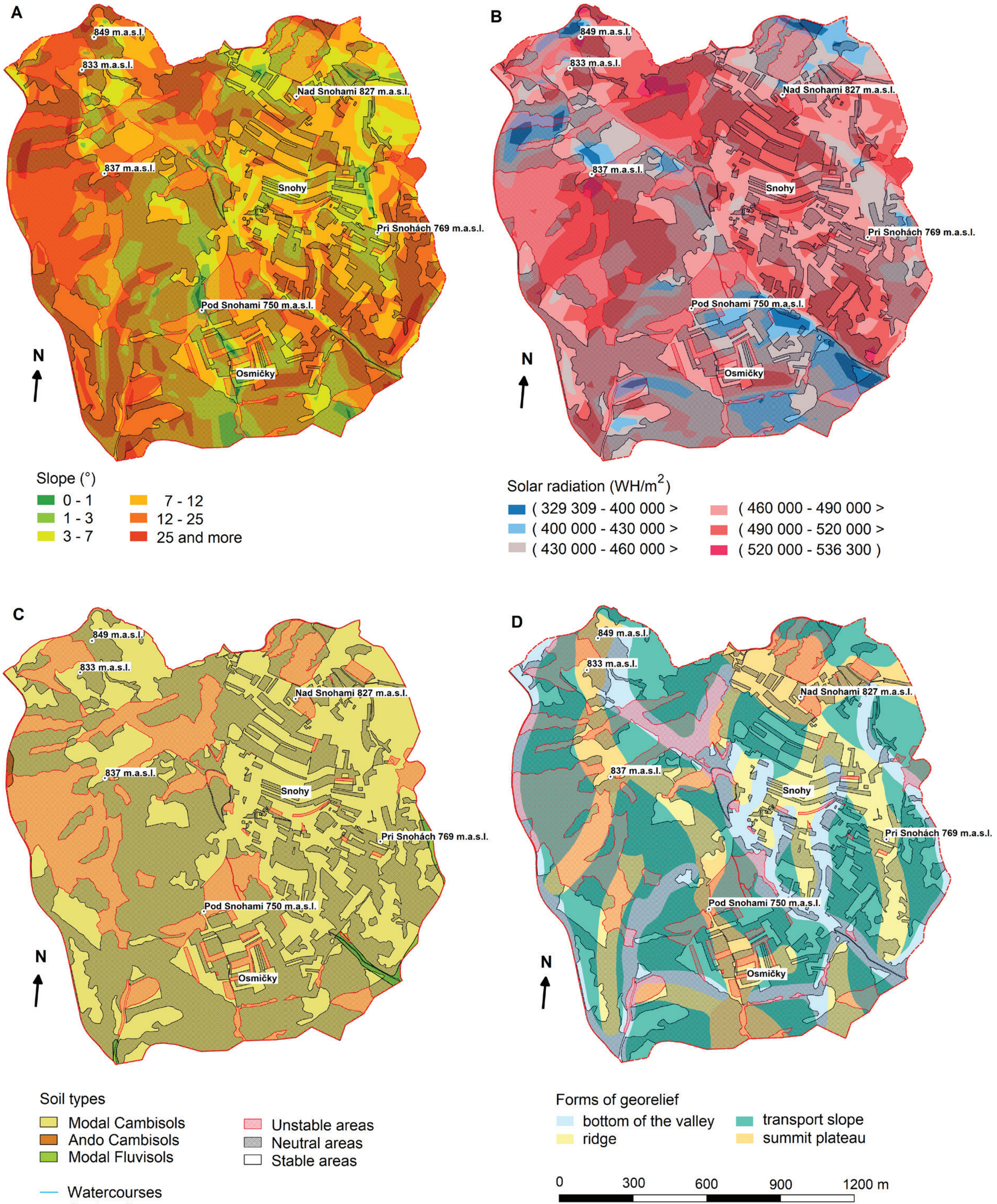

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Figure 7. Stability of land cover areas in relation to the **A** slope angle **B** insolation **C** soil types **D** form of georelief.

Almost 20% of the valley bottoms and ridges has been modified by similar processes. However, stable areas are connected mainly to the transport slopes and not to the ridges and valley bottoms.

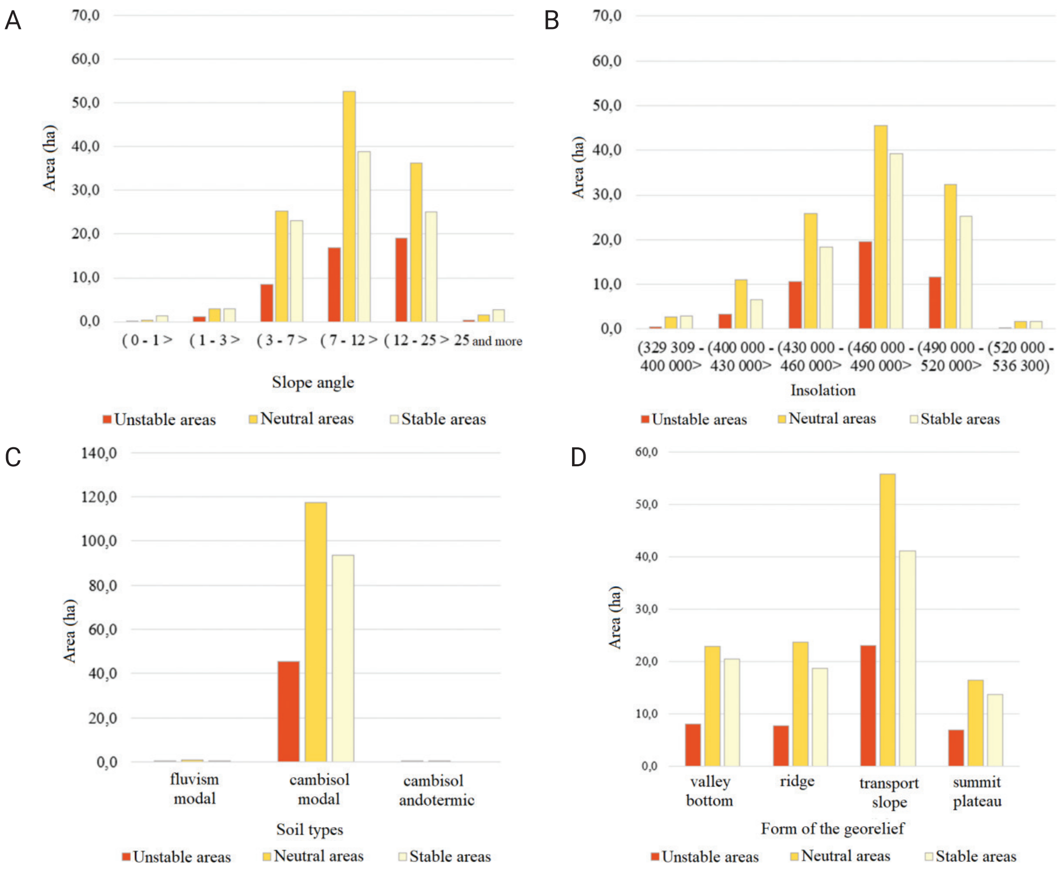

Figure 8. Stability of land cover areas in relation to the **A** slope angle **B** insolation **C** soil types **D** form of georelief.

Discussion

Long-term changes in land use also affect other environmental determinants, such as elevation, slope, insolation, or wetness of the soil (Druga and Falťan 2014). The drivers for the agricultural intensification and urbanization seem to be similar for both old democratic and post-socialist countries. In contrast, agricultural land abandonment in the old democratic countries was driven by technological, cultural and economic driving forces while in the post-socialist countries the political driving forces were mainly responsible (Skokanová et al. 2016).

According to several studies (Boltižiar and Olah 2009; Žigrai 2000), changes in the landscape and their spatial determinants are most intensively related to the most significant socio-political periods, namely socialism (1980–1990), post-socialism (1990–2000), accession to the EU (2000–2006), and EU membership (2006–2012). Pazúr et al. (2014) point to significant changes in the landscape management associated with institutional changes, especially in the post-socialist period. It was during this period that the protected landscape area of BR Poľana, established in 1981, was also included in the network of biosphere reserves in 1990, and until 2010, only its protective function was mainly ensured (Urban 2015). This was also reflected in a certain stabilization of the use of the territory and therefore roughly 70% of the territory was categorized as “no change” between 2003 and 2018 (Fig. 5B). The land cover changes in both evaluated periods took place mainly in forestry and agriculture, which, after the inclusion of Poľana in the network of biosphere reserves, are supported as close to nature forest management and sustainable agriculture, which is relevant also in our study area.

The territory that the Poľana biosphere reserve belonged to in 2014 was among the least urbanized protected areas in Slovakia. There were only three settlements (Iviny, Snohy and Vrchslatina) with a typical settlement (about 400 permanent residents in total) (Urban 2015). In 2016, Hriňovské lazy was also included in the transition zone. Scattered settlements were created in Hriňová due to the difficult availability of land from inhabited areas. In this area, horses and traditional agricultural tools are still used for agricultural work. This agroecosystem not only fulfils the function of a producer of food, feed and raw materials, but also supports a whole range of non-production functions of the country and thus becomes more sustainable (Prčík et al. 2019). The cadastral territory of Hriňová, as one of the few in Slovakia, was not affected by the collectivization of agriculture during the socialist period; it has preserved its unique landscape and traditional forms of farming. The preservation of these traditional forms of agriculture is mainly ensured by the work of the Coordinating Council of the Poľana Biosphere Reserve with stakeholders. In 2017, the Poľana Biosphere Reserve received the Michel Batisse Award for the Biosphere Reserve Management at the ceremonial event in the course of the ICC MAB, the highest possible award for the exceptional experience of man with nature.

Hanušin et al. (2020) claim that dispersed settlement with a residential or landscape area structure, significant building, and social and cultural peculiarities, create unique manifestations of material and spiritual heritage and genius loci. According to Woods (2005), a country with scattered population represents in most regions of Europe a specific type of rural landscape that has seen a significant change in recent times, predominantly during the twentieth and the beginning of the twenty-first century. Social and technological modernization has affected almost all areas of rural life. In Slovakia, regions with a dispersed settlement on mountain slopes have been changing their appearance and function for approximately the last 30–50 years. According to previous studies (Bezák and Halada 2010; Bezák and Mitchley 2014; Bezák and Petrovič 2006; Bezák et. al 2020), the main factors of changes were related to the onset of socialism after World War II, political and economic changes after 1989, and Slovakia’s accession to the EU. Socialism led to the establishment of State Property (SP) and United Agricultural Cooperative (UAC); narrow-band fields were consolidated into larger blocks. Increase in production and decrease in agricultural landscape diversity were significant. UAC was a type of agricultural cooperative in the Czechoslovak Socialist Republic until 1990. UACs were responsible for most of Czechoslovakia’s agricultural production. Any citizen could become a member after completing compulsory schooling. Upon joining the cooperative, a member of the cooperative handed over all the agricultural land he owned to the ownership and joint management of the cooperative. SPs were fully owned by the socialist state and they managed a smaller area of land than UACs in Slovakia.

After 1989 the population began to lose interest in agriculture, mostly in regions with dispersed settlements. The consequences for the landscape were thus significant: a large part of the agricultural land was abandoned, grassed-over, and overgrown with scrub. In Hriňová, due to the character of the landscape, there was no consolidation of offields during socialism. At the same time, the area of small-block, arable land with dispersed natural vegetation and coniferous forests with a continuous canopy managed by foresters gradually decreased in the Snohy study area from the middle of the 20th century. The area of gardens next to single-family houses, grass stands, and natural mixed forests has increased.

In the highly-fragmented agricultural patterns, which are typical of a traditional agricultural landscape, significant changes are not observable by conventional remote sensing approaches to land change measurement (Ellis et al. 2006). Specific research procedures included large-scale mapping based on aggregation data, such as historical records, field mapping combined with multiple level approaches that define land system – land cover – land use, cultural heritage features, and vegetation (Ellis et al. 2009; Kizos et al. 2010). Several detailed field investigations were carried out in the locality. The territory of the Hriňová dispersed settlement landscape became an object of interest for Mojses and Petrovič (2012, 2013). In addition to the changes in the land cover, they focused on other specific types of historical structures of the agricultural landscape, while the extensification of agriculture was recorded as the second most widespread type of change in the period from 1949 to 2010. The land cover changes recorded in this region were the result of several negative socioeconomic influences, such as unsuitable economic conditions, legislative and administrative obstacles, or the aforementioned loss of ties to the traditional way of farming. Krnáčová and Hreško (2014) came up with the suggested management and measures, which were based on the observed soil reaction of the management of agricultural biotopes, and described in detail the functions of vegetation and vegetational-anthropogenic elements of the agricultural landscape. With regard to the prepared site of NATURA 2000, it is necessary to preserve the agricultural management of meadows, gardens, and block fields in the Snohy area.

The development of mining and metallurgy in the 19th and 20th centuries caused significant logging and transformation of deciduous and mixed lowland forests into spruce monocultures (Urban 2015). The extracted wood was used in glassworks, in the production of charcoal or intended for export. More studies (Izakovičová 2013; Bezák and Mitchley 2014) link landscape changes with migration in Europe after World War II. The population tends to concentrate in bigger cities as a result of higher population density. This brings more opportunities for jobs, entertainment, and the social aspects of life (Antrop 2004). In the nearby surroundings of our study area, Detva and Hriňová, cities that attract the majority of the population, such as Zvolen, Banská Bystrica, or Brezno, can be found, which also provide better job prospects and improved cultural benefits while still being within reasonable distance of Poľana. A low population density without local services restricts viable employment opportunities (Lundmark et al. 2010). Generally speaking, the depopulation of dispersed settlements might have a serious effect on sustainable development of the region (Grau and Aide 2007). Although dispersed settlement has an irreplaceable ecological, cultural, and historical value, the interest of local owners in traditional farming is constantly decreasing in Slovakia (Pazúr et al. 2020). Olah et al. (2006) point out the impact of the decreasing number of farming inhabitants around Podpoľanie and the related reduction of the area of the managed permanent grasslands. Young people from Snohy also often left to work in nearby towns. The ecologically most stable areas lie in the lowest, southern, and south-western slopes in the region of the Poľana Biosphere Reserve (Olah 2003, 2006). Gallayová (2009) showed that overgrowths above 75% are clearly linked to the slopes higher than 15° in the territory of Poľana, which was also confirmed in previous research. It was similar in the analysis of soil types, where modal cambisol reached increased values in areas with a higher intensity of change or overgrowth. Galalyová registers overgrowth of over 75% in the areas oriented to the northwest, north and west. The smallest overgrowth was identified on the southern and eastern slopes. According to Žigrai (2000), distance plays an important role in the assessment of the relevant socioeconomic element.

Huba (2009) focused on the transformation of the dispersed settlement area on the example of Poľana. One type of secondary resettled population is the descendants of the original owners who decided to settle in this region and start farming. Another type are people who are looking for a second home or cottage, while at the same time carrying out agricultural and economic activities around the property. Nowadays, we can register a third type of residents, i.e., the descendants of the original owners, who live in either the original or newly-built houses, but are not interested in agricultural activities. This phenomenon is described also by Štefunková (2019). Therefore, land use changes are gradually taking place — overgrowth, conversion to recreational land, etc. This process is also evident by a slight increase of a discontinuous, built-up area with single-family houses in Snohy in recent years. Several authors have also reported changes in agricultural activities, which could create risks to the preservation of the traditional cultural landscape (Agnoletti 2007; Špulerová 2008; Van de Velde et al. 2010; Jakab and Petluš 2012). Another factor that potentially endangers the continuity of such areas is depopulation (Elbakidze and Angeltram 2007). Therefore, it is of utmost importance to find solutions to these problems, because research results confirm the irreplaceable role of HSAL (Deckers et al. 2005).

In the study of land cover changes around the villages of Malachov and Podkonice in Central Slovakia west of the Podpoľanie region, Druga and Falťan (2014) also confirmed a reduction of arable land during last century. Elevation and higher slope angle were related to the classes with lower intensity of use, such as forest land and extensive grassland, which is in our study too. Steep areas with a slope of more than 12°, despite having a higher amount of insolation, have been predominantly used for forestry after 1949. The trends of extensification of agriculture and afforestation have manifested more than urbanization in dispersed settlement localities. Löw and Míchal (2003) recorded fields from one settlement as having a maximum diameter of 1.2 km. In several studies (Hietel et al. 2004; Bárek et al. 2009; Martínez 2011; Tárnik and Igaz 2015), it has been stated that soils have minor or average influence on land cover changes. In our study, stability of land cover has been influenced more by georelief.

An interest in maintaining the integrity of traditional landscapes is also present in the European Landscape Convention (CETS No. 176 2000), because there are real threats of losing traditional landscapes and their genius loci. The recent CAP approach does not sufficiently reflect the specific local physical conditions of Slovak TALs. For example, small, remote meadows, and narrow fields with terraces and stone walls, typical for Podpoľanie area, are not included in the support system (insufficient size, difficult access). The system is financially demanding and inaccessible for many rural farmers. Administrative tasks are a huge barrier, especially for older people. This challenge of maintaining traditional agricultural landscapes is addressed to individuals, as well as to those politicians who make decisions on development policy (MRI).

Due to its location, Snohy and its wider surroundings have long been part of the Protected Landscape of Poľana, specifically falling into the transition zone. Logging is not prohibited in this zone, but practices of sustainable management of natural resources are promoted and developed in it. Compared to the unprotected forest land in the vicinity, the share of deciduous forests is gradually being restored here. The territory of Snohy is included in the upcoming part of the NATURA 2000 network, Special Area of Conservation SKUEV4026 Meadows of Poľana, which is supposed to protect valuable meadow biotopes: species-rich Nardus grasslands, on siliceous substrates in mountain areas and submountain areas in continental Europe, lowland hay meadows (*Alopecurus pratensis*, *Sanguisorba officinalis*), transition mires and quaking bogs and a species of European importance, *Campanula serrata* (https://www.minzp.sk/uradna-tabula/eu-uzemia/skuev4026-luky-polany.html). In 2021, negotiations began with the owners, managers, and tenants of the affected lands. According to Article 6(3) of the Habitats Directive 92/43/EEC1 (HD), any projects and plans within these sites or in their vicinity require an appropriate assessment to ensure that they will not have any significant impact on the integrity of the Natura 2000 site (Mockel 2017). This process is complicated in the territory of Podpoľania by the number of owners and users of the agricultural landscape. This region is very interesting from the point of view of landscape preservation, as well as for the monitoring and research of other connections.

Some studies have shown that the impact of settlement on landscape fragmentation in biosphere reserves has been more significant (Compas 2007; Liang et al. 2014; Li et al. 2023), and the interactions between settlement landscape patterns and natural ecosystems have been more complex (De Castro-Pardo et al. 2021). The area, number, density, connectivity, shape similarity, and spatial organization of patterns of settlements have had a significant impact on the size of biological communities and the species richness in biological reserves (Heider et al. 2018; Lu et al. 2018; Onilude and Vaz 2020; Wang et al. 2020). Woodland landscapes in mountainous areas have been characterized by high biodiversity and contain rich populations of plant and animal species (Bailey et al. 2002), so the influence of woodland landscape fragmentation is more complex, as compared to other types of landscapes (Pavlacky and Anderson 2007).

Our proposed methodology can be used in other Central European countries. Data for the soil maps creation is available in the portals of the forestry and agriculture research institutes. Digital elevation models, as a data for GIS georelief analyses, can be obtained according to the possibilities of state geodetic and cartographic authorities in different countries. Land cover mapping is realisable with the use of actual open remote sensing data and historical orthophotographs with interpretation procedures and nomenclature designed in the work Oťaheľ et al. (2017). The use of the CLC at the 5th level is methodologically possible throughout the European Union. The legend is usable in Central Europe, but of course, it can be expanded by adding specific types of areas (e.g. coast, forest-steppe, tundra, etc.). However, it should be noted that the process itself is more time-consuming compared to the use of satellite images for the CLC program and it is not very suitable for larger regions. The output of our work will be further used in the landscape management of the PLA Poľana, as the staff of the PLA administration requested them.

We perceive that globalization leads to the creation of a uniform society, as well as to the loss of the specificity of regions to varying degrees. This is gradually reflected in the formation of the landscape and scattered settlements. The landscape is changing into homogeneous units — peripheral settlements that had an agricultural and manufacturing function in the past are disappearing. Forms of anthropogenic relief in a traditional agrarian landscape are an important part of cultural heritage and, at the same time, a source of biodiversity. They were created over many centuries to improve the quality of the relief soil of agricultural land and contain specific features as a result of the local agrarian culture and specific natural conditions. The benefit of the research is a detailed evaluation of the development of the land cover of the territory with scattered settlements in the location of Snohy, which points to significant changes in the use and transformation of the landscape.

Conclusions

The area of Podpoľanie with the „lazy" dispersed settlement under the Poľana Biosphere Reserve, thanks to a specific method of management, has preserved several valuable historical landscape structures (e.g. terraced fields and fragments of non-forest woody vegetation). Our study introduced a new approach to deatiled research of land cover changes of dispersed settlement area in relation to soil and geomophological conditions. Since 1949, there has been a significant change in the land cover in more than half of the Snohy territory, mainly in the structure and composition of the forest communities. The relief factors were significantly dominated by the influence of the slope on land cover changes. Stable areas of forests were linked to slopes higher than 25°, transport slopes with a slope angle of 12–25° represented habitats with deforestation in the 20th century and afforestation in the 21st century. At the same time, the gradual extensification of agriculture was promoted. Research did not show any effects that would clearly indicate changes related to areas with very low insolation intensity. We can confirm the biggest changes occurred in the modal cambisol, and that the land cover areas linked to the fluvisol and andosol was more stable, regarding the soil types.

The city of Hriňová, in cooperation with the Poľana Biosphere Reserve, has implemented several arrangements to prevent the loss of diversity in this region. In 2016 the inclusion of the Hriňová dispersed settlements in the Biosphere Reserve marked another step forward in the sustainable development of the region. In 2016, Hriňovské lazy was also included in the reserve transition zone, where horses and traditional agricultural tools are still used for agricultural work. The main goal is to direct investments into the creation of new job offers, as well as into the creation of regional products and projects that will help preserve the customs and landscape character of the Podpoľanie region. The establishment of Special areas of Conservation (SACs) Meadows of Poľana, including locality Snohy, can significantly help preserve biodiversity in this area.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Climatic niche modelling and genetic analyses highlight conservation priorities for the Spotted Softshell Turtle (*Pelodiscus variegatus*)

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Abstract

The Spotted Softshell Turtle (*Pelodiscus variegatus*) has been recognised since 2019 from Vietnam and Hainan Island, China, but little information about its population status and distribution range is currently available. The species has been provisionally listed as Critically Endangered by the Turtle and Tortoise Working Group, although the status has not been officially accepted by the IUCN, due to the threats the species is facing, including habitat loss and degradation, overexploitation for food, competition with other non-native softshell turtles and pollution. To identify conservation priority sites for *P. variegatus* in mainland Indochina, this study combines molecular analyses and species distribution modelling. Our results show that, in Vietnam, Phong Nha-Ke Bang National Park has the largest suitable area and high probability of species occurrence, followed by Vu Quang National Park and Song Thanh and Ke Go Nature Reserves. In addition, the central provinces, from Thanh Hoa to Thua Thien Hue in Vietnam, constitute a key part of the species distribution and should be prioritised for conservation actions. According to the study's findings, although *P. variegatus* is possibly found in Laos, the probability decreases sharply at the border between both countries and there is also a gap in the occurrence of wetlands, arguing for strong natural barriers. Unfortunately, to date, only part of the species potential distribution is protected, while no records are known from protected areas, highlighting the need for extended or even new reserves. To recover natural populations of the species and following the IUCN's One Plan Approach to Conservation, breeding programmes have been established in Vietnam with a potential to expand to other facilities in the country and abroad. Once suitable sites are identified, offspring can be released into the protected areas to improve the current conservation status of this highly-threatened softshell turtle.

Key words: Cytochrome b, Laos, ND4, species distribution modelling, Vietnam

Introduction

The Chinese Softshell Turtle, *Pelodiscus sinensis*, was described nearly 200 years ago and was believed to represent a morphologically variable, geographically widespread taxon (from the Russian Far East through the Korean Peninsula, eastern and central China to Vietnam). The Northern Chinese Softshell Turtle, *P. maackii* (Brandt, 1857), was described 23 years later, but was then thought to be a synonym of *P. sinensis*. Only 35 years ago, the populations from the northernmost part of the *P. sinensis* distribution range were shown to represent a distinct species, based on osteological differences (Chkhikvadze 1987).

Two additional species of this complex from central China were described in the 90s, based on morphological differences: the Hunan Softshell Turtle (*P. axenaria*) and the Lesser Chinese Softshell Turtle (*P. parviformis*) (Zhou et al. 1991; Tang 1997). Recent molecular studies confirmed that the genus *Pelodiscus* constitutes a species complex (Fritz et al. 2010, see also Stuckas and Fritz (2011); Yang et al. (2011); Gong et al. (2018)). Based on genetic and morphological analyses, three new taxa were described: the Spotted Softshell Turtle, *P. variegatus* from northern Vietnam and Hainan (China), the Horse-hoof Softshell Turtle, *P. huangshanensis* from southern Anhui Province of China and the Chinese Stone Slap Softshell Turtle, *P*. *shipian* from Jiangxi and Hunan Provinces of China (Farkas et al. 2019; Gong et al. 2021; Gong et al. 2022).

Thus, the *Pelodiscus sinensis* complex at the moment comprises seven species, with six species being distributed in China and four endemic to the country (Gong et al. 2021). The revisions have consequences on the species conservation, as taxonomic splitting implies that the range size and number of individuals decrease for each species. However, these new research results have yet to be reflected in the conservation assessment of the genus. According to the IUCN (2023), only *P*. *sinensis* is listed as Vulnerable, but the data are greatly outdated with the last update in 2000 (Asian Turtle Trade Working Group 2000). The Turtle Taxonomy Working Group (TTWG) recently evaluated conservation status of six species in the genus and provisionally categorised three species as Data Deficient (*P*. *axenaria*, *P*. *huangshanensis* and *P*. *maackii*), one as Vulnerable (*P*. *sinensis*) and two as Critically Endangered (*P*. *parviformis* and *P*. *variegatus*; TTWG 2021). The last species, *P*. *shipian*, has not been assessed by any previous study.

The exact range of the recently-described *P. variegatus* is still largely unknown, but historical records suggest that the species occupies lowland areas of central and northern Vietnam and parts of southern China, viz. Hainan Province (Farkas et al. 2019; TTWG 2021). Recently, Ziegler et al. (2020) investigated whether natural populations of *P. variegatus* still exist, since the description of this species was mostly based on historical museum specimens. To find potential members of *P. variegatus*, this study focused on surveys of central lowland freshwater habitats, as well as local markets, restaurants and farms in Vietnam. Individuals with the species-specific dark blotched plastron pattern were identified and subsequently genetically screened to confirm their identity. By using this approach, Ziegler et al. (2020) demonstrated that

P. variegatus is still extant in the wild in Vietnam, both based on evidence from the trade and on surveys in the natural habitat. The study also showed that *P. variegatus* occurs in the central provinces of Thanh Hoa, Nghe An, Ha Tinh and Quang Binh, primarily in lakes with flat shores consisting of mud and soft soil, rivers in agricultural landscape and medium-sized streams in secondary forests.

As softshell turtles are common and prized as food where they occur, natural populations are threatened by local hunting, with further threats of habitat loss and competition with introduced softshell turtles (Shi et al. 2008; Le Duc et al. 2020). At the moment, there is little evidence of any viable population of the *P. variegatus* existing in the wild. It is, therefore, imperative to implement conservation measures in priority areas, where natural populations still likely survive. To establish conservation priorities for the species in Vietnam, the present study performed climatic niche modelling, based on comprehensive distribution data derived from detailed molecular analyses of existing and new samples collected from the country, the Emys system (emys.geo.orst.edu), the Turtle Taxonomy Working Group (TTWG 2021) and the Global Biodiversity Information Facility (GBIF).

Material and methods

Molecular analyses

To identify new samples collected in the field, we used a molecular approach. In total, 61 newly-collected samples were included in the analyses (Suppl. material 1). Sequences of other *Pelodiscus* species were downloaded from GenBank. Two species, *Dogania subplana* and *Palea steindachneri*, were employed as outgroups (Le et al. 2014). We used the protocols of Le et al. (2006) for DNA extraction and amplification. Two mitochondrial genes, the nearly complete cytochrome b (1110 bp) and partial ND4 (673 bp), were amplified using primers listed in Table 1. Successful amplifications were purified to eliminate PCR components using GeneJET™ PCR Purification Kit, Thermo Fisher Scientific (Vilnius, Lithuania). Purified PCR products were sent to 1st BASE (Selangor, Malaysia) for sequencing. Sequences generated in this study were aligned using De Novo Assemble function in the program Geneious v.7.1.8 (Kearse et al. 2012).

Data were then analysed using Maximum Likelihood (ML) as implemented in IQ-TREE v.1.6.7.1 (Nguyen et al. 2015) and Bayesian Inference analysis (BI), as implemented in MrBayes v.3.2.7 (Ronquist et al. 2012). For ML analysis, we used a single model and 10,000 ultrafast bootstrap replications. The optimal

Table 1. Primers used in this study.

Primer	Sequence	Reference
Gludg (f)	5'- TGACTTGAARAACCAYCGTTG - 3'	Palumbi (1996)
CB3 (r)	5'- GGCAAATAGGAAATATCATTC - 3'	Palumbi (1996)
CB534 (f)	5'- GACAATGCAACCCTAACACG- 3'	Engstrom et al. (2004)
Tcytbthr (r)	5'- TTCTTTGGTTTACAAGACC - 3'	Engstrom et al. (2004)
ND4 672 (f)	5'- TGACTACCAAAAGCTCATGTAGAAGC - 3'	Engstrom et al. (2002)
Hist (r)	5'- CCTATTTTTAGAGCCACAGTCTAATG - 3'	Arévalo et al. (1994)

model for nucleotide evolution employed in both methods was determined using jModelTest v.2.1.4 (Darriba et al. 2012). The optimal model for nucleotide evolution was set to TIM2+G for ML and single-model Bayesian analyses. For the Bayesian Inference, two independent analyses with four Markov chains (one cold and three heated) were run simultaneously for 10 million generations with a random starting tree and sampled every 1000 generations. Log-likelihood scores of sample points were plotted against generation time to determine stationarity of Markov chains. Trees generated before log-likelihood scores reached stationarity were discarded from the final analyses using the burn-in function. The posterior probability values for all clades in the final majority rule consensus tree were provided. The cut-off point for the burn–in function was set to 71 in the Bayesian analysis, as –ln*L* scores reached stationarity after 71,000 generations in both runs. Nodal support was also evaluated using ultrafast bootstrap (UFB) in IQ-TREE and posterior probabilities (PP) in MrBayes. PP and UBP ≥ 95% were regarded as strong support for a clade (Ronquist et al. 2012; Nguyen et al. 2015).

This study only employed mitochondrial genes to provide taxonomic identification of samples collected from the wild. Although maternally inherited mitochondrial loci cannot help detect hybridisation events, interbreeding between different species of softshell turtles has only been reported in turtle farms (Gong et al. 2018). In addition to genetic screening of captured animals, we morphologically identified the specimens using diagnostic characters.

Species records and environmental variables

For species records, we carefully checked potential records listed in recent studies, including, Le Duc et al. (2020), Pham et al. (2020), Ducottend et al. (2023), Pham et al. (2023a) and Pham et al. (2023b). However, the papers followed the older version of the Turtle of the World Checklist (TTWG 2018), which omitted information about *P. variegatus* because the species was only described a year later in 2019. As a result, the records were all assigned to *P. sinensis*, although the species is now considered only occurring in China and Taiwan according to the new checklist (TTWG 2021). In addition, much of the information came from interviews with local people and is not taxonomically robust. In Pham et al. (2023b), some photos clearly belong to the *P. sinensis* form, while the others did not show diagnostic characters to allow identification of these individuals. We, therefore, did not add the data to our analyses. In the final dataset, we used 54 unique, georeferenced and genetically confirmed locations located in unique grid cells, as shown in Fig. 2 (see below).

For environmental predictors, we used a combination of weather station-derived precipitation data and remote sensing data. The full list of variables including their interpretation is provided in Table 2. Average annual characteristics of precipitation regimes were obtained from the Worldclim database 2.1 as interpolated elements from different climate conditions collected over a period of 30 years (1970–2000) with a resolution of 30 arc seconds (Hijmans et al. 2005; Fick and Hijmans 2017). In order to characterise seasonal changes in land surface temperatures and in vegetation cover, we used 27 remote sensing predictors derived from the Moderate Resolution Imaging Spectroradiometer (MODIS) sensors of two NASA satellites available through the EDENext project (temporal resolutions: 8-day averages (MOD11A2) and 16-day averages (MCD43B4), spatial resolution 30 arc seconds) (Mu et al. 2007). Availability of wetlands as surrogate of suitable microhabitats was assessed using a recent assessment of tropical wetlands provided by Gumbricht et al. (2017) as a categorical variable. As the wetlands dataset lacks river networks which may provide suitable microhabitats for the turtle, we added a high-resolution water layer as additional category (GRDC 2020). The wetlands dataset was resampled to the spatial resolution of the remote sensing predictors and only grid cells including water bodies were considered.

Multi-collinearity amongst predictors may hamper successful model training and subsequent projection (Brun et al. 2020). Therefore, we computed pairwise Spearman rank correlations and selected each predictor pair with $\rho^2 > 0.75$ with only one predictor for final model training. The final set of variables comprised both climate and land-cover related variables, which are suitable to characterise the species range limits and microhabitat preferences. As these operate on very different scales, we used a two-step approach for modelling: (1) determining likely range limits with temperature and precipitation related variables and (2) using these range limits to train a second model using land-cover related variables to assess microhabitat preferences. For the first climate related model, the variables comprised the annual mean surface temperature (ED15078_bio1), maximum surface temperature of the warmest month (ED15078_bio6), surface temperature annual range (ED15078_bio7), annual mean precipitation (bio_12), precipitation of the driest month (bio_14) and precipitation of the warmest quarter (bio_18). Quantification of microhabitat preferences was derived from the annual mean Normalised Difference Vegetation Index (NDVI; ED1514_bio1), annual range of NDVI (ED1514_bio7), annual mean Enhanced Vegetation Index (EVI; ED1515_bio1), annual range of EVI (ED1515_bio7) and the categorical map of wetlands including the categories open water, mangroves, swamps, flood-out swamp, fens, riverine, floodplain, marshes, marshes—dryland/wetland and marshes—wet meadows.

Species distribution modelling

As the algorithm for climatic niche modelling development, we used Maxent v.3.4.0, which is specifically designed to derive potential distributions from presence-pseudoabsence data (Phillips et al. 2006; Phillips et al. 2017) and which can perform well even if the sample size is comparatively small (Hernandez et al. 2006). For the first model, we chose an area defined by a minimum convex polygon buffered with 5 km enclosing all species records as environmental background. Model selection followed the procedure described in Ginal et al. (2022). In Maxent, we allowed only linear, product and quadratic feature classes and used a regularisation multiplier of 0.8, as theses settings had, on average, the minimum delta AICc (689.4 with 8 parameters) and revealed the most realistic response curves. Using these optimal settings for feature classes and regularisation parameter and a bootstrap approach, we computed 100 models, each trained with 80% of the species records and using the remaining 20% for model evaluation via the area under the ROC (Receiver Operating Characteristic) curve [AUC] (Swets 1988). The average across all 100 replicates in cloglog format was used for further processing.

For the second land-cover niche modelling, we reclassified the potential distribution suggested by the first model applying the minimum training presence threshold, which was used as environmental background. Model selection followed again Ginal et al. (2022) and internal settings in Maxent were set to a regularisation parameter of 0.7 and linear, product and quadratic feature classes. The regularisation parameter of the categorical wetland predictor was set to 0.250. The average delta AICc was 760.6 with 10 parameters. Again, the average across all 100 replicates in cloglog format was used for further processing. The joint effects of climatic suitability and microhabitat suitability were estimated by rescaling both average predictions to a scale of 0–1 after applying the minimum training presence threshold and multiplying both. The resulting map highlights areas where both climatic and microhabitat suitability are highest.

Defining conservation priority sites

We merged the occurrence data with existing protected areas (Reserves, National Parks etc.) in the country. Information of protected areas was obtained from the world dictionary of protected areas/protected planet (https://www.protectedplanet.net). We selected the targeted area in north-central and central Vietnam because most of distribution records were reported from the region. In total, there were 42 potential conservation units within the general area and, for each Reserve, we computed the number of suitable grid cells, the sum of probabilities and the mean probability in QGIS 3.14. Map resolution ca. 1 km (30 arc sec).

Results

The molecular matrix contained 1921 aligned characters. Both BI and ML analyses showed that new samples belong to *Pelodiscus sinensis* and *P. variegatus*. The former species was only moderately supported (PP = 89%, UBF = 90%), while the latter received strong support from both BI and ML (PP = 100%, UBF = 99%). In total, 61 newly-collected and four GenBank samples were identified as *P. variegatus* (see Suppl. material 1: fig. S1, for the full tree). The localities of the samples were used for the species distribution modelling.

The niche modelling trained with only climatic variables had a good overall performance (AUC_{test} = 0.79; $AUC_{training}$ = 0.84, Suppl. material 1: fig. S2). The predictor with the highest explanative power was precipitation of the driest month (bio_14: 77%), followed by the maximum surface temperature of the warmest month (ED15078_bio6: 11.8%), precipitation of the warmest quarter (bio_18: 5.3%), surface temperature annual range (ED15078_bio7: 3.9%), annual mean surface temperature (ED15078_bio1: 1.4%) and annual mean precipitation (bio_12: 0.7%). Based on climatic conditions, the potential distribution covers major parts of central Vietnam, wherein the probability of occurrence sharply decreases towards Laos (Fig. 2A).

The niche modelling, trained with microhabitat variables, had a good overall performance (AUCtest = 0.73; AUCtraining = 0.80, Suppl. material 1: fig. S3). The predictor with the highest explanative power was the categorical map of wetlands (60.3%) (open water, mangroves, swamps, flood-out swamp, fens, riverine, floodplain, marshes, marshes—dryland/wetland and marshes—wet meadows), followed by annual mean NDVI (ED1514_bio1: 6.6%), annual mean EVI (ED1515_bio1: 3.6%), annual range of EVI (ED1515_bio7: 1.9%) and annual range of NDVI (ED1514_bio7: 1.7%). Density of suitable microhabitats is highest near the coast, with some suitable wetlands/water bodies in higher elevations towards Laos (Fig. 2B).

When integrating the probabilities of occurrence derived from climatic and land-cover variables, the most suitable habitats for *P. variegatus* are near the Vietnam coastline, where extensive freshwater wetlands exist (Fig. 2C). Rivers and other water bodies in mountainous areas are partly suitable, but they cover much less area.

Only part of the potential distribution for *P. variegatus* in Vietnam is protected and no known occurrence is directly located within reserves, although they are close, such as nearby Ke Go Nature Reserve and Vu Quang National Park (Fig. 2D). Table 3 has information on the relative ranking of protected areas, based on their IUCN status, the total area suitable for *P. variegatus*, the sum of potentially suitable sites and the average potential for the species. The Table is sorted with descending total suitable areas per Reserve.

Discussion

Farkas et al. (2019) stated that, in Vietnam, most records of *P. variegatus* fall within the “Northeast Lowlands Subregion” of Bain and Hurley (2011). The zoogeographical affinities of Hainan are closely related to this area as well as mainland south-western China, while the southern portion of the purported range forms part of the “Central– South Vietnam Lowlands Subregion” as defined by Bain and Hurley (2011). However, our molecular data show that most distribution localities of the species occur in the north-central region of the country, except for records from Dong Mo Lake (Fig. 1). It is likely that a majority of extant populations of the species is restricted to this region in Vietnam, although it is possibly found in Laos, based on climatic niche modelling results. However, the probability based on climate decreases sharply at the border between both countries and there is also a gap in the occurrence of wetlands. Thus, the border area may represent a strong natural barrier.

Our habitat suitability analysis predicts that two most important protected areas for *P. variegatus* include Phong Nha-Ke Bang and Vu Quang National Parks in Vietnam. Other protected sites with the largest suitable sizes consist of Song Thanh and Ke Go Nature Reserves and Ben En National Park (Table 3, Fig. 2). In terms of Average Probability, Hue Saola Nature Reserve receives the highest value (0.422), followed by Phong Dien Nature Reserve, Bach Ma National Park and Ke Go Nature Reserve (Table 3). The findings show that the central provinces from Thanh Hoa to Thua Thien Hue form an important part of the species distribution. Although one record in our study suggests that the species occurs in Laos (Fig. 2), its approximate field coordinates could not be used to absolutely confirm the species’ presence in the country. It is, therefore, essential to conduct field surveys at suitable sites in Laos to verify its presence or absence.

Prior to its discovery, *P. variegatus* was considered part of *P. parviformis.* The latter species was already assessed as threatened and included on appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). As its southern population has been split into a different species,

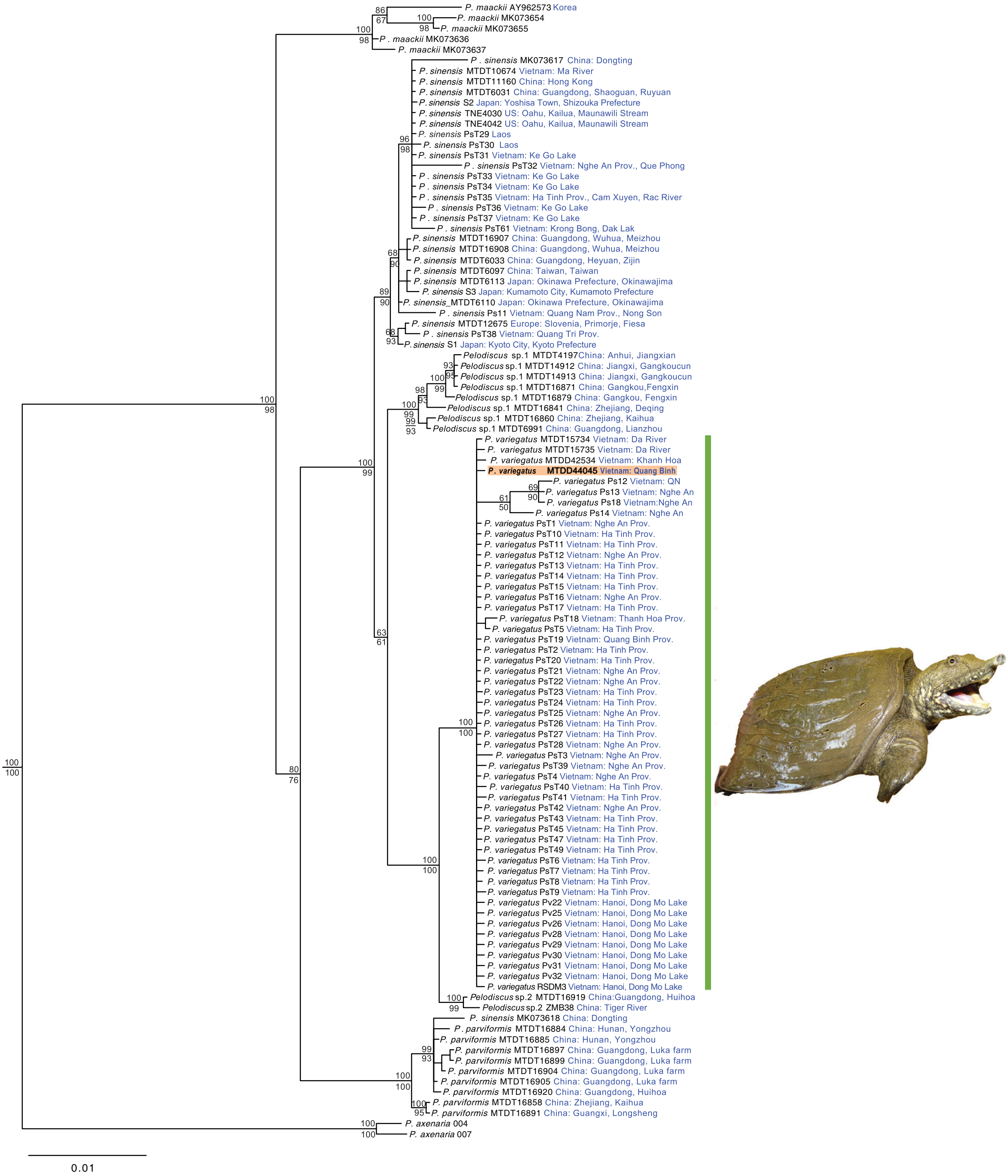

Figure 1. Trimmed phylogram, based on the Bayesian analysis. Number above and below branches of major nodes are Bayesian posterior probabilities and ML ultrafast bootstrap values, respectively. Sample highlighted in bold and orange is the paratype of *Pelodiscus variegatus*.

P. variegatus, the overall population of each species becomes even smaller than previously thought. Rhodin et al. (2018) regarded *P. parviformis* as "Critically Endangered (CR)". Consequently, TTWG (2021) provisionally categorised *P. variegatus* as CR, although the assessment has not been officially accepted by the IUCN Red List. The species was recently proposed to list as Vulnerable (VU) in the Viet-

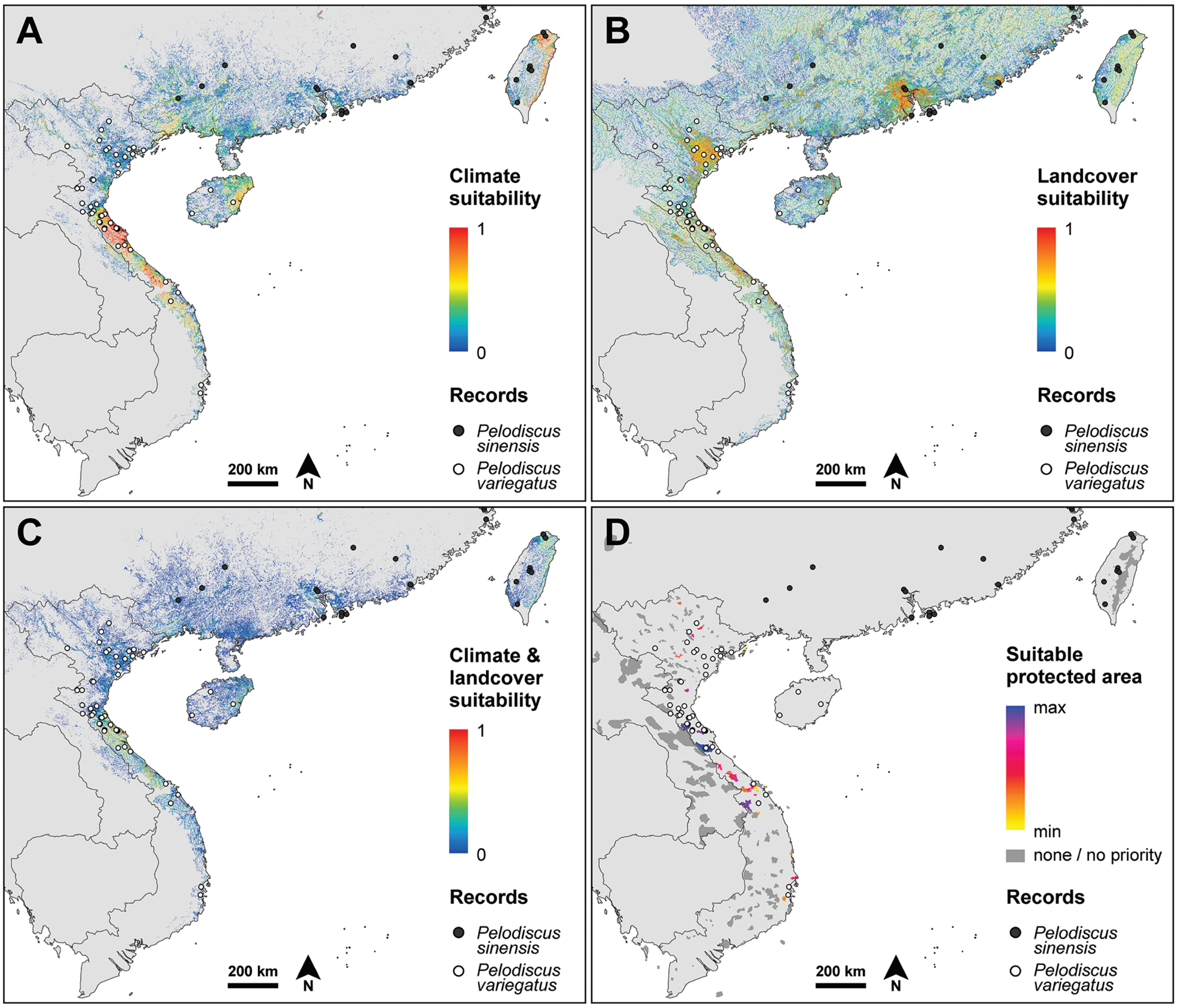

Figure 2. Potential distribution of *Pelodiscus variegatus* in Vietnam based on **A** climate **B** land cover **C** climate and land cover and **D** coverage with protected areas as number of suitable grid cells.

nam Red Data Book, based on estimation of population reduction approximately of over 30% in the past of 30 years (Nguyen, per. comm. 2023).

Ziegler et al. (2020) found during their market and trade surveys in Vietnam that human overexploitation of softshell turtles appears massive. In addition, interbreeding events were observed to occur amongst softshell turtle species in farms. Most of the inhabited freshwater bodies and their surroundings showed signs of human encroachment, such as fishing, vegetation transformation, conversion and pollution. Thus, both *in situ* and *ex situ* conservation measures seem essential for protecting *P. variegatus* from extinction. Some of the investigated freshwater habitats are already located inside protected areas, such as Ben En, Phong Nha-Ke Bang and Vu Quang National Parks and Ke Go Nature Reserve in central Vietnam. Some of the protected areas are well known internationally as either special bird areas (Ke Go Nature Reserve) or with spectacular mammals, including Saola (*Pseudoryx nghetinhensis*) and the Large-antlered Muntjac (*Muntiacus vuquangensis*) (Vu Quang National Park). Improving conservation in and around the protected areas will benefit a suite of critically-endangered and endemic species, including the *P. variegatus*.

Table 2. Variables used for climatic niche modeling computation. NDVI = Normalized Difference Vegetation Index, EVI = Enhanced Vegetation Index.

Abbreviation	Remote sensing variable	Bioclimatic variable	Derived variable	Source
bio_12	N/A	Annual Precipitation	N/A	Worldclim 2.1
bio_13	N/A	Precipitation of Wettest Month	N/A	Worldclim 2.1
bio_14	N/A	Precipitation of Driest Month	N/A	Worldclim 2.1
bio_15	N/A	Precipitation Seasonality (Coefficient of Variation)	N/A	Worldclim 2.1
bio_16	N/A	Precipitation of Wettest Quarter	N/A	Worldclim 2.1
bio_17	N/A	Precipitation of Driest Quarter	N/A	Worldclim 2.1
bio_18	N/A	Precipitation of Warmest Quarter	N/A	Worldclim 2.1
bio_19	N/A	Precipitation of Coldest Quarter	N/A	Worldclim 2.1
ED1514_bio1	MODIS V4 Band 14 Synoptic Months: NDVI	BIO1 = Annual Mean Temperature	Annual Mean of NDVI	EDENext
ED1514_bio2	MODIS V4 Band 14 Synoptic Months: NDVI	BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))	Mean Diurnal Range of NDVI	EDENext
ED1514_bio3	MODIS V4 Band 14 Synoptic Months: NDVI	BIO3 = Isothermality (BIO2/BIO7) (×100)	Isothermaility (BIO2/BIO7) (*100) of NDVI	EDENext
ED1514_bio4	MODIS V4 Band 14 Synoptic Months: NDVI	BIO4 = Temperature Seasonality (standard deviation ×100)	Seasonality of NDVI	EDENext
ED1514_bio5	MODIS V4 Band 14 Synoptic Months: NDVI	BIO5 = Max Temperature of Warmest Month	Max NDVI of Monthly Scores	EDENext
ED1514_bio6	MODIS V4 Band 14 Synoptic Months: NDVI	BIO6 = Min Temperature of Coldest Month	Min NDVI of Monthly Scores	EDENext
ED1514_bio7	MODIS V4 Band 14 Synoptic Months: NDVI	BIO7 = Temperature Annual Range (BIO5-BIO6)	Annual Range of NDVI	EDENext
ED1514_bio10	MODIS V4 Band 14 Synoptic Months: NDVI	BIO10 = Mean Temperature of Warmest Quarter	Mean NDVI of Warmest Quarter	EDENext
ED1514_bio11	MODIS V4 Band 14 Synoptic Months: NDVI	BIO11 = Mean Temperature of Coldest Quarter	Mean NDVI of Coldest Quarter	EDENext
ED1515_bio1	MODIS V4 Band 15 Synoptic Months: EVI	BIO1 = Annual Mean Temperature	Annual Mean of EVI	EDENext
ED1515_bio2	MODIS V4 Band 15 Synoptic Months: EVI	BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))	Mean Diurnal Range of EVI	EDENext
ED1515_bio3	MODIS V4 Band 15 Synoptic Months: EVI	BIO3 = Isothermality (BIO2/BIO7) (×100)	Isothermaility (BIO2/BIO7) (*100) of EVI	EDENext
ED1515_bio4	MODIS V4 Band 15 Synoptic Months: EVI	BIO4 = Temperature Seasonality (standard deviation ×100)	Seasonality of EVI	EDENext
ED1515_bio5	MODIS V4 Band 15 Synoptic Months: EVI	BIO5 = Max Temperature of Warmest Month	Max EVI of Monthly Scores	EDENext
ED1515_bio6	MODIS V4 Band 15 Synoptic Months: EVI	BIO6 = Min Temperature of Coldest Month	Min EVI of Monthly Scores	EDENext
ED1515_bio7	MODIS V4 Band 15 Synoptic Months: EVI	BIO7 = Temperature Annual Range (BIO5-BIO6)	Annual Range of EVI	EDENext
ED1515_bio10	MODIS V4 Band 15 Synoptic Months: EVI	BIO10 = Mean Temperature of Warmest Quarter	Mean EVI of Warmest Quarter	EDENext
ED1515_bio11	MODIS V4 Band 15 Synoptic Months: EVI	BIO11 = Mean Temperature of Coldest Quarter	Mean EVI of Coldest Quarter	EDENext
ED15078_bio1	MODIS V4 Band 07+08 Synoptic Months: day- & nighttime land surface temperature	BIO1 = Annual Mean Temperature	Annual Mean Temperature	EDENext
ED15078_bio2	MODIS V4 Band 07+08 Synoptic Months: day- & nighttime land surface temperature	BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))	Mean Diurnal Range of Temperature	EDENext
ED15078_bio3	MODIS V4 Band 07+08 Synoptic Months: day- & nighttime land surface temperature	BIO3 = Isothermality (BIO2/BIO7) (×100)	Isothermality (Bio2/Bio7) (*100)	EDENext
ED15078_bio4	MODIS V4 Band 07+08 Synoptic Months: day- & nighttime land surface temperature	BIO4 = Temperature Seasonality (standard deviation ×100)	Seasonality	EDENext
ED15078_bio5	MODIS V4 Band 07+08 Synoptic Months: day- & nighttime land surface temperature	BIO5 = Max Temperature of Warmest Month	Max Temperature of Warmest Month	EDENext

Abbreviation	Remote sensing variable	Bioclimatic variable	Derived variable	Source
ED15078_bio6	MODIS V4 Band 07+08 Synoptic Months: day- & nighttime land surface temperature	BIO6 = Min Temperature of Coldest Month	Min Temperature of Coldest Month	EDENext
ED15078_bio7	MODIS V4 Band 07+08 Synoptic Months: day- & nighttime land surface temperature	BIO7 = Temperature Annual Range (BIO5-BIO6)	Temperature Annual Range	EDENext
ED15078_bio10	MODIS V4 Band 07+08 Synoptic Months: day- & nighttime land surface temperature	BIO10 = Mean Temperature of Warmest Quarter	Mean Temperature of Warmest Quarter	EDENext
ED15078_bio11	MODIS V4 Band 07+08 Synoptic Months: day- & nighttime land surface temperature	BIO11 = Mean Temperature of Coldest Quarter	Mean Temperature of Coldest Quarter	EDENext

Table 3. Protected areas in Vietnam with projected proper climatic conditions and land-cover (Average Probability > 0.1) for *Pelodiscus variegatus*, sorted by size of suitable area. A full list of protected areas in China, Laos and Vietnam is presented in the Suppl. material 1.

Name	Type	IUCN category	Area [km^2]	Average Probability	Suitable Area [km^2]
Phong Nha-Ke Bang	National Park	II	1222.825	0.303	424.578
Vu Quang	National Park	II	591.661	0.275	168.536
Song Thanh	Nature Reserve	Not Reported	890.589	0.110	117.079
Ke Go	Nature Reserve	IV	239.724	0.364	91.145
Ben En	National Park	II	141.816	0.123	85.311
Phong Dien	Nature Reserve	IV	407.762	0.417	85.060
Bach Ma	National Park	II	375.506	0.389	79.732
Nui Coc	Cultural and Historical Site	V	90.263	0.200	60.395
Hoa Lu	Cultural and Historical Site	Not Reported	65.819	0.126	59.431
Bac Huong Hoa	Nature Reserve	Not Reported	235.057	0.162	56.869
Deo Ca-Hon Nua	Cultural and Historical Site	V	215.611	0.122	56.472
Than Sa-Phuong Hoang	Nature Reserve	Not Reported	136.517	0.112	35.724
Nui Thanh	Cultural and Historical Site	V	64.139	0.134	34.971
Dakrong	Nature Reserve	Not Reported	387.232	0.351	32.474
Dao Ho Song Da	Cultural and Historical Site	V	78.889	0.130	31.908
Bac Me	Nature Reserve	Not Reported	87.109	0.122	24.266
Hue Sao La	Nature Reserve	Not Reported	377.864	0.422	17.208
Hon Ba	Nature Reserve	Not Reported	198.213	0.146	14.560
Ngoc Linh (Quang Nam)	Nature Reserve	Not Reported	192.496	0.146	10.710
Quy Hoa-Ghenh Rang	Cultural and Historical Site	Not Reported	52.102	0.115	7.707
Ba Na-Nui Chua	Nature Reserve	IV	268.922	0.226	6.019
Nui Chung	Cultural and Historical Site	Not Reported	2.031	0.295	2.020
Bai Tu Long	National Park	Not Reported	64.906	0.214	1.401
Son Tra	Nature Reserve	Not Reported	38.368	0.198	1.213

As a first measure, based on the genetically-identified individuals, a conservation breeding programme has been established. This is following IUCN's One Plan Approach to Conservation, developed by the Conservation Planning Specialist Group (CPSG), which combines *in situ* with *ex situ* conservation measures for the optimum protection of a given species (Byers et al. 2013). For the build-up of the conservation breeding programme, the individuals identified as *P. variegatus* were transferred to the Melinh Station for Biodiversity of the Institute of Ecology and Biological Resources (IEBR), Hanoi. In the Station, located in Vinh Phuc Province, northern Vietnam, besides existing outdoor tank facilities, an exclusive softshell turtle breeding facility was constructed recently.

To maximise positive outcomes and for security reasons, viz., to extend the conservation breeding network, another group of the genetically-identified *P. variegatus* was provided to another softshell breeding facility in northern Vietnam. Successful breeding has already been observed in the colony and offspring are ready for release to the original habitat sites. To extend the conservation breeding programme and, thus, contribute to the build-up of a stable assurance colony and conservation breeding network, a plan has been developed to transfer a limited number of surplus offspring to other facilities in Vietnam and overseas. In addition to these *ex situ* conservation measures already being in place, focus should now be directed to improving *in situ* conservation of this beautiful, but threatened softshell turtle species.

Note added in proof

In the late 2023, 50 young and healthy spotted softshell turtles from the in-country breeding program initiated by the Institute of Ecology and Biological Resources (IEBR), Vietnam, together with the Cologne Zoo, Germany, were successfully released to a site in northern Vietnam.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

TZ, MDL, TTN, and TQN conceptualized the study; CTP, AVO, TEMM, and TTN conducted the fieldwork; DR, HTN, MDL, MHL, and TTN led the data analysis, data curation; TZ, MDL, DR led the writing and all authors edited and approved the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary data

Authors: Minh Duc Le, Dennis Rödder, Tao Thien Nguyen, Cuong The Pham, Truong Quang Nguyen, An Vinh Ong, Timothy E. M. Mccormack, Thang Tai Nguyen, Mai Huyen Le, Hanh Thi Ngo, Thomas Ziegler

Data type: docx

Explanation note: **table S1.** Samples used in this study. **table S2.** Relative importance of protected areas in China, Laos and Vietnam for *Pelodiscus variegatus* in terms of climatic conditions and land-cover. The Table is sorted according to the suitable area in each Reserve. **figure S1.** Full phylogram based on the Bayesian analysis. Numbers above and below branches of selected nodes are Bayesian posterior probabilities and ML ultrafast bootstrap values, respectively. **figure S2.** Summary of the receiver operating characteristic curve of 100 Maxent models for *P. variegatus* trained with climatic variables. **figure S3.** Summary of the receiver operating characteristic curve of 100 Maxent models for *P. variegatus* trained with land-cover variables.

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Review Article

Linking sufficiency and the protection of biodiversity: An issue of political implications, framing, descriptiveness and interdisciplinarity?

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Abstract

The dramatic loss of biodiversity is caused by the use of resources and land. One strategy aiming at reducing the use of resources and land is sufficiency, which consequently could be a strategy for protecting biodiversity. This article therefore examines the extent to which sufficiency in the context of biodiversity conservation is already being addressed by nature conservation associations and the scientific community. To this end, publications were analysed firstly with regards to the understanding of sufficiency, secondly with regards to the considered links between sufficiency and biodiversity as well as thirdly with regards to the considered fields of action. The systematic identification and evaluation of scientific publications (for the years 2017–2021) and publications by German and international nature conservation associations shows that few publications address the link between sufficiency and biodiversity. And when they do, the link often remains unspecific. Possible reasons are that sufficiency potentially has broader political implications, that the term is not descriptive and that other terms are used to describe similar strategies. Other potential explanations are that several framings for the need for sufficiency are possible and that linking sufficiency and biodiversity requires interdisciplinarity. Drawing on the results and the discussion, an argument in favour of using the term 'sufficiency' and further research is presented. Moreover, a sufficiency typology is developed and questions are raised that could form the basis for future research on linking biodiversity conservation and the various aspects of sufficiency.

Key words: Biodiversity conservation, biodiversity loss, sufficiency, sustainability strategies

Introduction

The loss of biodiversity, as described in the Living Planet Report (WWF 2020) or the Global Assessment Report on Biodiversity and Ecosystem Services (IPBES 2020), is dramatic. Land-use change, direct exploitation, invasive species, climate change and pollution are named as the main drivers of biodiversity loss (see e.g. IPBES 2020, p. 245). These are caused by human activities such as agriculture, forestry, fishing, farming, mining and energy production, among others (IPBES 2020). This means that the loss of biodiversity is largely due to human use of resources and land which consequently needs to be reduced.

One strategy that aims at an absolute reduction of resource consumption is ‘sufficiency’. As a term for a sustainability strategy, besides ‘efficiency’ and ‘consistency’ it was first used in the German-speaking world by Sachs (1993). He describes sufficiency as a “principle of self-restraint” (Sachs 2015, p. 4), which includes ‘deceleration’, ‘regionality’, ‘common good economy’, ‘reinvention of the commons’ and a modified or specific ‘art of living’. Linz (2002) explains that in the “narrower understanding [sufficiency] forms the counterpart to efficiency, is directed towards the reduced consumption of resources and is thus quantitatively oriented. The broader understanding [of sufficiency] is directed towards a new sense of prosperity and towards cultural change, which is both its precondition and its result” (Linz 2002, p. 13, own translation). Fischer et al. (2013) describe sufficiency as “changes in consumption patterns that help to stay within the Earth’s ecological carrying capacity, changing utility aspects of consumption” (Fischer et al. 2013, p. 13, own translation). Finally, the IPCC 2022 report defines sufficiency policy as “a set of measures and daily practices that avoid demand for energy, materials, land and water while delivering human wellbeing for all within planetary boundaries” (IPCC 2022, p. 31). Although there are a variety of definitions, the term sufficiency seems to stand for a strategy to reduce resource consumption, which can simultaneously include aspects of social change, social justice and the consideration of planetary boundaries.

The fact that, firstly, a reduction in resource consumption is imperative for the conservation of biodiversity and, secondly, sufficiency is a strategy that aims to reduce resource consumption suggests that sufficiency contributes to the conservation of biodiversity. This raises the question of the extent to which sufficiency as a strategy for the conservation of biodiversity is addressed by the scientific community, and in particular by the disciplines that deal with nature conservation, as well as by nature conservation associations. In order to investigate this systematically, scientific publications and publications by nature conservation associations were analysed under the following questions:

1. How is sufficiency understood or defined?
2. How are sufficiency and biodiversity linked?
3. Which sufficiency action field is the focus of the publication?

Following the answers to these questions, the results are discussed and a typology of sufficiency is proposed.

Methodology

To answer the research questions, a systematic literature review was conducted. The procedure is based on the content structuring analysis as described by Kuckartz (2018): Firstly, the publications to be examined are systematically identified and narrowed down, secondly, deductive-inductive categories are formed and a word environment analysis is carried out (cf. Fig. 1).

Identification of relevant publications

Both scientific publications and publications by nature conservation organisations were included in the analysis. The scientific literature was identified using the

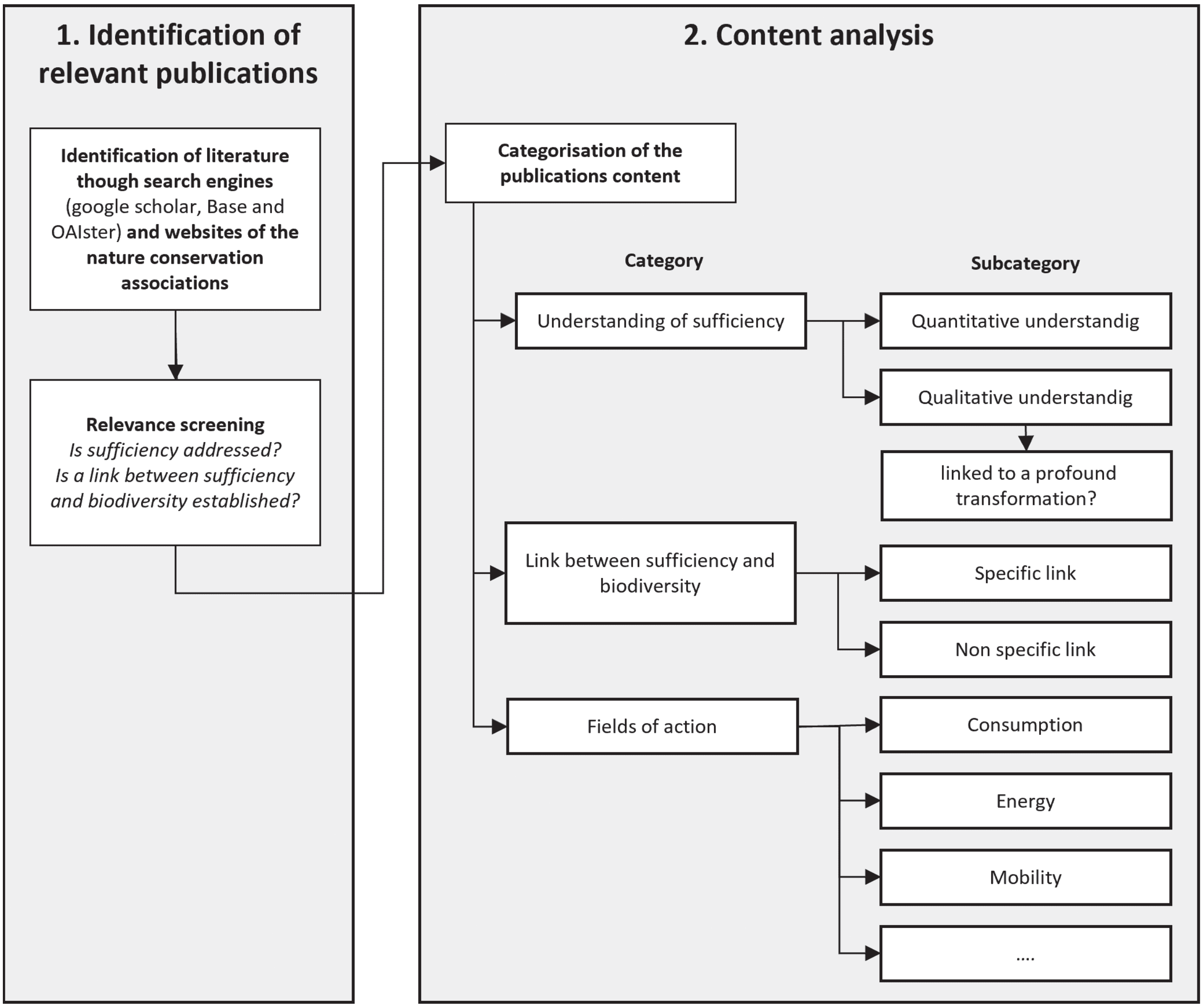

Figure 1. Methodology.

search engines BASE, OAIster and Google Scholar using the keywords ‘sufficiency’, ‘biodiversity’, ‘nature conservation’ and ‘consumption’ or ‘production’. Only publications that were published between 2017 and 2021 as well as written in German or English were evaluated. This temporal restriction was made in order to determine the current state of discussion. In order to filter out non-relevant publications, the search settings were adjusted (where possible) so that publications with terms such as ‘self-sufficiency’, ‘insufficient’ and ‘sufficiency of’ were not displayed.

Nature conservation associations were analysed both at the national level in Germany and internationally. To determine the publications of German nature conservation associations and foundations, the websites of German nature conservation organisations with a strong presence in the public debate and high membership numbers were analysed. These are: Naturschutzbund Deutschland (NABU), Greenpeace Deutschland, World Wide Fund for Nature Deutschland (WWF Deutschland) and Bund für Umwelt und Naturschutz Deutschland (BUND). This search was carried out via the websites of these organisations at federal and federal state level. In the search for publications of international nature conservation organisations and networks, the main pages of Greenpeace, WWF, International Union for Conservation of Nature (IUCN) and Friends of the Earth were searched. These organisations or networks were selected partly because of their size and partly because they are the 'umbrella organisations' of the German organisations surveyed. The IUCN is an exception to this but was selected additionally as assessing and protecting Biodiversity is a focal point of the organisation.

The keywords used in the search for publications of the nature conservation associations were 'Suffizienz' (German) and 'Sufficiency' (English) and 'Biodiversität' (German) and 'Biodiversity' (English). A restriction to a specific time period, as in the search for scientific publications, was not possible in the search on the websites of the nature conservation associations and was therefore not carried out. The evaluation was also not limited to specific years, as the year of publication was not specified in some cases.

The publications identified in this way were subjected to a relevance screening based on their tables of contents and summaries as well as a keyword ('sufficiency' as well as 'biodiversity') search. For the further evaluation, only publications were considered that firstly dealt with sufficiency as a sustainability strategy in the sense described above and secondly established a link between biodiversity and sufficiency.

Content analysis

For conducting the content analysis categories were defined. These were derived from the research questions mentioned above. The sub-categories were then developed deductively-inductively.

Category 'Understanding of sufficiency'

As mentioned above, sufficiency can be defined and understood in different ways. Therefore, it is also necessary to consider the respective understanding of sufficiency. Due to the large number of different definitions and following Linz (2002), a first step is to distinguish between a qualitative and a quantitative understanding of sufficiency.

A quantitative understanding of sufficiency refers to views in which sufficiency is aimed exclusively at the mere reduction of resource consumption. The qualitative understanding of sufficiency encompasses this quantitative aspect, but goes beyond it by also including socio-cultural change. This may involve a redefinition of prosperity, among other things.

In the next step, the qualitative understanding of sufficiency was further differentiated and it was examined whether the authors of the publications see sufficiency as being linked to a profound transformation of economic conditions and society. A critical attitude towards economic growth and capitalism, statements on the necessity of 'degrowth' and 'post-growth', as well as remarks on far-reaching changes in power structures and the distribution of property were evaluated as indications for such an understanding.

Category 'Link between sufficiency and biodiversity'

In this category, a distinction was made between specific and non-specific connections.

The subcategory 'unspecific connection' includes publications that mention biodiversity loss as a reason for the need for sufficiency or imply that sufficiency is necessary for the protection of biodiversity, without explaining this further. Publications that describe how sufficiency contributes to the conservation of biodiversity in greater detail are assigned to the category ‘specific context’.

Category ‘Fields of action’

The publications were – if possible - assigned to a field of action based on the system of Demuth and Heiland (2020). Fields of action are understood here as economic and socially significant areas that are distinguished from one another by functions or tasks. The fields of action were then supplemented by the inductive formation of subcategories.

The fields of action considered are: Consumption, energy, mobility, housing, work, agriculture and food, forestry, mining, travel and tourism, research and teaching, regional development and spatial planning, and nature conservation.

With regard to the field of action ‘nature conservation’, it should be noted that, since only publications that establish a connection between biodiversity and sufficiency were evaluated, the publications ultimately deal with the field of action ‘nature conservation’, since biodiversity conservation is a central aspect of nature conservation. However, the field of action ‘nature conservation’ as defined here encompasses the explicit engagement with social issues of nature conservation, nature conservation policy demands or nature conservation narratives.

The evaluation of the texts in relation to fields of action was carried out through the qualitative evaluation of the text sections dealing with sufficiency. In the case of the scientific texts, the one field of action that was considered was assigned. If several fields of action were considered as examples, no allocation was made. Several fields of action were not assigned to one publication, as the scientific publications did not take an in-depth look at several fields of action. It should, however, be noted that there are overlaps between the fields of action. For the purpose of classifying the content of the publications, and because almost all fields have points of contact with each other, a corresponding differentiation of the fields while at the same time making the classification unambiguous seems heuristically sensible.

The publications of the nature conservation associations were treated differently from the scientific publications with regard to the allocation of fields of action, as these publications often considered fields of action such as mining in relation to nature conservation. In the case of the evaluation of the publications of the nature conservation associations, the assignment to a further field of action was therefore made in addition to the assignment to the field of action nature conservation, provided that this second field of action was a central object of consideration of the publication.

Content analysis procedure

The paragraphs in which the terms biodiversity or sufficiency appeared were systematically analysed and the content was classified according to the categories described above.

In addition to the methodology described above, the translation assistance of DeepL, an AI assistant tool, was used in the preparation of the manuscript.

Results

The search for scientific publications yielded a total of 494 hits for the years from 2015 to 2021. These were narrowed down to 44 publications after the screening described above (see Suppl. material 1), which is strikingly low. In 20 of these publications, sufficiency and biodiversity or one of the two terms was mentioned only in passing, i.e., only once and/or without explaining it in more detail. Eleven publications are in English, whereby these are predominantly written by German authors, and 33 are in German. Furthermore, the type of documents differs: There are four articles in journals (three of which have gone through a review process), two anthologies with several contributions on the topic, seven contributions in anthologies, five dissertations, four master's theses, three bachelor's theses, six monographs and thirteen publications that fall into the category of 'grey literature' (research reports, conference/event documentation, etc.).

The search for publications by nature conservation associations yielded 286 hits. The screening reduced the material classified as relevant to twelve publications (see Suppl. material 1). This was due to duplications in the respective hit lists and the use of the term sufficiency in the sense of 'sufficient'. The material classified as relevant includes both web pages and pdf documents. These are published exclusively by NABU, BUND and WWF (Germany) and are written in German.

In the following, the results are presented first for the scientific publications and then for the publications and websites of the nature conservation organisations.

Understanding of sufficiency

Scientific publications

Sufficiency is understood quantitatively in eleven of the 44 publications according to the definition presented above, and qualitatively in 24 cases. In nine other publications the understanding remains unclear. Here, the term is mentioned without further explanation. Of the 24 scientific texts with a qualitative understanding of sufficiency, 13 mention biodiversity loss/biodiversity only once or twice and 19 only make a general connection between biodiversity/biodiversity loss and sufficiency.

Eleven publications mention that there is a conflict between sufficiency and economic growth (Keck et al. 2017; Pufé 2017; Schiemann and Wilmsen 2017; Sperfeld et al. 2017; Witt 2017; Zahrnt, 2017; Miehe 2018; Biermann and Erne 2020; Dallmer 2020; Berger et al. 2021; Wyborn et al. 2021), so it can be concluded that for them sufficiency is linked to a profound transformation.

Publications by nature conservation associations

In the twelve publications of nature conservation associations examined, sufficiency is understood quantitatively in five cases and qualitatively in four cases. Three publications cannot be clearly assigned.

The extent to which a profound transformation is seen as a prerequisite for sufficiency is not noted in the NABU publications. A BUND publication (2022c) states: "Policies that are primarily oriented towards the goal of economic growth are in stark contradiction to sustainable development and the world's limited resources" (BUND 2022c, own translation). The WWF publications do not address more fundamental changes in political and economic structures. One exception to this is WWF Germany's publication by Kind and Engel (2018) which states: "Alternative economic concepts to growth-oriented capitalism include the zero-growth, growth-reduction and sufficiency concepts" (Kind and Engel 2018, p. 76, own translation).

Link between sufficiency and biodiversity

Scientific publications

When looking at the links between sufficiency and biodiversity established in the scientific publications, it is striking that the necessity of sufficiency for the preservation of biodiversity is mentioned non-specifically in 32 of 44 publications.

The authors of twelve publications make specific links between the need for sufficiency in the face of biodiversity loss and the positive effects of sufficiency for biodiversity conservation: five publications describe the threat to biodiversity posed by agricultural practices and diets and consider sufficiency as a strategy for reducing this threat (Fehrenbach et al. 2017; Antos 2018; Fabricius 2018; Mok-Wendt 2020; Cohors-Fresenborg et al. 2021; Schlatzer et al. 2021). Three publications (Fehrenbach et al. 2017; Fabricius, 2018; Schlatzer et al. 2021) address the pressure on land use and sufficiency as a strategy to reduce this pressure. Other publications mention the positive and negative impacts on biodiversity that can result from sufficiency in tourism and recreational use (Schrader 2017; Zahrnt 2017; Antos 2018). Concrete positive correlations between sufficiency and biodiversity are shown in two publications for the forestry sector (Lippe et al. 2017; Creutzburg et al. 2020).

Publications by nature conservation associations

In contrast to the scientific publications, the publications of the nature conservation organisations more often consider the cause(s) of biodiversity loss and sufficiency as a way to combat those causes in more detail. For instance, they outline the negative impacts of chemicals (BUND 2022b), resource use in a bioeconomy (NABU 2022) and mining on biodiversity (BUND 2017; Kind and Engel 2018).

NABU publications also problematise the impacts of the energy transition on biodiversity: They highlight that the energy transition must be linked to energy sufficiency (Sothmann 2014; NABU Schleswig-Holstein 2019).

Fields of action

Fig. 2 shows the number of publications broken down by field of action. It should be noted that 17 scientific texts and two publications by nature conservation associations could not be assigned to a specific field of action. In these publications, fields of action are mentioned in passing. Haase (2020), for example, explains sufficiency in terms of mobility, Holzbaur (2020) in terms of agriculture and nutrition. The publications of the nature conservation associations

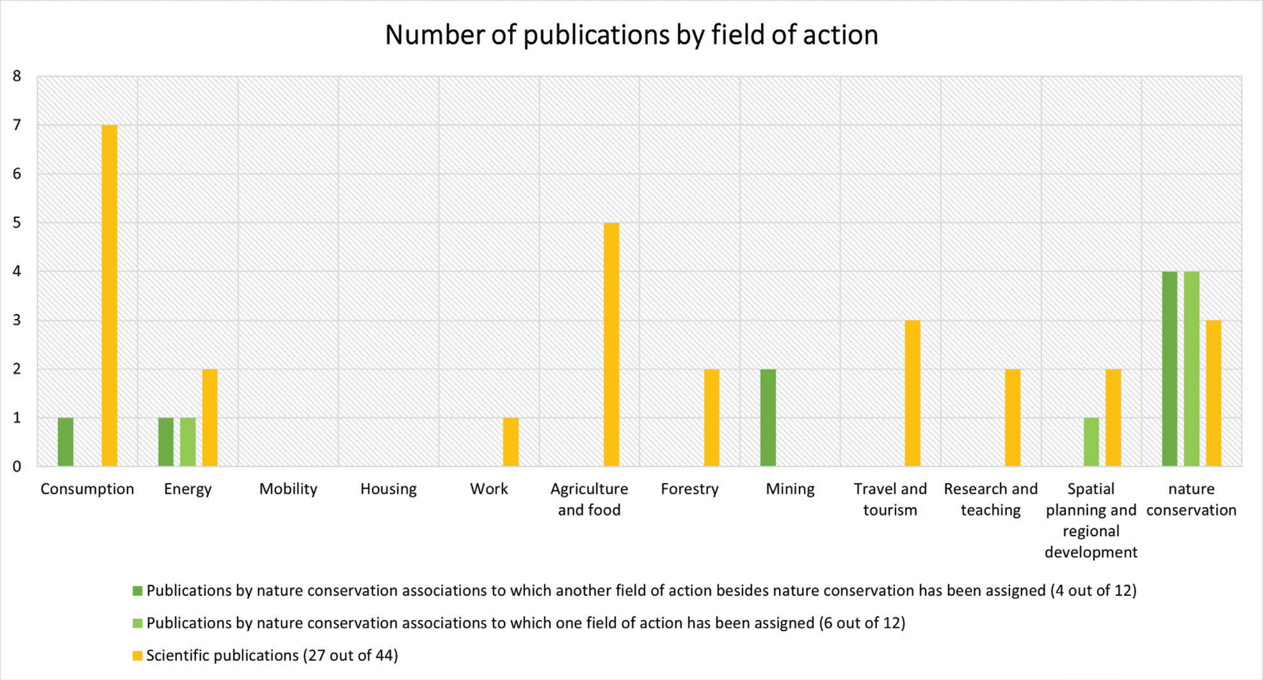

Figure 2. Fields of action.

that are not assigned to any field are a statement by BUND (2022a), and a text on the economy of transformation by the WWF (Zwiers et al. 2022).

The figure illustrates that in the publications of the associations in which sufficiency and biodiversity are linked the field of action 'nature conservation' is most frequently considered. In the scientific publications, the focus is most frequently on 'consumption' and 'agriculture and food'.

Statements and demands on the subject of sufficiency in publications on the field of action 'nature conservation'

A central aspect of this work is the consideration of the integration of sufficiency into biodiversity protection. As this is an essential task of nature conservation actors, central statements and demands on sufficiency from scientific publications on the field of action 'nature conservation' are presented below. Since biodiversity protection is also dependent on the spatial management of various anthropogenic land uses, the contents of publications on spatial planning are also listed.

- For effective environmental protection (as well as fair prices and wages), the "Western consumption model of constant increase cannot be continued" (Zahrnt 2017, p. 43, own translation). Thus, Sufficiency is necessary (see also Immovilli and Kok 2020, p. 21).
- Nature conservation (and conservationists) would have to discuss and reflect on its embedding in the imperial mode of living, "[f]or a sustainable social and economic system will not be achieved with efficiency and consistency strategies alone [...]. Sufficiency strategies are needed that pursue the goal of lower energy and material requirements and raise awareness of the non-material - i.e., emotional and social - dimensions of a 'good life'" (Leibenath et al. 2021, p. 147, own translation).

- Sufficiency will increase the importance of nature and thus increase the pressure on nature through uses such as domestic tourism and sport. Hence it is suggested to develop sufficiency strategies in the context of nature conservation as well as compensation measures for non-use (Zahrnt 2017, p. 43).
- For the implementation of sufficiency strategies, spatial planning and development, especially with regard to land-intensive economic and settlement developments, will play a decisive role (Hofmeister et al. 2021, p. 8).
- Sufficiency could reduce contradictions between climate protection and nature conservation caused by the energy transition (Sperfeld et al. 2017, p. 8).

Eight out of twelve texts from nature conservation associations focus on the field of action ‘nature conservation’. Concrete statements or demands of the associations that link biodiversity conservation and sufficiency can be summarised under the following keywords:

- Nature-friendly energy transition by saving energy (Sothmann 2014; NABU 2021).
- Absolute resource reduction targets (WWF Deutschland 2020; BUND 2022c).
- Putting a stop to deep-sea mining (BUND 2017).

The other demands or statements of the conservation associations such as “a stronger focus should be placed on social and ecological innovations (sufficiency) for a truly sustainable economy” (NABU 2022, own translation) are less concrete.

Nevertheless, these publications contain proposals such as “no-go areas” for the protection of biodiversity (Kind and Engel 2018, p. 67), which are, however, not linked to sufficiency by the authors.

Discussion

The discussion focuses on the one hand on possible reasons for the small number of publications dealing with the link between biodiversity and sufficiency and the fact that the description of the link, when addressed, often remains unspecific, and on the other hand on the critical reflection on categorisation for the understanding of sufficiency.

Number of publications and specificity of the links

The result show, that the overall number of publications linking biodiversity with sufficiency is low. This also becomes evident when comparing the sustainability strategies sufficiency and efficiency with regard to biodiversity: a search with Google Scholar and the keywords ‘sufficiency’ and ‘biodiversity’ yielded 1.410 hits, whereas a search with the keywords ‘efficiency’ and ‘biodiversity’ yielded 9.320 hits (as of September 14th 2023). What could be the causes of these discrepancies?

Political implications

One reason could be controversies about the political implications of sufficiency as suggested by the following statement by a BUND expert: “[S]ufficiency is […] questioning […] the current economic system and the growth paradigm. And that goes against the fundamental core logic of this society, this economy, this policy” (quoted in Huber 2023, p. 37). In other words, the term ‘efficiency’ is met with greater acceptance because it does not imply a restructuring of the existing economic and social conditions - and is therefore preferred. In contrast, the use of the term ‘sufficiency’ may imply this transformation. Spengler (2018) argues similarly: “In [...] several literature sources, there is talk of sufficiency ‘policies’, which are not policy instruments in the strict sense, but refer to broader socio-economic developments that would require a fundamental change in values and entire programmes of far-reaching political reforms. Examples are the “exit from growth policies”, the “reduction of working hours [...] and the reduction of social inequality in order to reduce luxury and ‘conspicuous’ consumption” [...], which could indeed have significant effects in terms of mainstreaming sufficiency” (Spengler 2018 p. 37).

Descriptiveness

The lack of clarity of the term sufficiency could be another reason why it is used much less frequently. Linz (2004) for example, writes: “To the uninitiated, [the term sufficiency] says nothing or something wrong” (Linz 2004 p. 47, own translation). For Linz (2004), the meaning of sufficiency is therefore unclear for people who are not familiar with the term, or a completely different meaning is attributed to it. Making the term effective in the public sphere is consequently unpromising in his eyes (Linz 2004, p. 47,).

In this context, however, it is noteworthy that the French government adopted a ‘plan de sobriété énergétique’ (energy sufficiency plan) in autumn 2022 (Gouvernement français 2022). Sufficiency is presented as one of three pillars of the decarbonisation strategy. Energy sufficiency is thus very much present in the French public. Hence, the mainstreaming of the term sufficiency, at least in the area of energy sufficiency, should be possible (cf. also Ore 2022). However, in this plan sufficiency is hardly associated with the need for a ‘deeper transformation’ or ‘growth critique’ by the government. Rather, the use of the term seems to focus primarily on a purely quantitative reduction in energy consumption. Moreover, France seems to be the only country in Europe with a focus on energy sufficiency (Messad 2023). Nevertheless, sufficiency policies are recently taken up more prominently in Germany as well, as publications such as those by Reese et al. (2023) on sufficiency and environmental law or by Nawothnig et al. (2023) on sufficiency as a ‘booster’ for reaching climate protection targets show.

Different terminology

Another possible explanation for the low number of publications dealing with sufficiency and its links to biodiversity is that scientists and/or conservation organisations write about the related issues without using the term ‘sufficiency’, either paraphrasing similar strategies and analyses, or using other terms. Examples of paraphrases without the use of other terms can be found, in Moranta et al. (2022) and Otero et al. (2020).

Moranta et al. (2022) write that economic growth, which is linked to human activity and resource use, is the main cause of biodiversity loss. In order to counteract this, a fundamental change in social values and a downsizing of the economy are required. Otero et al. (2020) make a similar argument. They point out, again without explicitly using the term sufficiency, that “economic growth contributes to biodiversity loss via greater resource consumption and higher emissions” (Otero et al. 2020 2). Therefore, they suggest that the conflict between economic growth and biodiversity conservation needs to be acknowledged in policies (Otero et al. 2020, 2). Thus, both Moranta et al. (2022) and Otero et al. (2020) put forward arguments that - given the diversity of the sufficiency definition - could also be put forward for sufficiency.

One example of a strategy with similarities to sufficiency is degrowth. Hickel characterises degrowth as “a planned, coherent policy to reduce ecological impact, reduce inequality, and improve well-being [by, inter alia, scaling] down ecologically destructive and socially less necessary production (i.e. the production of SUVs, arms, beef, private transportation, advertising and planned obsolescence), while expanding socially important sectors like healthcare, education, care and conviviality” (Hickel 2021, p. 1108). Another concept with similarities to sufficiency is the concept of consumption corridors. In accordance to Fuchs et al. (2021) consumption corridors “describe a space between minimum consumption standards that provide every individual with the ability to live a good life, and maximum consumption standards that keep individuals from consuming in quantities or ways that hurt others’ chances to do the same” (Fuchs et al. 2021 p. 4). It can be concluded from this that strategies with characteristics that could be attributed to sufficiency are discussed in the research without the term ‘sufficiency’ itself being mentioned. However, it is questionable whether the authors are familiar with the concept of sufficiency and whether or not they intentionally use other terms.

Framing and difficulties in in quantifying biodiversity loss

Another reason why only a small number of authors of scientific publications have used the term sufficiency to date could be that a discursive link between sufficiency and biodiversity has rarely been established. An expert from BUND Youth provides a possible explanation as to why this is the case: “This also raises the question of framing. So, on the one hand, what are the central problems we are actually referring to? Are we doing this for reasons of global justice, climate justice, biodiversity loss? All of those can be named. What does one refer to? And also: does one use the term sufficiency or not?” (cited in Huber 2023, p. 56). Thus, in addition to raising questions on terminology discussed above, this BUND Youth expert raises the question on how to frame sufficiency, suggesting that a variety of framings are possible. From this argumentation - and in view of the few publications that establish the connection between sufficiency and biodiversity - it can be concluded that the necessity of sufficiency is justified differently, i.e. other links are made between sufficiency and, for example, climate change. Is that the case? And if so, why?

When doing a Google search with the keywords ‘sufficiency’ and ‘climate change’ as well as ‘sufficiency’ and ‘biodiversity loss’ the number of hits for ‘sufficiency’ and ‘climate change’ is almost eleven times as high as the number for sufficiency' and 'biodiversity loss' (972.000 hits and 88.700 hits, as of December 18th 2023). The conclusion that the need for sufficiency is more frequently justified by climate change than by the loss of biodiversity thus appears to be substantiated, although it should be noted that the content of the hits just mentioned was not analysed.

One possible explanation for the difference in the number of hits is that it is easier to assess the benefits of sufficiency as a strategy for mitigating climate change by calculating the savings in greenhouse gas emissions or energy consumption (see for example Burke 2020; Cordroch et al. 2022). However, even with rather practical approaches to measure biodiversity loss caused by land use, like proposed by Durán et al. (2020), quantifying impacts on biodiversity is more complex. The complexity and the associated difficulty in quantifying the loss of biodiversity could therefore be another reason for the small number of publications that establish a link between biodiversity and sufficiency. Furthermore, this could also be a reason why, even when a link between biodiversity and sufficiency is established, it often remains unspecific.

Another explanation indicated by the keyword search mentioned above as well as by looking at the Google hits for the keywords 'biodiversity crisis' compared to the keywords 'climate crisis' (74.200.000 hits and 864.000.000 hits, as of February 21st 2024), is that the climate change crisis appears to be more anchored in the public consciousness than the biodiversity crisis, despite both crises being related and should therefore be considered together (Pörtner et al. 2021).

Need for interdisciplinarity

Another reason for general statements on the link between biodiversity and sufficiency may be that biodiversity as a topic is primarily researched by natural scientists, especially biologists. Sufficiency, on the other hand, is a strategy aimed at changing individual lifestyles and social lifestyles, i.e., it deals with social behaviour. Sufficiency is therefore first and foremost an object of study for sociology. The link between sufficiency and biodiversity therefore requires an interdisciplinary perspective.

However, various factors make an interdisciplinary perspective difficult. According to Russels (2022) and MacLeod (2018), these factors include:

- different methodological approaches and technical terminology
- difficulties in reading and receiving texts from outside the discipline
- different conceptions of what the object of study is (or should be)

Moreover, according to Russels (2022), experts tend to focus on what they know.

An additional explanation for general statements about the link between biodiversity and sufficiency that affect both disciplinary and interdisciplinary research is provided by Leipold et al. (2024): They argue that underlying values and disciplinary paradigms influence collective science, constraining it and thus limiting its potential to contribute to inform and shape societal changes. They therefore propose a reflection on values and paradigms through a 'narrative led dialogue' (Leipold et al. 2024).

Understanding of sufficiency – and the need for a comprehensive typology

The results of this study show that the term sufficiency is defined, interpreted and understood in different ways. In order to be able to systematically identify and discuss the similarities and differences of sufficiency, a typology of sufficiency is essential.

A comparison of the typology proposed here with that of Lage (2022), which was published after the content evaluation for this paper, shows that a differentiation of the 'quantitative understanding of sufficiency' would also have been interesting for the analysis of the publications.

According to Lage (2022), a distinction should be made between 'sufficiency as consumption corridors' and 'sufficiency as a pathway towards a post-growth economy'. According to this differentiation, a distinction is made between, firstly, "[s]ufficiency [...] concepts in the sense of having the minimum necessary to live well and as limits to social practices that cause ecological damage, especially to consumption" (Lage, 2022 p. 5) and, secondly, concepts that, in addition to limiting consumption through corridors, strive for the development of an "a-growth or degrowth society or a steady-state economy, where societal prosperity is independent of economic growth" (Lage 2022, p. 6).

Comparing those sufficiency goals with the categories that were used here it becomes clear that the aspect of having 'enough', aimed at with the goal of 'sufficiency as consumption corridors' has not sufficiently been considered. A modified typology would therefore be useful for future studies of sufficiency. Accordingly, a distinction would have to be made as to whether sufficiency includes the following aspects:

1. reduction of resource consumption and environmental damage
2. changes in lifestyles and the meaning of wealth (e.g., the 'decluttering' or 'deceleration' mentioned by Sachs (1993))
3. social justice (especially with regard to the right of all people to a materially secure life)
4. explicit critique of growth or aspirations for an economy that is not dependent on economic growth

According to this typology, an understanding of sufficiency that only includes the first aspect would correspond to the 'quantitative understanding of sufficiency' examined here, whereas all understandings of sufficiency that include another aspect in addition to aspect 1 would correspond to the 'qualitative understanding of sufficiency'.

Conclusion

The systematic identification and evaluation of scientific publications (for the years 2017–2021) and publications by various nature conservation associations show that very few publications to date have addressed the link between sufficiency and biodiversity. And when they do, this linkage often remains unspecific and thus superficial.

Possible reasons for why very few publications deal with sufficiency are its political implications, the lack of descriptiveness of the term as well as the use of other terms. Moreover, the lack of, or unspecific linkages between sufficiency and biodiversity could be due to the fact that, firstly, several framings of the need for sufficiency are possible and, secondly, that sufficiency and biodiversity belong to different 'scientific spheres'. Linking the two terms thus requires a reflective, interdisciplinary perspective.

In the author's opinion, however, it nonetheless makes sense to further explore the potentials of sufficiency. Reasons are:

1. The biodiversity crisis is primarily caused by land use changes and direct exploitation. It therefore is caused by the mode of living and can consequently be mitigated through a change of that mode of living and the associated consumption of resources. Hence through a strategy such as sufficiency.
2. Sufficiency, depending on how it is understood, also raises questions of justice and the meaning of prosperity, and thus enables a joint consideration of social and biodiversity conservation concerns. This joint consideration is necessary for a just transformation towards sustainability.
3. For the reasons already mentioned, an interdisciplinary, if not transdisciplinary, perspective is in any case necessary in order to preserve biodiversity.
4. The fact that different framings for the necessity of sufficiency are possible, for example for the mitigation of climate change, does not make it less, but more sensible to take sufficiency into account.
5. Moreover, the example of the French 'plan de sobriété énergétique' shows that a mainstreaming of the term is possible.

Accordingly, and in view of the small number of publications that deal with the topic, sufficiency in the context of biodiversity protection should be researched in greater depth in the future. Starting points for further research could be the presented results under 3.3. as well as the typology and research questions presented in the following table (Table 1):

Table 1. Research questions on the link between sufficiency and biodiversity.

	Sufficiency typology (as developed under 4.2)	Research questions
1	Reduction of resource consumption (incl. land use) and environmental damage.	To what extent, where and how must resource use (including land use and intensity of use) be reduced to avert (further) negative effects on biodiversity?
2	Changing lifestyles and the meaning of wealth (e.g., the ‚decluttering' or ‚deceleration' mentioned by Sachs (1993)),	How does a change in lifestyles affect biodiversity (e.g. increased pressure on nature) and how can precautions be taken against potential negative impacts?
3	Social justice (especially with regard to the right of all people to a materially secure life)	How can both social impacts and impacts on biodiversity be taken into account in the consumption/use of resources (incl. land)? How must social impacts be taken into account when reducing resource use (with the aim of protecting biodiversity and considering that an increase in resource use, e.g., in the global south might also be necessary)?
		Which groups of people are affected by the reduction of resource use? Are they already marginalised in material terms, in terms of access to resources (e.g. green spaces, water, etc.)?
4	Explicit critique of an economic growth paradigm or aspirations for an economy not dependent on economic growth	Where are conflicts between economic interests on the one hand and biodiversity protection on the other? Or: Where do economic interests prevent biodiversity protection?

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Analysed publications

Authors: Marianne Hachtmann

Data type: xlsx

Explanation note: This appendix contains information on the publications analysed as part of the literature review. It therefore contains both baseline information and information about the content. Baseline information includes information on the authors, the year of publication, the title, the document type and the search engine used to find the publication. The information on the content is organised according to the categories described in the Methodology section (see also Fig. 1). Thus it contains information on the link between sufficiency and biodiversity, the understanding of sufficiency and the field of action the publication focuses on.

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Review Article

Effects of management, habitat and landscape characteristics on biodiversity of orchard meadows in Central Europe: A brief review

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Abstract

Increasing agricultural intensification, combined with land transformation and fragmentation, poses significant threats to biodiversity. While extensively cultivated landscapes serve as vital refuges against biodiversity loss, they are modified by land abandonment and intensification. Orchard meadows in Central Europe represent traditional extensive land management systems, exhibiting high biodiversity. Comprising cultivated grasslands and scattered fruit trees, orchard meadows feature structures rich in different habitats supporting a diverse flora and fauna. However, their decreasing economic importance in recent decades has resulted in severe degradation or abandonment. Despite their importance for biodiversity conservation, there remains no comprehensive overview of orchard meadow biodiversity and management in Central Europe. This review aims to summarize existing knowledge on orchard meadows' role in biodiversity conservation and the effects of management practices on habitat diversity and quality at both smaller (structure and microhabitats, local scale) and larger scales (surrounding landscape, regional scale). The first part focuses on orchard meadow biodiversity, including both plants and animals and their link to landscape-scale factors. Biodiversity in orchard meadows is predominantly affected by patch size, determining species richness and composition, and connectivity to neighbouring orchard meadows, influencing species migration and recolonization success. The second part evaluates management impacts, illustrating differences in the benefits of mowing versus grazing across taxonomic groups. An intermediate management intensity for orchard meadows determines their conservation value in terms of species composition, varying among different taxonomic groups. To prevent area loss and abandonment of orchard meadows, we advocate for political and public support, along with incentives for farmers to maintain their biodiversity.

Key words: Extensively cultivated landscapes, extensive grassland, grazing, habitat connectivity, mowing, patch size, scattered trees, species richness, Streuobstwiese, structural diversity, traditional orchard

Introduction

Habitat loss and fragmentation are main drivers of biodiversity decline, affecting nearly all habitat types and species worldwide (Settele et al. 1996; Stuart et al. 2004; Haddad et al. 2015; Fletcher et al. 2018). Agriculture is a main contributor to habitat loss and fragmentation. In the European Union, for example, agricultural landscapes dominate with ca. 40% coverage (Eurostat 2021). They become increasingly intensified to produce higher yield leading to accelerating use of pesticides and fertilizer (Henle et al. 2008; Lécuyer et al. 2022). In addition, extensive and structurally heterogeneous cultivated land became rare due to abandonment, intensification or transformation to other land-use types. This change in land-use types leads to continuing fragmentation of remaining semi-natural landscapes as well as decreases in fragment size and increases in isolation to next suitable habitats (Haddad et al. 2015). As a consequence, intensive agriculture and biodiversity conservation are difficult to reconcile, representing one of the greatest challenges of global change (Henle et al. 2008; Egli et al. 2018; Lécuyer et al. 2022).

Only few organisms are adapted to intensively used agricultural landscapes, such as crop feeding insects or those adapted to high and continuing disturbance (Henle et al. 2004). The gross of species, however, cannot survive in highly intensified agricultural landscapes requiring natural or semi-natural landscapes (Henle et al. 2008; Guerrero et al. 2012; Tscharntke et al. 2012a; Lécuyer et al. 2022).

For conservation of farmland biodiversity, it is essential to maintain semi-natural habitats (Kleijn et al. 2011; Tscharntke et al. 2012b; Pe'er et al. 2014). This is especially the case for traditionally used landscapes, which can act as refuges, food source or nesting sites for many species (Lichtenberg et al. 2017; Eeraerts et al. 2019). Traditionally farmed orchard meadows in Central Europe present such a refugium for many threatened species (Kajtoch 2017; Schuboth and Krummhaar 2019; Henle et al. 2024). Their value for conservation and management recommendations will be the focus of the present contribution.

Orchard meadows are a combination of planted fruit trees in the overstory and extensively managed grassland in the understory (Stappen 2016; Degenbeck 2021). Orchard meadows as a habitat type is loosely defined (Henle et al. 2024) and they come in different forms and functions (Erlach 1994; Stappen 2016). Here, we concentrate on orchard meadows in the strict sense (Henle et al. 2024); that are, planted fruits trees with an herbaceous and/or graminoid understory that form two distinct strata composed of under- and overstory. It is important to note that orchard meadows are contrasted with fruit plantations by their style of management, which is mainly extensive with limited external input of fertilizer and pesticides in accordance with the EU regulation on ecological/biological agricultural production (EU 2018). Also, tree density is much lower, stem height is higher (at least 180 cm for newly planted trees) and age distribution wider in orchard meadows compared to fruit plantations.

Orchard meadows provide many important ecosystem services and functions, due to their multifaceted structure, the unique combination of two strata and the low to no external input of chemical substances (Bünger 1996; Hoff 2003; Krause et al. 2017; Henle et al. 2024). They have provisioning services like fruits for human consumption and fodder for livestock, but also regulating services like nutrient retention, carbon sequestration and flowering resources. Furthermore, the scattered density of trees in orchard meadows provide many different microclimatic conditions beneficial for animals and plants as trees offer alternating shaded and sunny patches and reduce wind speed. Fruit trees prevent fast surface flow of precipitation reducing soil erosion (Palma et al. 2007; Plieninger et al. 2010).

Despite these apparent values for food provisioning and biodiversity conservation, since the mid-19th century, orchards have declined substantially in

Europe (Herzog 1998; Plieninger et al. 2015; Forejt and Syrbe 2019; Žarnovičan et al. 2021). Also, their management has changed drastically (Poschwitz 2009; Žarnovičan et al. 2020) in line with many other extensively used traditional agricultural systems in Europe (Henle et al. 2008; Lécuyer et al. 2022). However, orchard meadows have received limited attention in scientific conservation biology and no comprehensive study has yet attempted to summarise existing knowledge and point out future research directions.

Here, we set out to fill this gap by reviewing the literature and distil patterns and drivers of species diversity in orchards, the effects of management on structural diversity of orchards and on species composition and the dependence of species in orchards to landscape characteristics that are likely to change when orchards get lost and fragmented. As studies are scarce on biodiversity in orchards, our baseline assumption is that diversity patterns recorded from fragmented habitats and factors affecting diversity in extensively used grasslands are transferrable to orchards. However, it is not our intention to provide a comprehensive literature review on grassland as this has been done in detail by other authors in the past (e.g., Dengler et al. 2014; Tälle et al. 2016). We point out differences and identify future research areas where further research is needed to identify drivers and effects of biodiversity in orchard meadows.

We concentrate on Central Europe as this region has comparable biogeographical, climatic and economic conditions and has a long and shared history of cultivation of orchard meadows (Beigel et al. 1995; Handlechner and Schmidthaler 2019) allowing for comparison of existing studies. However, we argue that similar processes may also affect diversity in similar systems outside Europe. Our review is structured in two main parts. In the first part, we focus on patterns of species diversity in orchard meadows across scales and their drivers. In the second part, we concentrate on management options to obtain high biodiversity of orchard meadows in a sustainable way. We introduce each part with ecological theories that explain observed patterns and then review to which extent these theories have been addressed for orchard meadows. Based on our key conclusions, we provide recommendations for further management of orchard meadows to support biodiversity in these unique and fascinating habitats. We further identify knowledge gaps for research to foster their conservation.

Methods

We conducted our literature search in English and German language. We searched across various databases, including the literature database of the German Federal Agency for Nature Conservation, literature references of the Federal Committee for Orchard Meadows of NABU, and searched in Web of Science, and Google Scholar. Our search included the following keywords ‘orchard meadows’, ‘orchard management’, ‘traditional orchards’, ‘grazing’, ‘pruning’, ‘mowing’, ‘landscape composition’ within the scope of ‘Central Europe’.

Because we encountered only a limited number of publications directly focused on the effects of orchard meadow management on biodiversity (totalling 20 publications), we broadened our scope to include studies related to recommendations for grassland management that could be used to understand biodiversity maintenance and change in orchard meadow systems. Our final dataset included 218 publications, which underwent thorough review (see Suppl. material 1 for complete literature list). Subsequently, we identified and selected 127 publications closely associated with orchard meadow management and conservation from this comprehensive set of references, which are cited in the main text.

Part I: Patterns of species diversity in orchard meadows across spatial scales and their drivers

In this part, we discuss the effects of different spatial scales and surrounding landscape configuration on biodiversity in orchard meadows. To this end, we first look at the importance of microhabitats and vegetation structure within orchards. Most species have specific requirements on their environment and their presence may depend on the availability and quality of microhabitats. Next, we turn towards the local scale to discuss the effect of size of individual orchard meadows as well as their isolation to other orchards on their biodiversity. As orchards share many species with other habitat types, such as grasslands, fallow lands and forests, we will look at the structure and diversity of the surrounding landscapes, which also play an important role on the biodiversity in orchards. Finally, we explore the diversity across multiple orchards on a regional or national scale.

Structure and microhabitats

The high amounts of multifaceted habitats and structural diversity of orchards are important drivers of their species diversity (Simon and Rühl 1992; Bösneck and Hampel 2013; Schuboth and Krummhaar 2019; Jagel et al. 2020). Here, an essential element of orchard meadows is the set of fruit trees of different ages. Young trees, planted at successive stages, secure the long-term continuity of orchard meadows. Younger trees with higher vitality also have higher numbers of flowers offering nectar, pollen and fruits for diverse flower visiting insects and fruit feeding vertebrates, compared to older or dying trees (Israel 2002; Steffan-Dewenter and Leschke 2003). Old and dying trees contain many important microhabitats for many arthropods, birds and small mammals like branch holes, cavities, bark injuries, crown and branch breaks, epiphytic mosses, cracks and crevices and deadwood (Braun et al. 2010; Grossmann and Pyttel 2016). Especially existing cavities and branch holes, e.g., created by woodpeckers or fungal heart rot, are essential for secondary cavity users like many bird species (e.g., *Athene noctua*, *Jynx torquilla*, *Otus scops*), mammals (e.g., *Dryomys nitedula*, *Glis*, *Eliomys quercinus*, *Myotis myotis*) and insect groups (e.g., Hymenoptera, Thysanoptera, Coleoptera) (Rösler 1992; Simon and Rühl 1992; Eichler et al. 2001; Israel 2002; Bauschmann 2005; Eckstein and Albrecht 2006; Burger and Creutzburg 2012; Grüebler et al. 2013; Henle et al. 2024). Low numbers of cavities decrease numbers of less competitive bird species, such as common redstart (*Phoenicurus phoenicurus*) and collared flycatcher (*Ficedula albicollis*), and they may lose breeding possibilities completely (Erlach 1994) if old and/or dying trees are removed from orchards. Therefore, dead branches or coarse woody debris on the ground should not be removed.

Dead wood, from trees or branches, is important for hibernating insects and small mammals like garden dormouse (*Eliomys quercinus*) (Ulitzka 2013; Godmann 2016). Insects, especially Thysanoptera, use bark crevices and space between dead bark and wood of older trees for hibernation (Ulitzka 2013), thereby offering food resource for woodpeckers and tree creepers (*Certhia* spp.) during winter. Wild bees and wasps benefit from dead wood as nesting and hibernation habitats (Saure 2016). Dead wood also benefits potential prey of saproxylic parasitoid wasps (Hilszczański 2018).

The heterogeneous habitat structure of orchard meadows favours specialist bird species like Eurasian hoopoe (*Upupa epops*), little owl (*Athene noctua*) and wryneck (*Jynx torquilla*), for which orchards are key habitats. Those species require both a structurally rich arboreal stratum for breeding (e.g., old trees with cavities) and open ground for foraging (Ullrich 1987; Kämpfer-Lauenstein and Lederer 2007).

However, orchard meadows also support generalist species, which feed on a broader range of host plants. For example, Herrmann et al. (2010) found that a structural diverse understory maintained by high plant diversity benefits generalist meadow spiders. Likewise, Szabó et al. (2022) found butterflies with generalist functional and life-history traits more abundant in orchard meadows compared to calcareous grasslands. In contrast, species adapted to more open habitats, such as farmland butterflies, occur in higher numbers and richness in calcareous grasslands (Ernst et al. 2017). It should be noted that the orchard meadows studied by Ernst et al. (2017) seemed to be more intensively managed, as farmland butterflies increased with abandonment, which possibly provide more herbs and flowers. This highlights that specialist species are tightly linked to management intensity, which will be discussed in the second part of this review.

In addition to habitat structure, particularly dead wood, edaphic conditions also significantly affect the diversity of plants and animals in traditional orchard meadows. According to the nutrition indicator values of Central European plants (Ellenberg et al. 2001), nutritionally rich orchard meadows exhibit species-poor plant communities, which in turn affect the species diversity of animal taxa dependent on a species-rich plant community (Kornprobst et al. 1994; Ružičková and Halada 2005; Žarnovičan et al. 2017). In contrast, orchard meadows that are dry and nutritionally poor, either naturally or due to usage-based biomass removal, show high plant and animal diversity (Kornprobst 1994; Thiem and Bastian 2014). Orchard meadows on nutritionally poor soils are rare in many regions of Central Europe (e.g., Kornprobst 1994; Denk and Wittig 1999).

The interplay of shadow and sun, influenced by the spatial distribution of trees and other structures (e.g., woodpiles, fences, and bowers), determines not only potential microhabitats for animals but also the composition of herbaceous plant communities (Langensiepen and Otte 1994; Denk and Wittig 1999; Žarnovičan et al. 2017). For example, Langensiepen and Otte (1994) observed that the number of spring geophytes increased with proximity to shade-providing fruit tree trunks, such as *Gagea lutea*, which only occurred in the vicinity of fruit trees. Spring geophytes grow before the fruit trees develop leaves, thus casting less shadow. They use this advantage in early-season growth over later-growing plants, which then grow in the shade of fruit trees, and their development is reduced by the shade of the fruit trees (Langensiepen and Otte 1994).

How management will influence the flora of orchard meadows will be discussed in the second part of this review.

When comparing the structural diversity of extensively cultivated orchard meadows with intensified fruit plantations, on plantations mainly low-trunk fruit trees are cultivated as those are easier to farm due to their homogenised low-trunk and dense plantation (Rösler 1992; Herzog 1998). Tree crowns start already at a height of <1 m. Tree care is intensive as well as the use of pesticides and fertilizers (Mader 1982; Rösler 2002). Low-trunk fruit trees have less microhabitats and offer less breeding sites for birds and arthropods. Vulnerable bird species and arthropods are more abundant in high-trunk, structurally rich orchards compared to plantations (Rösler 2002; Samnegård et al. 2019). Especially woodpeckers favour orchards with high-trunks (Rösler 2016). Mader (1982) found a much higher species diversity of carabid beetles and spiders in traditionally managed orchard meadow. The availability of ecological niches and habitats is a driver of high species diversity. Low-trunk trees and intensive tree care like use of pesticides are usually associated with low alpha diversity compared to extensively managed high-trunk fruit trees (Mader 1982; Rösler 2002; Samnegård et al. 2019).

Local scale: effects of patch size and isolation

Besides structural differences within orchard meadows, there are also large differences in species diversity and composition between orchard meadows in the same region. To understand these, we will now turn towards effects and drivers at local scale.

Orchard size and isolation from other similar habitats are important drivers of species diversity and composition. Core predictions for species diversity in habitat fragments, such as orchard meadows, can be derived from the Equilibrium Theory of Island Biogeography and metapopulation theory (MacArthur and Wilson 1967; Hanski and Gilpin 1997). Transferring island biogeography theory, which proposes that the number of species coexisting on an island depends on island size and isolation, to fragments on the mainland, it follows that patch size, and the diversity of different habitats therein, is an important factor how many species can coexist. Its distance to other suitable habitats determines how many species can recolonize if they become locally exinct. Similarly, metapopulation theory predicts that numerous smaller patches can secure the survival of a species in the landscape if they are not too isolated from each other, thus allowing constant multidirectional dispersal (Hanski and Gilpin 1997; Frank and Wissel 1998).

In line with these theories, it has been shown that the number of bird species and various insect groups (e.g., bees, wasps and their natural enemies) increases with patch size (Steffan-Dewenter and Leschke 2003; Bauschmann 2005; Amann 2007; Bailey et al. 2010). However, a positive species-area relationship was not found for species at lower trophic level like plants (Steffan-Dewenter 2003). Species-area relationships are assumed to be more pronounced for specialist species and species at higher trophic level (Holt 1996).

As expected, the abundance of solitary wrinkled wasps (Eumeninae) and bees increased with the size of the orchard. However, this was not the case for digger wasps of the family Sphecidae and the parasitisation rate also did not increase with area and connectivity, but depended only on the local and regional abundance of hosts (Steffan-Dewenter 2003). Why the abundance of some taxa and of functional relationships do not increase with patch area is still insufficiently understood.

Bailey et al. (2010) studied the effect of habitat isolation and size on snails, beetles, true bugs, spiders and breeding birds in traditional orchards and found that isolation was more important than patch size. Species richness and abundance decreased with increasing isolation, showing the importance of habitat connectivity. Further, predatory birds and spiders were more affected by patch isolation than herbivore beetles, true bugs and snails. The authors assumed that predators were more sensitive to isolation, which in turn benefit herbivores due to reduced control by predators, which supports the trophic level hypothesis (Holt 1996) and the mesopredator release theory (Henle et al. 2004). This is in line with a study by Herrmann et al. (2010), who found a positive effect of patch isolation on canopy spiders in relation with lower predation pressure of insectivorous birds and spider wasps (Pompilidae and Crabronidae).

Orchard meadows are usually of small size, e.g., ranging from 0.08 to 5.8 ha in southern Lower Saxony, Germany (Steffan-Dewenter 2003), possibly acting as strong filter for species with larger area requirements. Similarly, smaller orchard meadows are more prone to edge effects possibly affecting species composition. While small, isolated orchard meadows can be important as resting and feeding habitats for bird species, only species tolerating anthropogenic disturbance or coping with habitat edges, like blackbirds (*Turdus merula*) or great (*Parus major*) and blue tits (*Cyanistes caeruleus*), breed in small and isolated orchards (Bauschmann 2005). Species with larger area requirements and poorly adapted to edge effects like wryneck (*Jynx torquilla*), little owl (*Athene noctua*) and spotted flycatcher (*Muscicapa striata*) prefer large, interconnected orchards in combination with adjacent structurally similar habitats (Bauschmann 2005; Amann 2007).

The number of studies testing the effects of area and isolation of orchard meadows on the composition and abundance of different taxonomic groups and species within a taxonomic group is still very limited. Clearly, more studies are required to fully understand the contrasting reactions that have been observed for some species and groups in the studies carried out so far. Also, we need more studies that allow derivations of minimum sizes of orchards for providing breeding sites and the longer-term persistence of populations, especially for threatened species and species for which orchard meadows belong to their favoured habitats.

Surrounding landscapes

The surrounding landscape can be an important driver for the community composition in orchard meadows (Ernst et al. 2017). Orchard meadows share many habitats with other, larger ecosystems in Central Europe, such as grasslands, forests and moderately modified anthropogenic landscapes like gardens and suburban habitats. Orchards can draw species from such habitats, and their abundance and configuration in the surrounding landscape likely have a strong effect on the species composition and diversity in orchards. At the same time orchards surrounding other (semi)-natural habitats can serve as temporary refuges for some species when their habitat changes (e.g., deciduous woodlands for saproxylic beetles) (Horák 2014a). However, only few studies investigated the effect of surrounding habitats on orchard meadow diversity, making general claims difficult. The few available studies indicate that surrounding land use types similar in structure to orchard meadows benefit species richness of birds, butterflies (though not for butterflies that are typical for forests) and plants in orchards (Horák et al. 2013). For some bird species (e.g., *Coccothraustes*, *Dendrocopos major*, *Muscicapa striata*), orchard meadows may even complement woodlands or grassland as they can act like transition areas (Šálek et al. 2010; Horák et al. 2013). For snails, the results are contradictory. While terrestrial snails were affected by land use in the surroundings in a study in the Czech Republic (Horák et al. 2013), the surrounding landscape had no effects on snail species richness in a study in Switzerland (Bailey et al. 2010).

Further, it is important to note that the effects of patch size and surrounding landscape composition can be tightly interlinked (see also above). Patch size, matrix quality and amount of suitable patches in the surroundings determine metapopulation dynamics and the survival of species in fragmented habitats (e.g., Settele et al. 1996; Hanski and Gilpin 1997; Frank and Wissel 1998). The sensitivities of species to these effects differ between generalist and specialist species (Henle et al. 2004) and between the core and the periphery of the distribution area of species (Prieto-Ramirez et al. 2020). Specialist insects, like aboveground-nesting bees and eumenid wasps, appear to be more sensitive to habitat fragmentation than unspecialized insects (Steffan-Dewenter 2003).

Here, we regard the lack of studies specifically targeting landscape effects on orchard diversity as the main hindrance to reach clear conclusions. However, such knowledge is of paramount importance to formulate effective conservation measures for orchards at landscape scale. Also, the link of landscape effects on species composition in orchards presents a promising avenue for future research. For instance, it is poorly understood whether orchard meadows with high structural diversity may support non-arboreal species with different traits than tree-less meadows and meadows in intensively used plantations that may only support species with limited trait diversity.

Regional scale

The regional scale describes species diversity across many different orchard meadows, such as in a region, state or country. At this scale, orchards are among the most biodiverse cultivated landscapes in Central Europe (Saure 2016; Schuboth and Krummhaar 2019). For instance, in the German state of Saxony-Anhalt, one third of the nationwide species of wild bees, 19% of wasp species and 22% of hoverfly species (Syrphidae) were found in orchard meadows (Saure 2016). The area of orchards covers approximately 60–70 km^2 (Henle et al. 2024), which is approximately 0.3% of the area of Saxony-Anhalt. A similarly high richness in orchards was reported for the German state Baden-Württemberg and for Upper Austria for wild bee species with >40% and 23% of the state's overall diversity respectively (Schwenninger and Wolf-Schwenninger 2012; Ockermüller 2018). Among these species, 21% of the bee species listed as endangered in Baden-Württemberg were recorded in orchards (Schwenninger and Wolf-Schwenninger 2012).

For birds, the numbers are similarly high. In Austria, about one third of the 200 species of regular breeding birds, and half of the 103 songbird species were recorded in orchard meadows (Erlach 1994).

Schuboth and Krummhaar (2019) provide a detailed list of species occurring in orchard meadows in the German state Saxony-Anhalt. In their study, the authors recorded a total of 3,623 species in ten orchard meadows, of which

359 species are considered endangered in Germany [Figures from Henle et al. (2024), who used slightly different data from the individual chapters of a few taxonomic groups]. Taxa like mosses (Bryophyta), lichens (Lichenophyta) and fungi were recorded with 95, 72 and 326 species, respectively. The highest diversity was from the phylum Arthropoda, in which the class of Coleoptera dominated with 899 recorded species, followed by butterflies (368), Arachnida (365 species), and Hymenoptera (351 species: bees, ants, wasps).

A study by Zarabska et al. (2009) compared lichens among apple orchards in Poland, Slovakia and Italy and found highest diversity in Slovakia (52), followed by Italy (43) and Poland (32). The authors concluded that each orchard creates its own conditions, leading to a different number of species, as some species favour specific conditions over others (Zarabska et al. 2009).

Only few similar studies exist for Central European orchard meadows, all of them showing a high species richness for many taxonomic groups [see Henle et al. (2024) for studies in Germany]. Most comprehensive studies focussed on one or very few taxonomic groups, mainly on birds, pollinating insects and/or plants. As a consequence, for most regions and other taxonomic groups occurring in orchard meadows, much less is known (Henle et al. 2024). This bias in taxonomic coverage makes it difficult to evaluate whether other taxa, like mammals and other insect groups, are similarly diverse in orchard meadows from the local to the regional scale, which species depend on orchard meadows and how the availability of orchard meadows at the regional scale affects their abundance.

The high species diversity at regional scale can be explained by different factors. Generally, it is important to note that there is no standard appearance among orchard meadows at local scale. Orchard meadows have different sizes, managements, fruit tree compositions, and environmental site conditions, e.g., soil properties and topographic location (e.g., Glück et al. 2004; Bauschmann 2005; Grüebler et al. 2013; Žarnovičan et al. 2017). Differently structured orchard meadows offer more diverse habitat types, food resources and nesting sites, resulting in higher species diversity (Erlach 1994; Wiche et al. 2015; Kajtoch 2017; Tonelli et al. 2017; Schuboth and Krummhaar 2019). Similarly, the surrounding landscape is an important driver for high beta diversity at the regional scale (Horák et al. 2013). All these different local characteristics lead to higher species spatial turnover among orchard meadows contributing to the high alpha diversity observed at regional scale.

However, it is important to note that only few studies assessed the overall species diversity at regional scale and especially across scale. More knowledge is needed to (i) gain better understanding of which taxa are particularly diverse in orchards locally and regionally and (ii) to compare diversity among regions and taxa. This knowledge is important to guide conservation actions in orchards and could potentially serve as a baseline for future monitoring of biodiversity changes in orchards over time. This is of high relevance, since the total area of orchard meadows has declined substantially in most regions of Central Europe since the 1950s (Ullrich 1987; Rösler 1996; Herzog 1998; Žarnovičan et al. 2017; Henle et al. 2024) and their quality as habitat is also in decline due to eutrophication, which refers to nutrient enrichment that can degrade habitat quality (e.g. Kornprobst 1994; Wassen et al. 2021).

The loss of orchard meadows and the reduction of their quality threatens the biodiversity of orchard meadows at the regional scale. This has been well documented for bird species that breed primarily in orchard meadows (Henle et al. 2024). In the region of Havelland, Germany, for example, the population of the hoopoe (*Upupa epops*) has declined sharply and all but a few pairs of the little owl (*Athene noctua*) have disappeared despite support measures (Putze et al. 2009). In Bavaria, wrynecks (*Jynx torquilla*) and ortolan (*Emberiza hortulana*) have declined by more than 50% in the last 25–30 years (Kilian 2016). The declines were probably due to the extensive loss of orchards and rows of fruit trees in the arable farming areas of Franconia (ortolan) and the loss of the meager, patchy vegetation for the wryneck. Species, for which orchard meadows remain the last remaining refuges, such as *U. epops*, *A. noctua* and woodchat shrike (*Lanius senator*) (Ullrich 1987), became threatened with extinction regionally or even extinct nationally (Ullrich 1987; Kilian 2016). Given the large number of threatened species of other taxonomic groups that have been observed in orchard meadows (Henle et al. 2024), similar regional declines likely also occurred for many species in other taxonomic groups but in the absence of targeted long-term monitoring it is difficult to evaluate.

For plants, eutrophication of orchard meadows has led to a rather low species richness of plants across large parts of Franconia in Bavaria (Kornprobst 1994). Global nitrogen-based production of fertilizer has increased by one order of magnitude since the 1950s (Smil 2001). Central Europe is particularly heavily impacted by nitrogen deposition (Ellenberg 1991), which has contributed to a reduction of plant species richness in many parts of Europe (Stevens et al. 2010; Wassen et al. 2021). The understory vegetation in orchard meadows, which strongly influences many taxonomic groups, likely contributed to regional declines in diversity and abundance of numerous animal species due to eutrophication.

Part II: Management of orchards

Extensive management of the under- and overstory is a major driver of the biodiversity of orchard meadows. In this part, we will review and discuss the effects of different management practices on species diversity in orchards. We provide recommendations on the management of orchard meadows for maintaining and increasing biodiversity.

In Central Europe, most open grasslands and scattered tree landscapes are artificial and maintained by people (Mühlenberg and Slowik 1997). Traditionally managed grasslands support high species richness, with one third of all native vascular plant species in Central Europe occurring in grazed or mowed agricultural grassland (Leuschner and Ellenberg 2017). To maintain open grassland, interventions are necessary as otherwise those areas would disappear, driven by succession towards forests. Management interventions in the form of fertilization, grazing and mowing are disturbances affecting species composition and diversity (Leuschner and Ellenberg 2017).

The intermediate disturbance hypothesis by Connell (1978) describes that to a certain (intermediate) degree, disturbance can promote establishment of new and less competitive species into a system and rearrange species composition by changing competitive interactions. For example, vascular plant species richness peaks at moderate grazing levels rather than at complete abandonment of livestock (Yuan et al. 2016). Even for some semi-natural grasslands protected under the Natura 2000 framework in Central Europe, it is assumed that current grazing and mowing intensities are too low compared to the previous traditional management or that the timing of management is not suitable for many species with strong effects on species richness and composition (Diekmann et al. 2019). However, spatio-temporal shifting of management could ameliorate this problem (Kleyer et al. 2007).

In general, too high intensification in the form of high fertilizer use (N fertilization >120 kg N ha^{-1}: eutrophic) either intensive grazing and mowing (3–6 times a year) can lead to decreasing species diversity of grassland (Leuschner and Ellenberg 2017); and presumably also in orchards. The transition from high-diversity grasslands to less diverse plant communities likely has similar effects on the fauna, which may become less diverse and dominated by generalist species (Siemann et al. 1998; Ebeling et al. 2018). For the overstory, tree management is mandatory for maintaining high biodiversity. Appropriate management increases microhabitats in fruit trees that, in turn, leads to high biodiversity, especially in arthropods and birds (see above for the effects of structure and microhabitats on species richness, Asbeck et al. 2021).

Maintenance of structural diversity of the understory

The understory of orchard meadows, which consists of grasses and herbs, can be managed through grazing, mowing, fertilization, mulching, or a combination of these methods. Each management form has specific effects on the plant community composition of the understory and likely also its fauna. It is important to note that orchard meadows can include dry, moderate, or wet grasslands, and nutrient poor or eutrophic grasslands (Kornprobst 1994), which changes the plant community and subsequently the meadow management. However, due to the scarcity of literature, we did not differentiate between grassland types (such as gradients in wetness of nutrients) in orchard meadows within our management categorization. This gap in knowledge is a distinct need for future research.

Grazing mainly promotes species richness of grasses, whereas mowing increases richness of herbs (Steffan-Dewenter and Leschke 2003). Characteristic plant species found in extensive managed orchard meadows are grasses such as *Arrhenatherum elatius*, *Dactylis glomerata* and in warmer stands *Bromus erectus* (Langensiepen and Otte 1994; Denk and Wittig 1999; Čejka et al. 2018). However, it is important to note that plant communities highly depend on the location (and its associated environmental conditions) and management which determine the species composition and plant community in the understory. For example, in grazed orchards, the herb layer is typically lower than in mown ones, mainly comprising low grasses (such as *Cynosurus cristatus*, *Lolium perenne*), along with herbs tolerant to grazing and trampling (e.g. *Bellis perennis*, *Plantago major*, *Prunella vulgaris*). While on nutrient-rich soils, species of the *Molinio-Arrhenatheretea* class are common (e.g. *Acetosa pratensis*, *Festuca pratensis*, *Ranunculus acris*), nutrient-poor and semi-arid meadows will feature more *Festuco-Brometea* species (e.g. *Galium verum*, *Medicago lupulina*, *Securigera varia*) (Denk and Wittig 1999).

Fertilization or mulching can be important for nutrients repatriation (Degenbeck 2021). All methods have their pros and cons, and these depend on their type and intensity (Fig. 1).

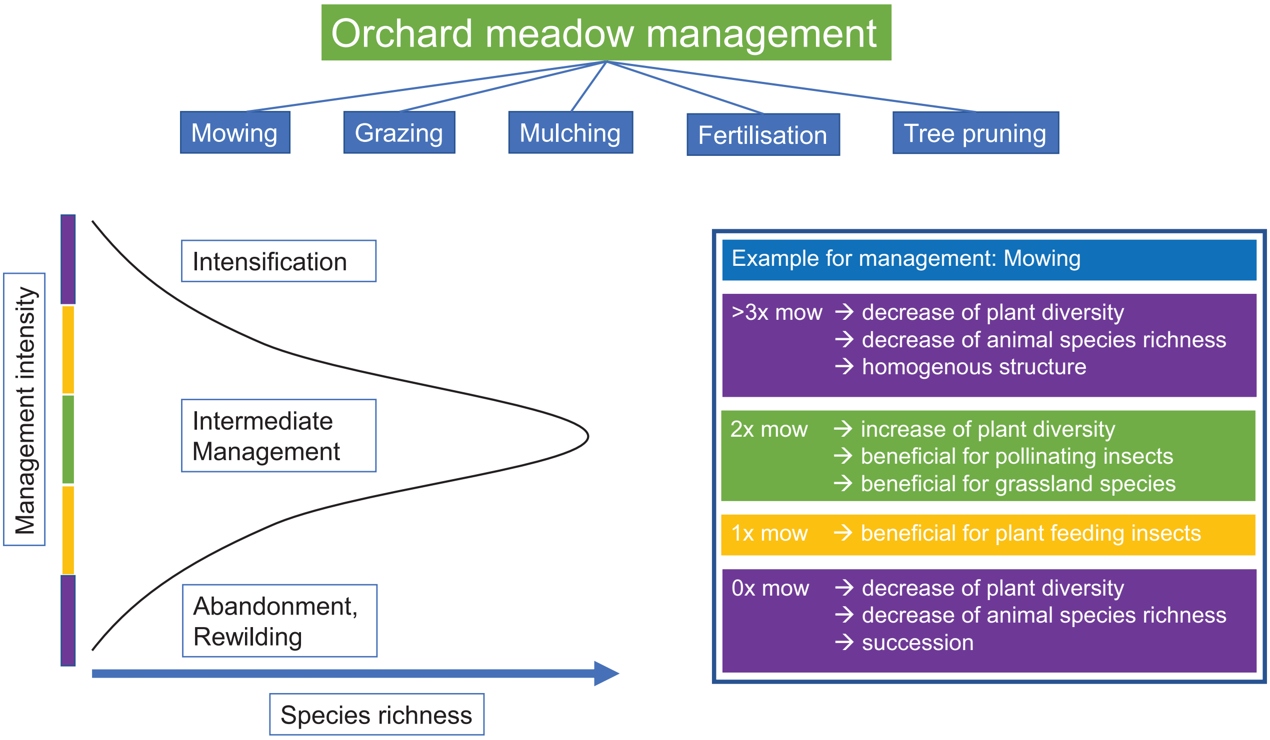

Figure 1. Schematic figure illustrating the effects of management intensification on species richness. The graph illustrates the potential effect of management intensity (from high over intermediate to abandonment/rewilding) on species richness in orchard meadows. As an example, the effect of mowing intensity on species richness is shown in a box.

Grazing as management option

Grazing can ensure that meadows remain structurally diverse by creating a mosaic of damaged and undamaged vegetation (Schoof et al. 2019). Cattle, sheep, horses and goats have different feeding behaviour, with different effects on vegetation (Carvell 2002; Öckinger et al. 2006; Rook and Tallowin 2011; Schoof et al. 2019). Sheep and goats bite off vegetation, whereas cattle tear it off, leaving more vegetation intact (Schoof et al. 2019).

Öckinger et al. (2006) found that grasslands being grazed by sheep support less plant and butterfly species compared to grasslands grazed by horse or cattle. These results were mirrored by Carvell (2002), who found that cattle grazed grasslands supported higher bumble bee abundance than those grazed by sheep. Sheep have a highly selective feeding behaviour, which can lead to a floristic impoverishment as only certain plant species are affected. Also, temporal scale of grazing is crucial for plant species composition. A short time period of grazing with a high number of sheep can lead to an evenly grazed vegetation. In contrast, a small number of sheep grazing over a long time period in the same meadow can lead to selective feeding behaviour (Zahn and Tautenhahn 2016). Selective grazing by sheep can be partly compensated by combining sheep with goats or cattle as not only sheep-selected plants are grazed (Zahn and Tautenhahn 2016). Goats can modify their feeding behaviour depending on the seasonal change of vegetation. They also feed on woody plants suppressing the potential development of shrubs and therefore prevent succession (Elias and Tischew 2016). Cattle, however, are unselective in their choice of plants and support higher structural and floristic diversity (Zahn and Tautenhahn 2016). Low to moderate cattle grazing can be beneficial for butterflies by creating more structural diversity and potentially be used as conservation tool for disturbance-dependent grasslands (Bussan 2022) like orchard meadows. Furthermore, a structurally diverse meadow created by extensive grazing with areas excluded for livestock promotes orthopteran diversity and abundance (Gardiner 2018).

Timing of grazing can also highly influence the grassland community. Paesel et al. (2019) found that when the grazing period starts late and fast-growing competitive species spread and reach a certain height (120 cm and more), grazers most likely avoid them due to lignification of plant tissue. Therefore, in their study, grazing did not increase vegetation heterogeneity (Paesel et al. 2019).

The intensity of grazing and thus the number of livestock is crucial when aiming at high biodiversity. Grazing intensity measured on sward height (Jerrentrup et al. 2014) showed that an intermediate lenient grazing (12 cm) by cattle results in higher species richness of grasshopper and butterflies compared to moderate (6 cm) and very lenient (18 cm) grazing intensity. The authors recommend a stocking rate of ~1 SLU ha^{-1} [standard livestock unit (SLU) = 500 kg] to maintain heterogenous sward structure, which is beneficial for less mobile insects and insects sensitive to grassland structure like grasshoppers (Jerrentrup et al. 2014).

The management should aim towards a structurally diverse understory created by intermediate grazing intensity and meadow areas excluded for livestock to offer undisturbed areas for, e.g., breeding birds. However, if the whole meadow is grazed by livestock, a grazing break of 2–4 months should be included to create regeneration time for fauna inhabiting the understory (Zahn and Tautenhahn 2016). In any case, tree protection (e.g., bite protection by fencing off trunks) is recommended when orchard meadows are grazed by livestock as goats, cattle and sheep debark trees (López-Sánchez et al. 2020). Debarking can lead to damage of fruit trees, which in turn can harm crown health and development (López-Sánchez et al. 2020).

Dung of livestock can be an important fertiliser and is also crucial for dung living and visiting organisms like several dipteran families (e.g., Syrphidae, Dolichopodidae, Muscidae) and dung beetles (e.g., Scarabaeidae, Geotrupidae) (Young 2015; Schoof and Luick 2019). Orchard meadows are usually not treated with pesticides or other chemical input (Rösler 1992; Erlach 1994). However, husbandry of livestock is mostly associated with the use of veterinary medicine, like, e.g., antiparasitics and antibiotics, which are highly debated due to, e.g., antibiotic resistance and their environmental impact on ecosystems (Van Puyvelde et al. 2018; Sebestyén et al. 2018; Lalouckova and Skrivanova 2019).

Veterinary medicine or their metabolites in dung of livestock negatively affects dung living insects (Tonelli et al. 2017; Schoof et al. 2019). It can reduce biomass, abundance, functional diversity and species richness of dung beetle communities, which are important decomposers and also serve as food source for a range of animals (Tonelli et al. 2017; 2020). Numerous bird species (e.g., *Turdus merula*, *Lanius collurio*), bats (e.g., *Rhinolophus ferrumequinum*) and hedgehogs (*Erinaceus europaeus*, *Erinaceus concolor*) feed on dung living insects [see Young (2015) for a comprehensive list of predators of dung living insects], which in turn are affected by the reduction of dung organism.

Römbke et al. (2019) recommended risk mitigation measures to protect dung and soil organisms from antiparasitics. The following recommendations were made to protect dung organisms: (i) a selected instead of strategic use (e.g., common practice is that prophylactically all animals are treated) of anti-parasitic treatments as well as (ii) restricting strategic treatments to seasons when diversity and abundance of dung organisms are not at their highest. There should be (iii) no treatment of livestock on the same pasture in the successive season (e.g., spring and summer of the same year). If possible, (iv) animals should be in the shed during the treatment period. The effect of veterinary medicine on biodiversity of dung living insects and other non-target organisms is little studied and generally not considered in conservation management plans yet (Römbke et al. 2019; Schoof et al. 2019).

Management by mowing

When the understory of orchards is managed by mowing, the timing and frequency of mowing strongly determines vegetation structure. Ideally, plants should have reached seed maturity or be capable of vegetative propagation by the time of cutting. This will increase their chances of persistence and propagation under a mowing regime with a constant temporal sequence. Plant species not adapted to frequent mowing, either due to low build-up of energy reserves, damage before seed production or sudden change in microclimate, may not persist long-term under unfavourable mowing management. However, plant species with low competitive ability, such as slow growing species or those adapted to high disturbances, depend on regular clearing or removal of more competitive, fast-growing plants for their survival (Oppermann and Briemle 2009; Schoof et al. 2019).

In orchard meadows, the type and structure of the understory has a strong effect on faunal diversity. Hence, mowing time and frequency also determines which animals persist and establish. For wild bees, early mowing that removes flower buds of spring flowers depletes important flowering resources, such as pollen and nectar (Schwenninger and Wolf-Schwenninger 2012). Therefore, Schwenninger and Wolf-Schwenninger (2012) recommended mid of June as orientation date for the first mowing occasion and from the end of August for the second mowing date, when most summer plants already withered.

Besides timing, the frequency of mowing is also crucial. Intermediate cut frequencies, such as twice a year, support high species richness of vascular plants (Fig. 1) (Socher et al. 2013). Subdominant plant species can establish, as resources essential for them, such as light, become available. However, Wiche et al. (2015) found that mowing twice a year already negatively affected species richness of cicada. Mowing, in particular, enhances the survival of smaller, less competitive plant species on which relatively few cicada species are specialised. Therefore, the authors recommended a single annual mowing to maintain high cicada species richness. To maintain high wild bee diversity, in contrast, Schwenninger and Wolf-Schwenninger (2012) suggested staggered and twice mowing to maintain high flower supply. However, Steffan-Dewenter and Leschke (2003) found that above-ground nesting bee and wasp species were less effected by mowing frequency. The authors assumed that the studied insect communities are only indirectly dependent on the vegetation layer as food supply as flowering fruit trees provide additional pollen and nectar as well as attract prey for hunting wasps. This was similarly observed in a study of Horák (2014b) who found that butterfly species richness was not associated with management (mowing) but with flowering intensity. The author suggested that flowering fruit trees and the surrounding areas might compensate for the effect of mowing on the understory vegetation. Nonetheless, as tree blossoms are temporary and mainly in spring, targeted understory management for bee, wasp and cicada fauna is necessary.

To account for the diverging effects of different mowing schemes on different taxonomic groups, a spatial and temporal mosaic of mowing regimes could be implemented (Kleyer et al. 2007; Johst et al. 2015). For example, a spatio-temporal mowing scheme benefits the survival of the scarce large blue butterfly (*Phengaris teleius*) in grassland systems (Johst et al. 2006). Likewise, asynchronous mowing of grassland likely is a key process governing the high density of white stork in an extensively managed farming landscape of east-central Poland (Golawski and Kasprzykowski 2021). Johst et al. (2015) developed a model to assess the effects of different spatial-temporal mowing schemes on butterfly and bird species. This approach was extended by Sturm et al. (2018) towards a decision-support software, which calculates the effect of grazing and mowing regimes on endangered bird and butterfly species, to determine ecologically and cost-effective agri-environment schemes. Although spatial-temporal mosaic mowing schemes are occasionally implemented in the management of orchards at small scales (Fig. 2), we are not aware of any study applying this model to orchards or that analysed the effects of spatio-temporal mosaic mowing schemes for species in orchards.

Figure 2. Example of a spatio-temporal mosaic mowing regime in an orchard in Rutesheim-Perouse, southern Germany. Photo: Klaus Henle.

To date, there are no studies comparing the combined effect of different management methods in orchard meadows. Similarly, most studies focus on management effects on plants, birds and pollinating insects, which makes it difficult to develop conservation strategies that also account for the needs of the wide range of species from other taxonomic groups or ecological guilds for which orchard meadows are important (Mader 1982; Schuboth and Krummhaar 2019; Henle et al. 2024). The focus on the conservation of a single species group can be detrimental for other taxa. As most studies cited by us were done in grasslands lacking trees, there is an urgent need for studies covering simultaneously several taxa inhabiting orchard meadows for the development of management guidelines that account for synergistic and antagonistic needs of different taxonomic groups and for interactions between the understory and the tree story.

Effects of abandonment and management intensification

Orchard meadows that are neither grazed nor mowed and left fallow rewild and lose their typical structure of semi-open grasslands with scattered trees. Important habitats disappear, floral as well as faunal species richness decrease (Žarnovičan et al. 2017). Grass and herbal cover of the understory decrease and succession starts to develop (increase of shrub and tree abundance) (Steffan-Dewenter and Leschke 2003; Wiche et al. 2015; Vowinkel 2017). Such forms of abandonment of orchard meadows can lead to a short increase of butterfly and bird diversity as additional resources like forbs and shrubs as well as dead wood become available (Ernst et al. 2017; Kajtoch 2017). In the long term, however, open structures vanish followed by habitats and forage resources unsuitable for species requiring (semi-)open environments (Ernst et al. 2017; Čejka et al. 2018). Horák et al. (2018) found that the number of lichens, butterflies, beetles, and orthopteran species increased when abandoned orchard meadows were restored. These results show that maintenance or restauration has positive effects on species biodiversity and successional changes argue against rewilding of extensively used agricultural landscapes like traditional orchard meadows.

Intensification of orchard meadows towards fruit plantations is the opposing effect of abandonment. Intensification aims at increasing economic output by increasing external inputs like fertiliser and pesticides and by more intensive management like removing old and/or less productive trees. Permanent transition towards intensive grazing and/or high disturbance by mowing in combination with high nutrient input leads to a decrease of taxonomic and functional diversity of pollinating insects (e.g., species of Hymenoptera, Lepidoptera and Diptera) and orthopteran species as well as in alteration of vegetation communities toward highly competitive and disturbance-adapted species (Gardiner 2018; Rakosy et al. 2022). Similarly, high fertilizer rates and frequent mowing can lead to homogenisation of plant communities (Kornprobst 1994; Hammel and Arnold 2012; Socher et al. 2013; Kilian 2016). Addition of fertiliser favours dominant plant species; especially threatened species are lost by high N and P inputs (Harpole et al. 2016; Hautier et al. 2020; Wassen et al. 2021) as many of the Central European threatened plant species depend on nutrient poor soils (Ellenberg 1991; Ellenberg et al. 2001) and are threatened because of the substantial increase of N-emission and P-input across most areas in Central Europe (Ellenberg 1991; Wassen et al. 2005).

Similarly, accumulation of dung due to overstocking of livestock and mulching (cut vegetation left on the meadow) can lead to accumulation of nutrients and rotting processes. This in turn can lead to a change and homogenisation of the flora and vegetation structure in the longer term (Pavlů et al. 2016). However, mulching or fertilising can be important for the replenishment of nutrients for fruit trees, which can have an undersupply of phosphor, potassium and magnesium if, e.g., regularly harvested (Degenbeck 2021). Pavlů et al. (2016) found no significant changes of nutrient concentration in herbs or soil when cuttings were either left or removed in upland grassland in the Czech Republic. Nonetheless, the authors reported a tendency towards higher nutrient concentrations in grasslands treated by mulching than grassland with cuttings removed. Mulching or fertilising should be considered based on the location and abiotic factors, e.g., soil properties. While mulching can benefit plant species and functional trait diversity in a nutrient-poor mountain meadow (Doležal et al. 2011), it can decrease plant species richness in an nutrient-rich upland grassland (Gaisler et al. 2019). Beside the need to change from intensive to extensive cultivation, a grassland study in Germany showed that variation of land-use intensification (mowing, grazing, fertilisation) across years can be a complementary strategy to enhance biodiversity (Allan et al. 2014).

If extensive management like grazing or mowing with cut vegetation removed cannot be maintained, temporarily mulching twice a year seems to be a good option to conserve plant diversity and depress succession (Römermann et al. 2009; Gaisler et al. 2019).

Effects of extensive tree management on species richness

The diversity of microhabitats is a useful indicators for species richness as it is assumed that microhabitats such as dead wood, cavities and branch holes, correlate with the abundance and diversity of organisms living on and in trees (Grossmann and Pyttel 2016; Asbeck et al. 2021). Therefore, the maintenance of high-trunk trees in form of occasional pruning is very important as pruning promotes the formation of natural cavities beneficial for cavity users like many bird and insect species (see chapter structure and microhabitats) (Eckstein and Albrecht 2006; Grüebler et al. 2013; Henle et al. 2024). Especially, removing of main branches leads to large pruning wounds, which in turn lead to decay cavities (Grüebler et al. 2013). Extensive tree care, such as leaving dead branches, promotes deadwood and notably increases decay-induced tree cavities (Eckstein and Albrecht 2006).

Quality and quantity of microhabitats further depend on the tree species and tree associated properties, such as bark structure and trunk diameter. Eckstein and Albrecht (2006) found that trunk and branch hollows were mostly associated with apple trees whereas moss cushions were mostly found on pear trees. Lichens are most common on pear and plum trees. Grossmann and Pyttel (2016) found more microhabitats in walnut trees compared to apple trees and a correlation of tree diameter with microhabitats. Larger tree diameter is associated with more microhabitats as trees are usually older. Older trees are more likely to be exposed to natural disturbances resulting in injuries or rot infestation, which favours development of microhabitats (Bobiec 2002; Vuidot et al. 2011; Grossmann and Pyttel 2016). Although walnut trees seem to offer many microhabitat structures because of their high tree diameter, they are not common in orchard meadows in Central Europe (Degenbeck 2003; Schuboth and Krummhaar 2019).

The importance of different tree species and varieties is also shown by different susceptibility to diseases. Apple trees are affected by higher rates of fungal heart rot infestation. This, in turn, attracts high numbers of woodpeckers and consequently leads to higher numbers of woodpecker-cavities (Grüebler et al. 2013). Other studies also showed that especially apple trees contain higher numbers of tree holes (Amann 2007), which might be because of the softer bark of apple trees, which is easier to penetrate compared to other fruit trees (Eckstein and Albrecht 2006). To obtain a high quantity of microhabitats new tree plantings should include a high proportion of apple trees (Grüebler et al. 2013). At the same time, nesting possibilities in the form of artificial nesting boxes are also an important conservation strategy to promote birds, small mammals and insects (Amann 2007).

Summary conservation and management recommendations

The species diversity of orchard meadows is very closely connected to the maintenance and management of the under- and overstory, which determines structural diversity. However, it is evident from the studies reviewed above that management recommendations depend on and differ among targeted taxa, and thus each may be detrimental for non-target taxa (Wiche et al. 2015; Schoof et al. 2019). In any case, rewilding, a strategy for biodiversity conservation recently increasingly promoted also for cultural landscapes in the form of land abandonment (Navarro and Pereira 2015), is not an appropriate conservation strategy for orchard meadows. Rewilding will lead to the disappearance of plants and animal species that depend on the combination of open diverse grassland with trees (Ernst et al. 2017; Žarnovičan et al. 2017). Likewise, intensification with high interventions will be detrimental to many taxa and will result in the loss of the biodiversity conservation value of orchards (Pavlů et al. 2016; Gardiner 2018; Rakosy et al. 2022).

Similar to the understory, the maintenance of the overstory by extensive tree pruning leads to high numbers of microhabitats, which offers manifold ecological niches for different species. Management of trees prevent premature ageing. For the maintenance of orchard meadows adding young trees of different species and varieties are important. Similarly, dead wood, e.g., standing trees contain many microhabitats and are crucial for cavity users (Eckstein and Albrecht 2006; Grüebler et al. 2013; Grossmann and Pyttel 2016). Although fruit trees contain many microhabitats, nesting boxes should be provided to support secondary cavity users like several bird, mammal and insect species (Amann 2007).

Grazing, mowing and tree maintenance are key management aspects for biodiversity in orchard meadows. However, there is no “silver-bullet strategy” for an optimal management regime that fits all taxa as it is highly dependent on the location and the targeted species groups. For instance, a mowing frequency of twice per year can already lead to a decrease of cicada species (Wiche et al. 2015), whereas bee species richness may benefit (Schwenninger and Wolf-Schwenninger 2012). As such, we recommend a combination and time-shifted implementation of different management regimes, like grazing and mowing, as this seems to be a promising way to create an understory suitable for taxa with different ecological requirements as grasses and herbs will be in different growth stages. A caveat is that such schemes can be implemented effectively only in very large orchards or at the landscape scale with many orchards in close vicinity to each other.

Compared to other livestock, cattle, with their unselective feeding behaviour, seem to have the best effect on plant, butterfly and bee diversity (Carvell 2002; Sheil and Wunder 2002; Öckinger et al. 2006; Schoof et al. 2019). However, when livestock is involved, fruit trees should be protected with fences to avoid debarking (López-Sánchez et al. 2020). Similarly, risk mitigation measures, e.g., a selected use of antiparasitic treatments, to protect dung living insects should be considered when using veterinary medicine (Römbke et al. 2019).

Mowing times should be adjusted to the surrounding landscapes (e.g., timing of mowing in the neighbouring landscape) to prevent synchronous mowing and maintain alternative areas for the fauna (Kleyer et al. 2007; Johst et al. 2015; Golawski and Kasprzykowski 2021). Especially habitats similar to orchard meadows seem to be beneficial for species richness and should be considered as well (Horák 2014b; Ernst et al. 2017). Generally, conservation schemes should take place on the landscape scale rather than the local scale to promote interconnectivity and landscape diversity. This will provide flower resources during a longer time period for flower visiting insects and structural diversity thereby promoting nesting, foraging or hunting sites for different taxa (e.g., grassland birds, small mammals). If mowing or grazing cannot be afforded, temporarily mulching is also an option to maintain plant diversity and prevent successions (Römermann et al. 2009; Gaisler et al. 2019).

Further, traditional ecological knowledge about orchard meadows in a specific region can be very helpful in optimizing management regimes, as it has been verified by generations of farmers (Babai and Molnár 2014).

Conclusions

To conserve and halt the decline of orchard meadows in Central Europe, we argue that it is paramount to acknowledge their importance for biodiversity at a political, cultural and societal level. There is a need for a clear definition of orchard meadows to create a common term in Europe, which would make the assessment as well as their protection more straightforward (see Henle et al. 2024). Orchard meadows should be listed in the Habitats Directive of the Council of the European Union and farmers should get incentives for maintaining orchard meadows; see Henle et al. (2024) for comprehensive conservation recommendations at the political, economic, cultural and societal level from the local to the European level.

While we highlighted the important ecological role of orchard meadows in Central European landscapes (Table 1), our review also points out the limitation of available studies on orchard meadows in Central Europe. Most studies focused on plants, birds and pollinating insects, and only a few studies are available on other taxonomic groups. Even for plants, the knowledge depends to a large extent on an extrapolation from studies of tree-less grasslands to orchards, which contains uncertainty on the extent of the robustness of the extrapolation. Conservation strategies on single taxonomic groups, e.g., bird

Table 1. Key conclusions.

1. Biodiversity in Orchard Meadows	**Selected References**
Orchard meadows are one of the most biodiverse agricultural habitats in Central Europe explained by their high structural diversity. (See chapter: Structure and microhabitats)	Kilian (2016), Saure (2016), Schuboth and Krummhaar (2019)
Species richness in orchard meadows increases with patch size. (See chapter: Local scale: effects of patch size and isolation)	Amann (2007), Bauschmann (2005), Steffan-Dewenter (2003)
Habitat surroundings, landscape composition and connectivity of orchard meadows determines species composition. (See chapters: Surrounding landscapes, regional scale)	Ernst et al. (2017), Horák et al. (2013), Steffan-Dewenter (2003)
2. Management in Orchard Meadows	
Extensive grazing and mowing promote structural diversity of the understory. (See chapter: Maintenance of structural diversity of the understory)	Schoof et al. (2019), Jerrentrup et al. (2014), (Gilhaus et al. 2017)
Fertilization and intensive mowing or grazing leads to homogenisation of plant communities. (See chapter: Effects of abandonment and management intensification)	Hammel and Arnold (2012), Kilian (2016), Leuschner and Ellenberg 2017
Extensive tree management promotes animal species richness. (See chapter: Effects of extensive tree management on species richness)	(Rösler 2002), Samnegård et al. (2019), Erlach (1994), Eckstein and Albrecht (2006), Grüebler et al. (2013)

or pollinator insect species, might be detrimental for other taxonomic groups. Studies on the management effects on neglected taxonomic groups is a major research need for improving applied conservation of biodiversity in orchard meadows as is the effect of spatially and temporally asynchronous understory management. Similarly, very few studies investigated the landscape scale effects on orchard meadows, and comparisons with other extensive agricultural landscapes are lacking. Those studies could help to understand the function of orchard meadows as extensive agricultural habitat on the landscape scale and what effect it has for other land use types and vice versa.

Here, we will briefly outline future research directions.

Research gaps and directions:

- Alpha diversity is closely linked to structural diversity of orchard meadows highlighted by the high diversity of different taxa inhabiting the under- and overstory of orchard meadows. However, to this date there are only a limited number of studies comparing orchard meadows to other extensive cultivated landscapes like grasslands or woodlands in the same landscapes. Comparing other extensively cultivated landscapes to orchard meadows could help to better understand species composition in orchard meadows, especially for which species orchard meadows belong to their preferred habitats. Studies on the effects of the presence of trees with their shading, leaf fall and fruit availability on diverse taxonomic groups, and how extensively managed landscapes can substitute or supplement each other at a landscape scale are very rare. Similarly, knowledge about the interactions of orchard meadows with the surrounding matrix in the conservation of species is rather limited, including an assessment of the relative importance of orchard meadow and the matrix for the presence of species in orchard meadows. There is evidence that orchard meadows can be important for specialist and generalist species.
- We see an urgent need to invest in research addressing area requirements of different species. Great promise lies in the discipline of functional ecology

where species occurrences in orchards of different size and isolation can be linked to their traits and other indices of their life-history strategy. For instance, the link of species traits and their occurrence in differently sized and isolated orchard meadows is largely unknown. Here, future research may test whether species with high dispersal capacity, such as winged insects or birds, are overrepresented in small and/or isolated orchard meadows.
- Area requirements of species in orchards are poorly known, rendering it speculative whether species with large area requirements are mostly absent in orchards. Future research should link such questions with habitat connectivity of different orchard meadows. For instance, even smaller but well-connected orchard meadows may allow species with larger area requirements to persist more likely than isolated but larger orchard meadows. Such questions are also of great relevance for conservation and management strategies that we discuss in detail under conservation and management recommendations.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

CS and KH conceived the ideas. CS and MLH conducted literature research. CS wrote the main text of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

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Research Article

The greater the proportion of *Robinia pseudoacacia* in a stand the greater its effect on the population characteristics of *Erythronium dens-canis*

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Abstract

Management of invasive alien plants is an increasing problem throughout the world. In some cases native rare or protected species can appear or even prefer habitats dominated by invasive alien plants, which raises questions about the optimal treatment of such areas. *Erythronium dens-canis* in Hungary is a protected species which only have several occurrences in the country and a number of these populations situated in *Robinia pseudoacacia* stands developed after harvesting native forests. In this study a total of five populations of *E. dens-canis* were surveyed between 2020 and 2022 in southwestern Hungary examining and comparing the ongoing demographic changes under native and *Robinia* stands by monitoring individual plants. Two populations were situated in forests composed of native tree species, two in *Robinia pseudoacacia*-dominated stands and one in a *Robinia*-native tree species mixed stand. We categorized the plants into five age-state categories: dormant, seedling, juvenile, vegetative adult, and reproductive adult. We found some considerable differences (e.g. leaf size, reproduction rate) between the populations situated in native and in *Robinia* stands, whereas the population in mixed forest showed intermediate character in most examined factors. Based on our results, *R. pseudoacacia* have a significant effect on the phenology and life history of *E. dens-canis*, and this effect is greater with higher proportion of *R. pseudoacacia* in a forest stand where the *E. dens-canis* occurs.

Key words: Endangered species, habitat transformation, Hungary, invasive alien species, population dynamics

Introduction

One of the most problematic aspects of invasive alien plant species is their potential ability to transform ecosystems in which they are introduced to (Richardson and Rejmánek 2011). They can affect plant functional traits (Sitzia et al. 2018), influence community structure and composition (Daehler and Strong 1994; Hejda and Pyšek 2006; Gaertner et al. 2009; Nascimbene and Marini 2010) together with altering soil characteristics and nutrient cycling (De Marco et al. 2013; Medina-Villar et al. 2015). Biological invasions can lead to homogenization of native communities (McKinney and Lockwood 1999; Rooney et al.

2004), loss of biodiversity, including altered ecosystem functioning (Pimentel et al. 2001; Didham et al. 2007; Vilá et al. 2010).

In Hungary, *Robinia pseudoacacia* L. (black locust or false acacia) is one of the invasive alien plant species with the greatest impact on natural ecosystems (Mihály and Botta-Dukát 2006). *R. pseudoacacia* belongs to the legume family (Fabaceae), it is a light-demanding pioneer species native to North America. In its native range it rapidly colonises forest gaps and is gradually replaced by other tree species after 15–30 years. In contrast, in secondary habitats its populations can persist for longer periods, displacing native communities (Cierjacks et al. 2013) and therefore *R. pseudoacacia* is considered an invasive species throughout Europe, which resulted in the inclusion of this species in national blacklists and other lists summarising alien species (Norway: Gederaas et al. 2012; Czech Republic: Pyšek et al. 2012; Pergl et al. 2016; Germany: Seitz and Nehring 2013; Italy: Celesti-Grapow et al. 2009; Switzerland: FOEN 2010).

It was one of the first American tree species to be introduced to Europe in the early 17th century (Vadas 1914; Vítková et al. 2016) due to its numerous economically positive properties (Vadas 1914; Göhre 1952; Straker et al. 2015). Since then, *R. pseudoacacia* has played a major role in forest management in Hungary, resulting in a significantly higher proportion of black locust forests than in other Central European countries (Vítková et al. 2017). The share of *R. pseudoacacia* in the managed forested areas is the highest (nearly 460 thousand hectares, about 24%; KSH 2023) of all tree species, and its area is still increasing (KSH 2023).

Several authors have shown that the herbaceous level of *R. pseudoacacia*-dominated forests differs significantly from that of native forests in Europe (Wendelberger 1954; Montagnini et al. 1991; Peloquin and Hiebert 1999; Von Holle et al. 2006; Taniguchi et al. 2007; Vítková et al. 2016). Under the canopy of *R. pseudoacacia*, conditions are more favourable for shade-tolerant and nitrophilous species (Hruška 1991; Cierjacks et al. 2013; Vítková et al. 2016).

In the first half of the growing season, two different phenological aspects of the herb layer are observed in *R. pseudoacacia*-dominated forests (Vítková et al. 2017). In early spring, before the appearance of *Robinia* leaves (March-April) geophytes and ephemeral annuals are observed. In late spring (May-June), shade-tolerant annuals, common nutrient-demanding plants and grasses appear. In the second half of the growing season, annuals and geophytes disappear and the herb layer often dries out (Vítková et al. 2017).

As agricultural intensification in Europe resulted in substantial loss of natural habitats (Tilman et al. 2001), there has been a gradual selection towards species that can survive in secondary habitats or on the edge of cultivated fields, especially when the management is extensive (Perrino et al. 2014). This process, although in a slower pace, is still in progress, and therefore the conservational value of semi-natural habitats is gradually increasing, as for example for annual meadows of the *Thero-Brachypodietea*, that give refuge to some rare and endangered plant species (Brana et al. 2014; Perrino et al. 2022, 2023), and are considered a priority habitat of the Directive 92/43/EEC. In Hungary, several rare and endangered species occur in *Robinia*-dominated stands, such as *Erythronium dens-canis* L., *Crocus reticulatus* Steven ex Adams or *Sternbergia colchiciflora* Waldst. Et Kit. (Bagi et al. 1998; Pacsai et al. 2022). As *E. dens-canis* only has a few large populations in Hungary and a number of these are situated in

Robinia forests, the better understanding of the species biology and ecology in this specific situation is essential to plan suitable treatments of these habitats. Relatively little is known about the population biology of *Erythronium* species in general, a few long-term studies have been carried out so far with *E. japonicum* Decne. (Kawano et al. 1982) and *E. americanum* Ker Gawl. (Holland 1981), and two study concentrated on *E. dens-canis*, one in Italy (Pupillo and Astuti 2017), and one in Ukraine (Kricsfalusy et al. 1995) where the detailed morphological and demographic properties of multiple populations were monitored through five years.

In our study we started a long-term monitoring of five *E. dens-canis* populations occurring in forests with different compositions: natural forests consisting of native tree species, intermediate, mixed and semi-natural, *Robinia*-dominated stands to follow the demographic and structural changes taking place in each population. During the first year of the study we noticed considerable differences in phenology and demography of the *E. dens-canis* plants in native and in *Robinia* stands (significantly different-sized individuals in same age-states, different ratio of flowering and pollination), which prompted us to expand our study with more, intermediate type sites to investigate these differences between populations situated in these two types of habitats in more detail.

Materials and methods

The dog's tooth violet (*Erythronium dens-canis* L.)

E. dens-canis is a monocotyledonous, perennial geophyte species, belonging to the lily family (Liliaceae). Mature specimens of the species are 10–30 cm tall (Király 2009). It flowers before trees leafing out, starting in mid-February. The vegetative plants produce one leaf, reproductive ones produce two leaves, varying in shape from ovate to lanceolate, slightly fleshy, ashy green, with brown spots that fade by the end of the growing season (Király 2009; La Rocca et al. 2014). It produces its characteristic, pink (rarely white) flowers with recurved petals with dark purple (rarely yellow) stamens, usually solitary, on 8–25 cm peduncles. The fruit is a tricarpic capsule, in which the seeds ripen in May. The seeds have an elaiosome, which is dispersed by ant species (myrmecochoria) (Guitián et al. 2003). It occurs in dry to mesophilic, usually slightly acidic soils, in hornbeam-oak woodlands, beech woodlands, mountain meadows, less frequently in wet meadows, but mainly in older, more open forest stands (Király 2009, Vacek et al. 2020).

All members of the genus *Erythronium* are native to the northern temperate zone. In Europe, only one of them, *E. dens-canis* is native. In Asia, three more species [*E. caucasicum* Woronow, *E. sibiricum* (Fisch. & C.A.Mey.) Krylov and *E. japonicum* Decne.] are present, and 23 species occur in North America (Kawano 2005). Despite the fact that *E. dens-canis* is listed as a threatened species in several European countries [Hungary: near threatened (Király 2007), Austria: regionally endangered (Niklfeld and Schratt-Ehrendorfer 1999), Slovakia: vulnerable (Turis et al. 2014), Romania: vulnerable (Hurdu et al. 2022)] or only small local populations occur [Czech Republic (Vacek et al. 2020), Ukraine (Tykhonenko et al. 2017), Hungary (Nagy et al. 2019)], few studies have investigated the life history, ecology and population dynamics of the species (Kricsfalusy and Mihaly 1993; Kricsfalusy et al. 1995; Guitián et al. 1999; Mondoni et al. 2012; Pupillo and Astuti 2017).

Similar studies have been carried out mainly on species occurring in America (*E. americanum*, *E. grandiflorum* Pursh) and Japan (*E. japonicum*) (Holland 1974; Muller and Bormann 1976; Yokoi 1976; Kawano et al. 1978; Holland 1980; Kawano et al. 1982; Hughes 1992; Ruhren and Dudash 1996; Sawada et al. 1997; Takada et al. 1998; Kawano 2005; Tessier 2019). As these species have similar life histories, they are occurring in similar habitats and genetically close to *E. dens-canis* (especially *E. japonicum*, which some authors describe as a subspecies of *E. dens-canis*), we used literature related to these species as well in the design and preparation of the present research.

Study area and permanent plots

In 2020 we installed permanent quadrats (1×1 m) along transects at three locations (one near Becsehely and two near Lispeszentadorján villages) for long-term monitoring of *E. dens-canis* populations. The corners of these quadrats were marked with nails and numbered aluminium plates to ensure the accurate positioning of the 1×1 m frames (with 10 cm wire grids) which helped us in repeated locating of individuals. Two of the studied *Erythronium* populations situated in native forest stands (Native 1, Native 2: Lispeszentadorján 1 and 2; abbreviations: N1, N2) and one in a *R. pseudoacacia*-dominated, secondary forest (*Robinia* stand 1: Becsehely 1; abbreviation: R1). In 2021 two more set of permanent quadrats have been installed, one in a *Robinia*-dominated stand (*Robinia* stand 2: Becsehely 2; abbreviation: R2) whose population was discovered in 2020, and one in a stand composed of approximately half *Robinia* and half native tree species (Native-*Robinia* mix: Lispeszentadorján 3; abbreviation: NR) (Table 1). In each area, we installed enough quadrats (their number varied between 7 and 16 per site) to have included at least 100 individuals per sample area. The positions of each *Erythronium* individual within the quadrats were recorded by 1 cm accuracy and they were marked individually by nails equipped with numbered aluminium plates. This way we were able to find the same individuals repeatedly throughout the years.

Since it is very difficult to determine precisely the age of some bulbous species, demographic-population dynamics studies often classify individuals into age-state categories based on various physical parameters (Rabotnov 1985). In our study, we used methods commonly used for population dynamics studies of geophyte species and age-state classification based on leaf morphology

Table 1. Description of the sample sites.

Sample sites and their abbreviations in parentheses	dominant tree species	locality (WGS84, DD; X,Y)	elevation (m a.s.l.)	no. of censused plants between 2020 and 2022 (min–max)
Lispeszentadorján 1 (**N1**)	*Quercus robur*, *Carpinus betulus*	46.52987°N, 16.70998°E	225	110–185
Lispeszentadorján 2 (**N2**)	*Fagus sylvatica*, *C. Betulus*	46.53225°N, 16.71440°E	220	146–172
Lispeszentadorj 3 (**NR**)	*Robinia pseudoacacia*, *C. Betulus*, *Q. Robur*	46.52972°N, 16.71071°E	230	80–104
Becsehely 1 (**R1**)	*R. pseudoacacia*	46.46072°N, 16.79269°E	210	112–294
Becsehely 2 (**R2**)	*R. pseudoacacia*, *C. Betulus*	46.46271°N, 16.78128°E	245	276–396

measurements, which have been proven suitable for other *Erythronium* species (Yokoi 1976; Kawano et al. 1982; Sawada et al. 1997) as we assessed this method in our earlier study on *E. dens-canis* (Pacsai et al. 2022). We measured maximum length (without petiole) and width of each leaf of every individual (besides seedlings, as their length were recorded only), and presence or absence of reproductive organs at the end of the flowering period (April) between 2020 and 2022.

Data analysis

Leaf area was estimated using a coefficient derived from proportions of leaf areas to leaf length and width ratio measured by image analysis of 76 leaves of 56 *E. dens-canis* individuals, photographed in 2020 at the study sites (Pacsai et al. 2022).

One of the main difficulties in the case of perennial species is the separation of juvenile individuals and vegetative adults. As we wanted to study the long-term life history of individuals of this species and it is also protected by law in Hungary, we used only non-destructive methods during the data collection. Therefore we couldn’t examine the bulbs of the individuals which otherwise could have provide significant help in categorizing the plants into age-states (Kricsfalusy et al. 1995). We used leaf area of the smallest (but statistically not outlier) reproductive individuals as the boundary line between the juvenile and vegetative adult age-state categories. As the time of measurement and environmental factors affect plant growth, this limit should be estimated for each year and each site separately (Jeong et al. 2022; Pacsai et al. 2022).

Since it cannot be determined whether a plant is dead or dormant at this point (the possible length of the prolonged dormant period is not yet known), we considered dead only seedlings which did not appear in the following years. Beside these instances, we categorized the plants as dormant when they did not produce aboveground organs. Calculating population growth (λ) without including mortality rates obviously results in skewed values, but it still makes it possible to compare each population with some certain limitations.

Numerical analyses (descriptive statistics and one-way ANOVA with post-hoc Tukey-tests) were carried out using IBM SPSS 22.0 and R version 4.1 (R Core Team 2015). The figures presented in this paper were prepared with R and ggplot2 package (version 3.4.2). Calculation of deterministic growth rates (λ) was done in R with package popbio (version 2.7, Stubben and Milligan 2007).

Results

The number of individuals present at a sample site showed notable changes between years (Table 1), which is partly due to individuals which did not appear aboveground in some years (we found such plants in all sites and in all years after the baseline survey), but more influenced by the high fluctuations in the number of seedlings each year. In 2020 there were no seedlings present in the surveyed quadrats, we found only a very few around the sample sites. In contrast, at most sites, in 2021 a high number of seedlings appeared which was followed by a lower, but still considerable amount in 2022. Only the NR site showed a different trend, where the number of seedlings was higher in 2022 (Fig. 1).

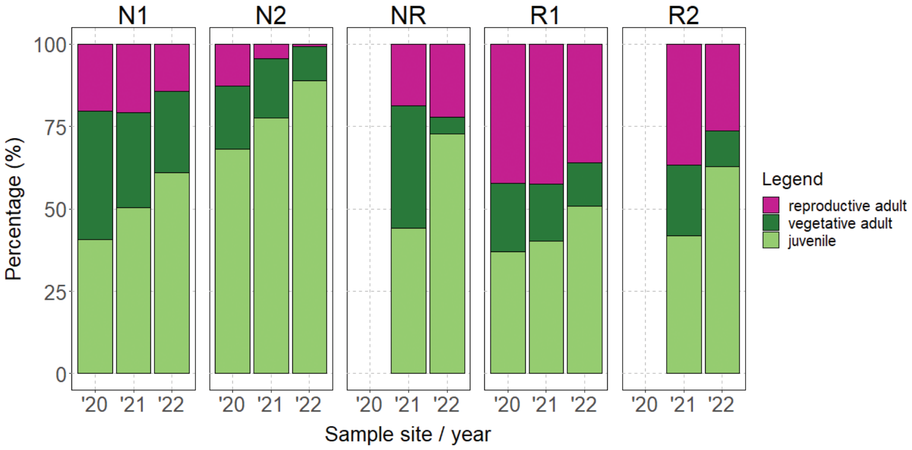

Figure 1. Population structure at each sample site between 2020 and 2022, seedlings included.

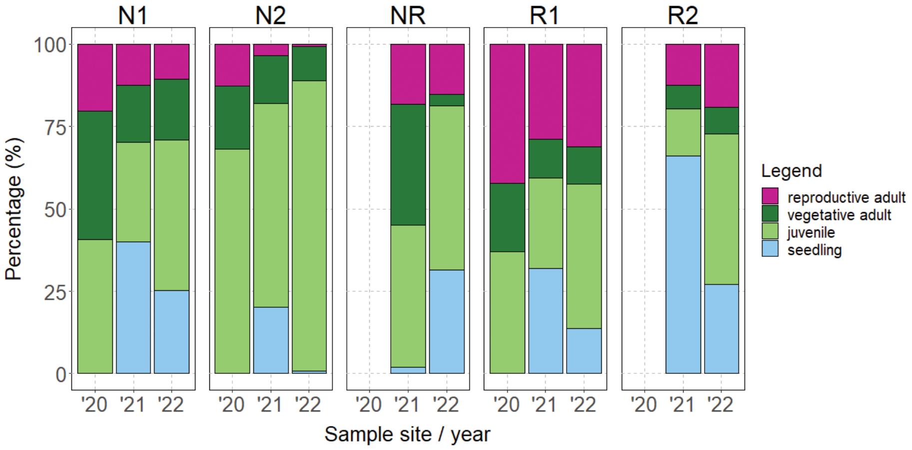

Figure 2. Population structure at each sample site between 2020 and 2022 with the exclusion of seedlings.

With the highly fluctuating seedlings category omitted, the population structure at each sample site during the three years show some uniform trends (Fig. 2). The fraction of juvenile individuals gradually increased at all sample sites during the study years. This was mainly caused by the substantial increase in the number of juvenile individuals, but in the case of N1, N2 and NR the decrease in the numbers of vegetative and reproductive adult individuals also contributed to this rearrangement in population structure. At sites with *Robinia pseudoacacia* dominance (R1, R2) the proportion of reproductive adults in the population and the ratio of vegetative/reproductive adults were notably higher than at sites with native tree species (N1, N2) for each year. The population structure of *E. dens-canis* population in native-*Robinia* mix stands (NR) was similar to native sites (N1, N2) in 2021, while in 2022 it was close to *Robinia* dominated sites (R1, R2).

Although seedling lengths were quite similar in 2021 and 2022 at each sample site (Fig. 3), we found significant differences between different sites in both years. Sites separated into two groups by statistical analyses (one-way variance analysis followed by Tukey-tests), with the R1 and R2 sites together with

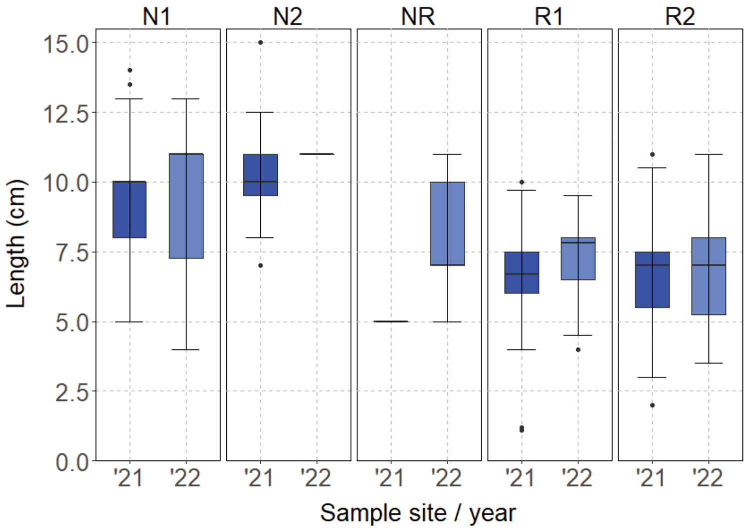

Figure 3. Length of seedlings at each sample site between 2021 and 2022.

Table 2. Number of cases (N) and means of vegetative characteristics of different age-states at the five sample sites between 2020 and 2022. The grouping results of ANOVA followed by Tukey tests is indicated in uppercase.

	Year	N1		N2		NR		R1		R2	
		N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Seedling length (cm)	**2020**	0	–	0	–	n.a.	n.a.	0	–	n.a.	n.a.
	2021	74	93.74^{b}	35	98.86^{b}	2	50.00^{a}	63	66.83^{a}	266	66.47^{a}
	2022	26	96.35^{b}	0	–	27	79.82^{a}	28	71.29^{a}	99	70.95^{a}
Juveniles leaf area (cm^{2})	**2020**	40	6.17^{a}	75	6.10^{a}	n.a.	n.a.	36	15.45^{b}	n.a.	n.a.
	2021	56	6.50^{ab}	107	4.95^{a}	45	6.42^{ab}	60	8.72^{b}	57	6.81^{ab}
	2022	48	2.50^{a}	128	3.06^{a}	43	6.23^{b}	89	4.13^{a}	167	2.43^{a}
Vegetative adult leaf area (cm^{2})	**2020**	38	28.10^{a}	21	27.50^{a}	n.a.	n.a.	20	60.69^{b}	n.a.	n.a.
	2021	32	34.39^{b}	25	25.13^{a}	38	35.33^{b}	17	48.85^{c}	29	47.56^{c}
	2022	19	17.70^{a}	15	26.78^{ab}	3	46.95^{c}	23	39.14^{bc}	29	34.27^{bc}
Reproductive adult leaf area (cm^{2})	**2020**	19	44.92^{a}	9	35.44^{a}	n.a.	n.a.	37	60.82^{b}	n.a.	n.a.
	2021	22	43.46^{ab}	5	29.39^{a}	19	51.69^{bc}	56	52.74^{bc}	50	71.33^{c}
	2022	8	28.63^{a}	0	–	3	55.48^{ab}	58	58.01^{b}	64	68.53^{b}

NR forming one ($p = 0.201$ in 2021 and 0.177 in 2022) and N1 and N2 sites forming the other in 2021 ($p = 0.966$). In 2022 the latter group consisted of only N1 since the absence of seedlings at N2 in that year (Table 2).

Leaf areas of juvenile plants showed a similar decreasing trend in all sample sites during the three years (Fig. 4), most remarkably in R1. Although in 2021 and 2022 there were marked differences between the maximums of leaf areas, their means were much closer to each other (Table 2).

In the case of adult individuals (both vegetative and reproductive), their leaf areas were the largest at R1 and R2 sites, followed by NR, while the two sites with natural habitats had the smallest leaves in all three years. The extent of these differences varied between years (Table 2).

In all three sample sites which were surveyed over the three years, in 2022 we found adult individuals (10 vegetative and 2 reproductive) which were not

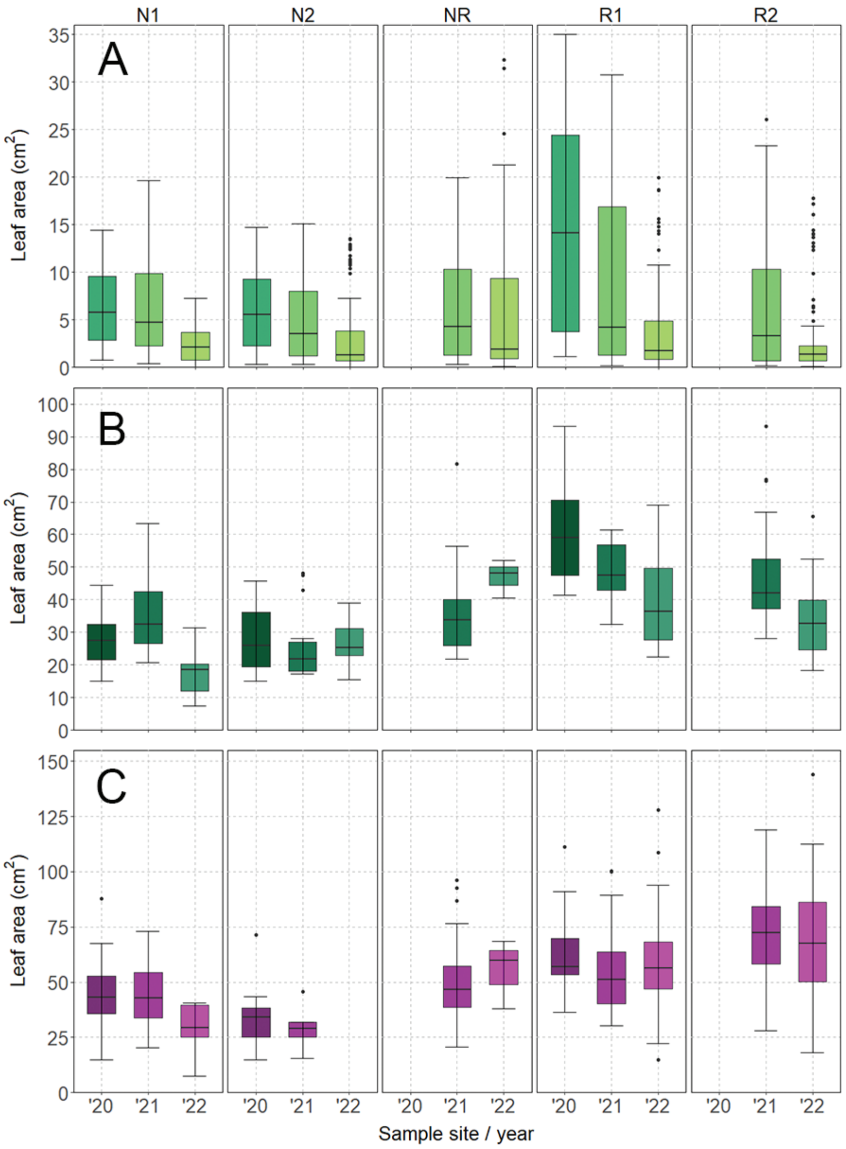

Figure 4. Leaf area of juvenile (**A**), vegetative adult (**B**) and reproductive adult (**C**) individuals at each sample site between 2020 and 2022.

recorded before, which suggests that *E. dens-canis* could become dormant for at least two years. Transition matrices also reveal that all age-states are prone to dormancy (Suppl. material 1). At sites with *Robinia*, reproductive plants had much higher tendency to flower again in subsequent years (32–86%) and juvenile plants developed into vegetative adult category in higher percentage. In terms of recruitment we did not observe notable trends as the rate of recruitment at each site varied greatly in some cases. The overall growth rates (λ) of the populations also show some differences between sites (Table 3). During the first transition (2020–21) the one site with *Robinia* cover (R1) had the lowest growth rate by far (0.792) of the three surveyed sites, the two populations situated in native forest stands (N1, N2) had a λ close to 1 which is a characteristic of stable populations. However, during the 2021–2022 transition, this trend reversed, the two populations under *Robinia* (R1, R2) had the highest growth rate, while the ones under native stands (N1 and N2) had the lowest (still close to 1) and the mixed stand (NR) had a λ between the values of these two groups.

Table 3. Projected population growth rates (λ) of each site.

Sample site	2020–2021	2021–2022
Native 1 (N1)	0.929	1.046
Native 2 (N2)	0.978	1.001
Native-Robinia mix (NR)	–	1.067
Robinia 1 (R1)	0.792	1.128
Robinia 2 (R2)	–	1.365

Discussion

Between 2020 and 2022 the number of individuals surveyed in the quadrats at sites with native tree species were more constant than at sites with *R. pseudoacacia*. These differences were mainly caused by the more pronounced recruitment in some years at the latter sites. Besides this difference in most sites the gradual increasing proportion of juvenile individuals in the populations was observed. Comparing the demographic characteristics of the studied populations with literature data we found that at the N1 and N2 site the demography of the *Erythronium* populations is very similar to what Kricsfalusy et al. (1995) described as “left-sided”, which means the dominance of young individuals, the highly dynamic seedling category and marginal proportion of adult plants in the populations. The populations situated in mixed forests showed somewhat similar trends, but with the growing proportion of *Robinia* in the forest stands (NR, R2, R1) the demographic distributions of the *Erythronium* populations gradually changing towards the “right-handed” state described by Kricsfalusy et al. (1995) with the difference that the seedling category is also significant and highly fluctuating. Despite the fact that prolonged dormancy (not producing aboveground shoots during one or more growing seasons even though the plant is still alive) has been reported in several genera of the lily family (Tyler and Borchert 2002; Delvallée et al. 1990; Miller et al. 2004; Tatarenko 2019), we found no mention in the literature about the observation of this phenomenon regarding any species of the genus *Erythronium*. We found only mention of ‘senescent’ plants by Kricsfalusy et al. (1995) as old, dying bulbs without leaves. In contrast, at all sites in both years after the baseline survey we found individuals from multiple age-states which were not present aboveground in the previous one or two years. The relatively high number of such plants indicates that this phenomenon is most likely not only the result of occasional damage occurring to plants, but a natural characteristic of the species.

The average size of adult individuals was significantly greater in the populations under *Robinia* than at the sites in native forests in all three years. Such a difference would hardly be explained by the location, exposure or geology of the sample sites, and it is therefore assumed that differences in the composition of the forest stands in the sample sites may be the cause of this phenomenon. It is known that *R. pseudoacacia* can significantly increase the amount of nitrogen available for uptake by *Rhizobium* bacteria (Rice et al. 2004), often resulting in a significant increase in the leaf area of species in its understory (Guo et al. 2021).

Although the mean of leaf areas of juvenile plants decreased in all areas throughout the three years, the lower limit of the leaf area of reproductive individuals did not change as much. Thus, even smaller plants became adults in each following year, which could be the result of gradual environmental changes or just a coincidence in weather patterns.

One transition does not tell much about recruitment or growth rate of the population (Crawley 1990), as these values are highly variable, likely depending on environmental factors among others as well. However, it is notable that although the λ value of the one population under *Robinia* (R1) during the 2020–21 transition was the lowest among all sites, in the next transition the λ values of *Robinia* stands were the highest by far. The populations situated in native stands had a similar growth rate (close to 1) in both transitions. As in 2020 we could not find any seedlings in any surveyed quadrats, and in 2021 and 2022 there were numerous in some sites, these great differences in recruitment rates likely caused by annual variances in weather. Great differences between recruitment rate at different sites have been observed in the case of this species (Kricsfalusy et al. 1987) but in our study these contrasts cannot be attributed to differences in geographical conditions as all our study sites are situated in the same landscape and the NR site which is just 50 metres in distance from the N1 site shows the highest similarities with the R2 site (9 km away). The low λ value of R1 in the 2020–21 transition means that the population without recruitment is rapidly declining even with the mortality rates omitted during the calculation of deterministic growth rates. In the 2021–22 transition the presence of recruitment made λ considerably higher than the 2020–21 value, which suggests that in *Robinia*-dominated habitat type *E. dens-canis* is highly relying on recruitment and these populations could be characterized by a more dynamic demography which is generally uncommon in long-lived herbaceous species (Eriksson 1989), but not unknown for *E. dens-canis* (Kricsfalusy et al. 1995).

In contrast with the R1 and R2 populations, the *E. dens-canis* populations situated in native forests have more stable demographical characteristics, their growth rate was close to 1 with or without recruitment. The *Robinia*-native tree species mixed stand (NR) showed an intermediate growth rate, which also suggests that the greater the ratio of *Robinia* in a stand the greater is its effect on the *Erythronium* population as well. Since these values were also calculated without the mortality rates of most age-states, the growth rates are likely lower. It could easily change the growth rate of populations in native forest stands from stable or slowly growing to a declining category.

Conclusions

Nitrogen pollution originating from agricultural activities (fertilization, production of leguminous crops) is a common potential threat to biodiversity, especially to endangered species (Hernández et al. 2016). The habitat-transforming capability of *R. pseudoacacia* is already known, which is mostly caused by increasing nitrogen input into the ecosystem (Buzhdygan et al. 2016; Vítková et al. 2016), however its effect on endangered plant species is a less studied topic. Our results agree with previous studies (Buzhdygan et al. 2016) that *R. pseudoacacia* accelerates environmental processes, as *E. dens-canis* populations under *R. pseudoacacia* show much more volatile deterministic growth rates and higher turnover compared with populations situated under native tree species and this effect was greater in sites with greater ratio of *Robinia* in the tree cover. Such variations in the life cycle of plants may support the dynamic heterogeneity of populations which in turn ensures their stability in different environmental conditions and management regimes (Kricsfalusy 2016). In most aspects, where we found differences among populations, the extent of these differences was in correlation with the proportion of *R. pseudoacacia* in the forest stands, as the population at the NR site often showed intermediate characteristics between the native and *Robinia*-dominated stands.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: JB, BP. Data curation: VL, BP, EAB. Formal analysis: BP. Funding acquisition: JB. Investigation: BF, EAB, BP, VL. Methodology: JB. Project administration: JB. Resources: EAB. Supervision: JB. Validation: VL, BF. Visualization: BP. Writing – original draft: BP, JB. Writing – review and editing: JB, BP.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Transition matrices of *Erythronium dens-canis* populations between 2020 and 2022

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Data type: docx

Explanation note: Transition matrices of *Erythronium dens-canis* populations in five sample sites (2 in native forests, 2 in *Robinia pseudoacacia*-dominated stands and 1 in mixed, native-*Robinia* stands) in Hungary between 2020 and 2022.

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Research Article

Prioritising areas for conservation within Tropical Important Plant Areas of the British Virgin Islands, Caribbean

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Abstract

Oceanic islands are particularly vulnerable to the global decline of biological diversity, suffering disproportionally large losses of endemic species. A primary tool for mitigating species loss is the establishment of protected area networks. The 2030 Global Biodiversity Framework target calls for the protection of 30% of Earth's land surface by 2030. This study identifies areas within the Tropical Important Plant Areas network of the British Virgin Islands (BVI), to better inform the expansion of the current protected area network with the objective of conserving the BVI's unique flora. We identified and applied conservation targets for five threatened habitats and 34 species of conservation concern, including four endemic to the BVI. A total of 5,248 georeferenced plant records for the 34 species collected through decades of collaborative work between the National Parks Trust of the Virgin Islands and the Royal Botanical Gardens, Kew, along with the distribution of five threatened habitats were used within the decision support system MARXAN to identify four spatial portfolios to guide the expansion of the BVI's current protected area network. Highlighting the need to expand the current (2007–2017) Protected Areas System Plan in the BVI, we found that the current Plan only covers 15% of terrestrial land and does not meet the conservation targets for plants and habitats. The portfolios identified in our analysis efficiently expand the current Protected Areas System Plan to strategically expand coverage for all conservation features, with two main portfolios reaching all defined conservation targets for protection. Notably, to evaluate options not requiring land purchase, we extracted areas within state-owned Crown land from two main identified portfolios and found that the two Crown land-portfolios could protect 28% and 23% of the BVI, respectively, while meeting the targets for most plants of conservation concern. However, to reach 30% land protection and meet the conservation targets for all plant species, including endemics, private land would need to be considered for this inclusion within the protected area network. Our results provide science-based guidance for the selection of candidate protected area expansion sites that include threatened plants and habitats for reaching the 2030 Biodiversity Framework targets. While systematic conservation planning can provide guidance on protected area expansion, it is important to evaluate and prioritise conservation

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actions, based on multiple solutions and available resources. We recommend similar approaches are applied more broadly throughout the Caribbean and other archipelagos across the world.

Key words: Caribbean, hotspots, MARXAN, protected areas, threatened habitats, threatened plant species

Introduction

Anthropogenic demand for space and resources has led to biodiversity loss, primarily driven by habitat loss and land-use changes (Chaudhary et al. 2015). Notably, the Convention on Biological Diversity (CBD) found habitat loss and degradation to be the greatest threats to the world's biodiversity (Secretariat of the Convention on Biological Diversity 2010). Without actions to reduce the drivers behind loss of biodiversity, one million species are at risk of extinction, several within decades and the acceleration of species loss will continue which has been estimated to being ten to a hundred times larger than the average over the past 10 million years (IPBES 2019). Thus, conserving adequate and representative habitat space is one of the most crucial challenges we are faced with in environmental management policies (Tjørve 2010). Threshold estimates have led to the suggestion that conservation action is needed when the amount of remaining functional habitat falls below ~ 30% (Banks-Leite et al. 2014). Responding to this, the 2030 Global Biodiversity Framework sets a minimum conservation target of 30% of Earth's terrestrial, inland water, marine and coastal areas by 2030. It states that areas crucial for biodiversity and ecosystem functions and services should be prioritised. This involves effective conservation as well as management of ecologically representative, well-connected and equitably governed systems of protected areas or employing other effective area-based means (CBD 2022). Despite the expansion of protected areas over the past decades (Johnson et al. 2017), the Protected Planet Report 2020 claimed that terrestrial and inland waters protection stands at 17% of the terrestrial land surface (Bingham et al. 2021), emphasising the need for active measures towards protecting additional land to reach the 2030 Global Biodiversity Framework targets (CBD 2022).

Landscape connectivity is crucial for the exchange of species and genes between habitats (Daigle et al. 2020). The challenge is not only to declare sufficient land for conservation, but also design efficient protected area networks that capture key biodiversity dynamics. Notably, it has been suggested that protected areas that are small, isolated or poorly designed do not perform well when it comes to biodiversity conservation (Dudley and Parish 2006). The question of efficient protected area network design is summarised in the ongoing Single Large Or Several Small (SLOSS) areas debate (Tjørve 2010). Fukamachi et al. (1996) argued that more species could be supported with several small areas, but few large areas could preserve a greater number of rare species. Greater diversity of habitats can be reached with several small areas, but this results in extensive boundary lengths, which can require larger management costs, reduced connectivity and more edge effect (Ball et al. 2009). Edge effect could result in areas becoming more impacted by invasive species and extreme weather conditions (Saunders et al. 1991) and, in general, edges are unfavourable for a majority of species, although more generalised species benefit from edge effect (Ardron et al. 2010). Ardron et al. (2010) further suggest that compact reserve systems, with less edge-to-area ratio, benefit from a smaller number of reserves, lowering management and transactional costs. These observations underline that there is no single best solution in designing protected area networks and the importance of choosing parameters wisely, as well as prioritising between outcomes, is recommended for effective conservation planning.

Due to the high ratio of endemic species and impacts caused by invasive species, islands are critical places to prioritise conservation and mitigation efforts. Although oceanic islands comprise only 5.3% of the Earth's landmass, they are home to 17% of plant, 19% of bird and 17% of rodent species, resulting in islands having 3.6 times as many species per km^2 than continental areas (Tershy et al. 2015). Importantly, many island species are endemics that are found nowhere else on earth (Whittaker et al. 2017). Moreover, 37% of species listed as Critically Endangered (IUCN 2022) occur on islands (Tershy et al. 2015). However, Whittaker et al. (2017) found that islands have contributed to more than 60% of the global terrestrial species extinction since 1500 CE.

The Caribbean Region is considered a global biodiversity hotspot, with many endemic species and large rates of habitat loss (Myers et al. 2000). The British Virgin Islands (BVI) lies within the Puerto Rican Bank Floristic Province, interconnecting the Greater Antilles and the southern inner arc volcanic islands of the Lesser Antilles (Dalsgaard et al. 2014). The BVI stretches over 153 km^2 and consist of more than 50 rocks, cays and islands, of which 16 are populated. Most of the human population in the BVI live on Tortola, which is also the largest island, with the highest peak, Sage Mountain, rising to an altitude of 543 m (The BVI TIPAs National Team 2019b). In area, Tortola is the largest (57 km^2) followed by Anegada (40 km^2) and Virgin Gorda (22 km^2) (The BVI TIPAs National Team 2019a). In September 2017, the BVI experienced catastrophic damage from category five Hurricane Irma, which emphasised the need to account for climate change mitigation when designing protected area networks (Gore et al. 2019). The BVI has approximately 648 native plant species, including four endemics. Many plant species in the BVI are threatened (Dani Sanchez et al. 2021) and face several threats, including invasive species, pests and diseases, grazing and urbanisation (Bárrios et al. 2021). In addition, island species are under greater pressure in coastal areas due to land clearing activities to accommodate expanding human needs, as sea levels rise (The BVI TIPAs National Team 2019b). The four endemic plant taxa on the BVI are: *Vachellia anegadensis* (Britton) Seigler and Ebinger, *Metastelma anegadense* Britton, *Pitcairnia jareckii* Proctor and Cedeño-Mald. and *Senna polyphylla* var. *neglecta* H.S. Irwin and Barneby (The BVI TIPAs National Team 2019a; Bárrios et al. 2021). All four endemics face extinction risk in the wild (IUCN Standards and Petitions Committee 2019).

To advance the identification and protection of Important Plant Areas (IPAs) in tropical regions and mobilise both current and newly-acquired plant data, The Tropical Important Plant Areas (TIPAs) programme (https://www.kew.org/science/our-science/projects/tropical-important-plant-areas) was launched in 2015 (Darbyshire et al. 2017). In 2018, a total of 18 TIPAs were identified within the BVI, based on decades of joint botanical activity between the Royal Botanic Gardens, Kew and the National Parks Trust of the Virgin Islands (Dani Sanchez et al. 2021). The identification of TIPAs within the BVI provides a framework for shaping future policies and providing information for the protection and conservation of regional and national biodiversity, as well as endemic plant species and their habitats (The BVI TIPAs National Team 2019a). Although the BVI has declared 21 terrestrial National Parks, the identification of TIPAs showed that most native flora are under-represented within the current protected area network (The BVI TIPAs National Team 2019b; Dani Sanchez et al. 2021). Dani Sanchez et al. (2021) further argued that as the TIPAs network was both too large to have all areas protected and that some TIPAs were on private lands, further analysis should be conducted to find areas within the TIPAs to declare as protected areas.

One way of expanding protected area networks is through the six-step approach of systematic conservation planning (Margules and Pressey 2000; Wiersma and Sleep 2016). The first three steps provide information for the expansion of protected areas, by firstly collecting biological data, secondly identifying conservation goals and conservation features and thirdly by reviewing the performance of the current areas. The fourth step is the actual reserve selection. The final steps are to implement the conservation actions and provide on-going maintenance (Margules and Pressey 2000; Wiersma and Sleep 2016). The reserve selection practice can be done with various decision-support systems and reserve-selection algorithms, where MARXAN (Ball et al. 2009) is believed to be the most used worldwide, both for terrestrial and aquatic ecosystems (Ardron et al. 2010; Schill and Raber 2011; Wiersma and Sleep 2016). MARXAN has been used in the designation of the marine protected areas of the BVI and protected areas in Jamaica, as well as the wider Caribbean Basin (Huggins et al. 2007; McPherson et al. 2008; Woodfield-Pascoe et al. 2013). MARXAN identifies the overall best or most efficient portfolio of planning units (Huggins et al. 2007), while seeking to meet conservation goals and minimise costs (Schill and Raber 2011). Systematic conservation planning using MARXAN can, thus, help design a biologically-resilient and redundant network of protected areas.

This study supports the 2030-Global Biodiversity Framework (CBD 2022) by identifying important areas for expanding terrestrial protection within the TIPAs of the BVI, based on a synthesis of a comprehensive species dataset and habitat maps that are integrated into the MARXAN reserve selection tool. As suggested by Cowling et al. (2003), our study intertwines expert knowledge and algorithms into conservation practice and, as suggested by Dani Sanchez et al. (2021), we provide information for the expansion of the protected areas of the BVI within the TIPAs. Our results have implications for the conservation of Caribbean biodiversity and will guide the strategic expansion of new protected areas across the BVI and thereby help conserve threatened plant species, including the BVI's four endemic plant taxa.

Methods

Conservation features and targets

We selected 34 plant species and five threatened habitats as conservation features for the analysis. The 34 plant species originated from a list of 35 Species of Conservation Concern as defined by the UKOTs Team (2021), based on their restricted range and endemism (The BVI TIPAs National Team 2019a; Dani Sanchez et al. 2021) which were used for the identification of TIPAs in the BVI. One plant species, *Picrasma excelsa* (Sw.) Planch., had no recorded locations, thus the analysis did not consider this species. For the 34 Species of Conservation Concern, a total of 5,248 high accuracy (+/- 10 m) georeferenced location records were included in the analysis (Appendix 1: Table A1). Data were available for terrestrial seed plants only. All plant records were treated as presence/absence data, as the population size was not available for all records. For full details on data collection, see Dani Sanchez et al. (2021).

In addition to geographical plant records, we examined five threatened habitats. The BVI national list of threatened habitats consists of coastal shrubland, dry salt flats, mangrove, semi-deciduous gallery forest and upland evergreen forest (The BVI TIPAs National Team 2019a). These habitats meet the TIPAs criteria of being either natural or semi-natural habitats that support higher vascular plants and each currently cover less than 10% of BVI terrestrial land or is present in three or fewer islands (Darbyshire et al. 2017). Further, to be considered a threatened habitat, a continuous decline of the habitat has to have been observed, estimated, inferred or projected for the BVI (The BVI TIPAs National Team 2019a). Threatened habitats were mapped using QGIS (QGIS.org 2021) from two satellite-derived land-cover datasets: (1) The BVI Habitat map 2020 (Scarth and Pike 2020) derived from Sentinel-2 imagery was used primarily, due to its high spatial resolution of 10 × 10 m; (2) the Landsat-7 dataset in Kennaway et al. (2008) with a 30 × 30 m spatial resolution was used for the semi-deciduous gallery forest class as it was not present in The BVI Habitat map 2020.

Authors from the British Virgin Islands TIPAs National Team and University of Copenhagen held workshops to determine conservation targets for all conservation features (a percentage to protect for each plant population or habitat extent), making use of their collective practical knowledge on the conservation features, their spatial distributions and levels of threat. The threatened habitats were assigned a conservation target of 30% each in accordance with the 2030 Global Biodiversity Framework (CBD 2022). Targets for each conservation feature are shown in Appendix 1: Tables A1, A2.

Reserve selection through MARXAN

We investigated where to strategically expand the current protected areas of the BVI within the boundaries of the TIPAs (The BVI TIPAs National Team 2019b; Dani Sanchez et al. 2021), using the decision-support system MARXAN (Ball et al. 2009). MARXAN seeks to identify near optimal solutions through a simulated annealing algorithm, by selecting areas (portfolios of planning units) that efficiently achieve conservation targets, while minimising costs (Ball et al. 2009). The cost parameters were provided by an overall boundary length and an Environmental Risk Surface (ERS; see below). The boundary length is controlled by setting a Boundary Length Modifier (BLM), which allows the user to change the compactness of the final solutions and thereby address the SLOSS approach (Ardron et al. 2010). Lower BLM values will result in the BLM to have less influence and, therefore, select portfolios with increased fragmentation (Schill and Raber 2011). It is important to note that since MARXAN applies a simulated annealing algorithm, it produces several near-optimal solutions (portfolios of planning units to protect) and a summed solution, rather than one single best solution. In terms of practical conservation planning, these portfolios would then have to be evaluated with respect to practical and/or political perspectives (Ball et al. 2009).

Planning units and MARXAN analysis

A grid of planning units with a size of 30 × 30 m was used, based on the fine scale of available input data (accuracy of +/- 10 m) and the relatively small land areas of the BVI and the TIPAs, making a compromise between data scale and practicality of implication and management (Schill and Raber 2011; Mo et al. 2019). We used the borders of the BVI Habitat map 2020 (Scarth and Pike 2020) to create the grid surface of planning units, removing only the Open water class to cover all land areas of BVI, as well as mangrove habitats. No planning units were clipped to the coastlines nor political boundaries within the BVI, as this would result in small, fragmented planning units. The final area of planning units covering the BVI (~ 166 km^2) was, therefore, slightly larger than the official land area of the BVI (~ 153 km^2) (The BVI TIPAs National Team 2019b).

In order to design a biologically resilient network of protected areas, the planning units were divided into three geographic strata, named after the largest islands in each specific strata: Anegada, Tortola and Virgin Gorda (Fig. 1). The introduction of these strata, as opposed to running MARXAN on all of the BVI at once, were used to drive the optimal portfolio selection across the archipelago rather than being centred in a single area of the BVI. This design follows the previous marine protected area analysis within the BVI, which also employed three strata to force the selection of conservation features beyond Anegada's large reef system (Woodfield-Pascoe et al 2013). Similarly, the use of strata in a terrestrial planning context would influence portfolio selection beyond Anegada's rich terrestrial biodiversity. The use of strata also allowed different application of adaptive targets to plants which were more abundant in one stratum than another. MARXAN was run individually on each stratum and results were combined into collective portfolios for all of the BVI. A total of 184,791 planning units were analysed and assigned a status of "available" for MARXAN selection if they fell within the TIPAs boundaries and a status of "unavailable" for those outside the TIPAs boundaries. This allowed the prioritisation of areas within TIPAs for future protection. Planning units already part of the Protected Areas System Plan were assigned a "locked" status to ensure inclusion in the portfolios. Each planning unit was assigned a cost provided by an ERS (see below). For each planning unit, we calculated the total amount of each conservation feature within, either in number of occurrences (plant taxa) or area (habitats).

Protected area solutions that are too fragmented can be difficult to implement and are ecologically less functional. Following a BLM sensitivity analysis, we settled on a BLM value of 0.1. This achieved an optimal degree of planning unit clustering and delivered a smaller total area with accepted initial coverage and connectivity. For each of the three strata of the BVI, 100 runs were executed using 1,000,000 iterations per run. MARXAN generates two standard outputs: the best solution, as well as a summed solution. The best solution portrays the portfolio of planning units with the lowest cost score, found in all the good combinations of planning units (portfolios) selected by MARXAN (Ball et al 2009). As we executed 100 runs, the best solution was found within one of these. The latter portrays the selection frequency, i.e. the number of times each planning unit was selected in each of the 100 runs, which Ardron et al (2010) suggests being one of the most useful outputs. We therefore examined two main portfolios of planning units; (1) the best solution output (hereafter 'Best Solution'); (2) the planning units with a selection frequency of a 100, thus a portfolio of planning units that were selected in each of the 100 MARXAN runs (hereafter 'SF100'). From those portfolios, we extracted two Crown land portfolios: (3) the best solution output that fell within Crown lands, thus not private land (hereafter 'Best Solution CL'); and (4) the planning units with a selection frequency of 100 that fell within Crown lands (hereafter 'SF100 CL'). The extraction of overlapping areas within the main portfolios and Crown land allowed us to examine portfolio areas that would not require land purchase. Planning units within the current Protected Areas System Plan were included in all four portfolios.

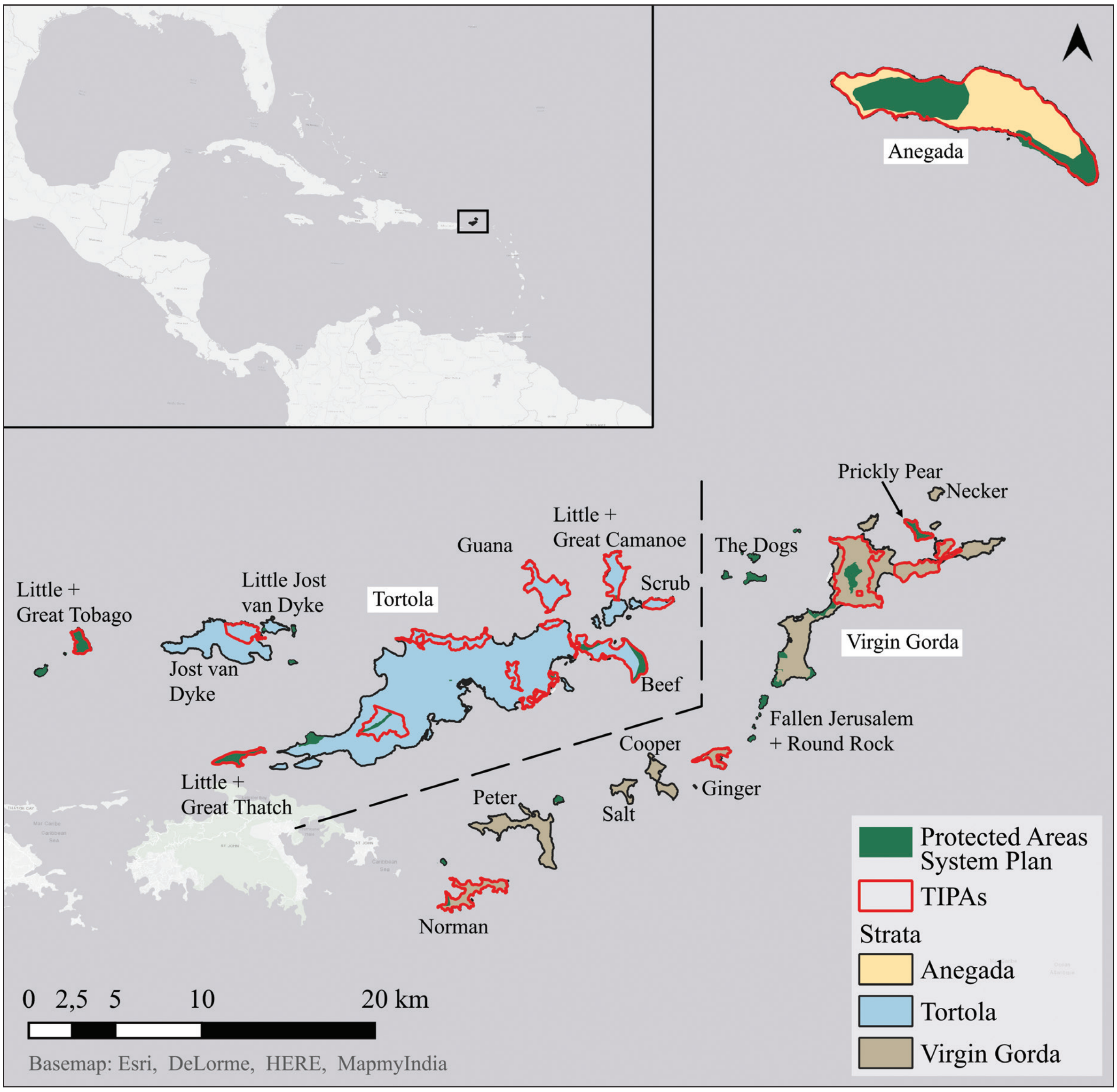

Figure 1. Study area. Reference map showing the location of the BVI in the Caribbean Sea, as well as the strata division, named after the largest island in each specific area: Anegada; Tortola; and Virgin Gorda. Tropical Important Plant Areas (TIPAs) are shown, as well as areas within the current Protected Areas System Plan. A dashed line is added to show the distinction between islands allocated to the Tortola and Virgin Gorda strata.

Environmental Risk Surface as a cost parameter

To estimate the level of threat to the conservation features and to provide MARXAN with a cost parameter beside the BLM, an Environmental Risk Surface (ERS) was produced to assign a cost value to each planning unit under consideration (Schill and Raber 2011). We assessed the locations of possible threats to biodiversity (e.g. an airport). The threats were drawn as polygons in GIS using a combination of OpenStreetMap layers (OpenStreetMap contributors 2021), the BVI Habitat map 2020 (Scarth and Pike 2020) and ground reference information acquired from Google Maps satellite imagery (Google 2021a, b), to capture the outlines of the threat features. Each threat was assigned an intensity score and an influence distance (Appendix 2: Table A3, Fig. A1). The latter expresses the intensity of the threat outside the immediate area, with the intensity decreasing the further away from the threat, until it no longer poses a threat to the conservation features. We examined two published assessments that applied the ERS methodology in Jamaica (McPherson et al. 2008) and across the Caribbean (Huggins et al. 2007) to understand the relationships between threat intensity scores and decay distances. In MARXAN, all costs are seen in relation to the other costs, so intensity scores in the modelled ERS are within a relative scale (Schill and Raber 2011). We selected four levels of intensity scores within the range of 0–99: 99 (high), 66 (medium), 33 (low) and 10 (very low). The scale was chosen to reflect fractions of thirds, followed by a lower intensity score of 10 to quantify the lower intensity for agriculture (Huggins et al. 2007; McPherson et al. 2008). To ensure MARXAN only selected planning units that were crucial for the portfolios, the minimum cost was set to a very low, non-zero, value (e.g. 0.01) to all planning units that did not overlap with the ERS modelled values. For each individual threat feature, multiple buffers were drawn outside the borders, until they reached the length of the defined decay distance. For each threat feature with multiple buffers, each buffer was assigned intensity scores from the aggregation of intensity scores and decay distances of the threat and the specific buffer's distance to the threat, with the outermost buffer having the intensity score closest to zero. The ERS layer was turned into vector points in GIS, reflecting the grid surface of 30 × 30 m, with the maximum intensity score assigned for overlapping buffer values. The vector points were ultimately joined to the planning units as cost values.

Results

We identified two main portfolios covering 39% ('Best Solution) and 32% ('SF 100') and two Crown land portfolios covering 28% ('Best Solution CL') and 23% ('SF 100 CL') of the total area of the BVI planning units of 166.3 km^2, respectively (Fig. 2, Table 1; Suppl. materials 1–3). It was possible to reach all defined conservation targets during the analysis for the two main portfolios, 'Best Solution' and 'SF100'. Protection of individual conservation features improved for all four portfolios of planning units compared to the current Protected Areas System Plan, which covers 15% of the total area of the BVI (Table 1).

The current Protected Areas System Plan of the BVI meets the conservation target for only 12 plant species and two habitats, but meets none of the targets for endemic species. The current plan covers 15% of the terrestrial land area of the BVI and 31% of the combined area of threatened habitats. The main 'Best Solution' and 'SF100' portfolios both cover more than 30% of the BVI (39% and 32%, respectively). Despite the 'Best Solution' including more planning units than 'SF100', the two main portfolios covered the exact same species and threatened

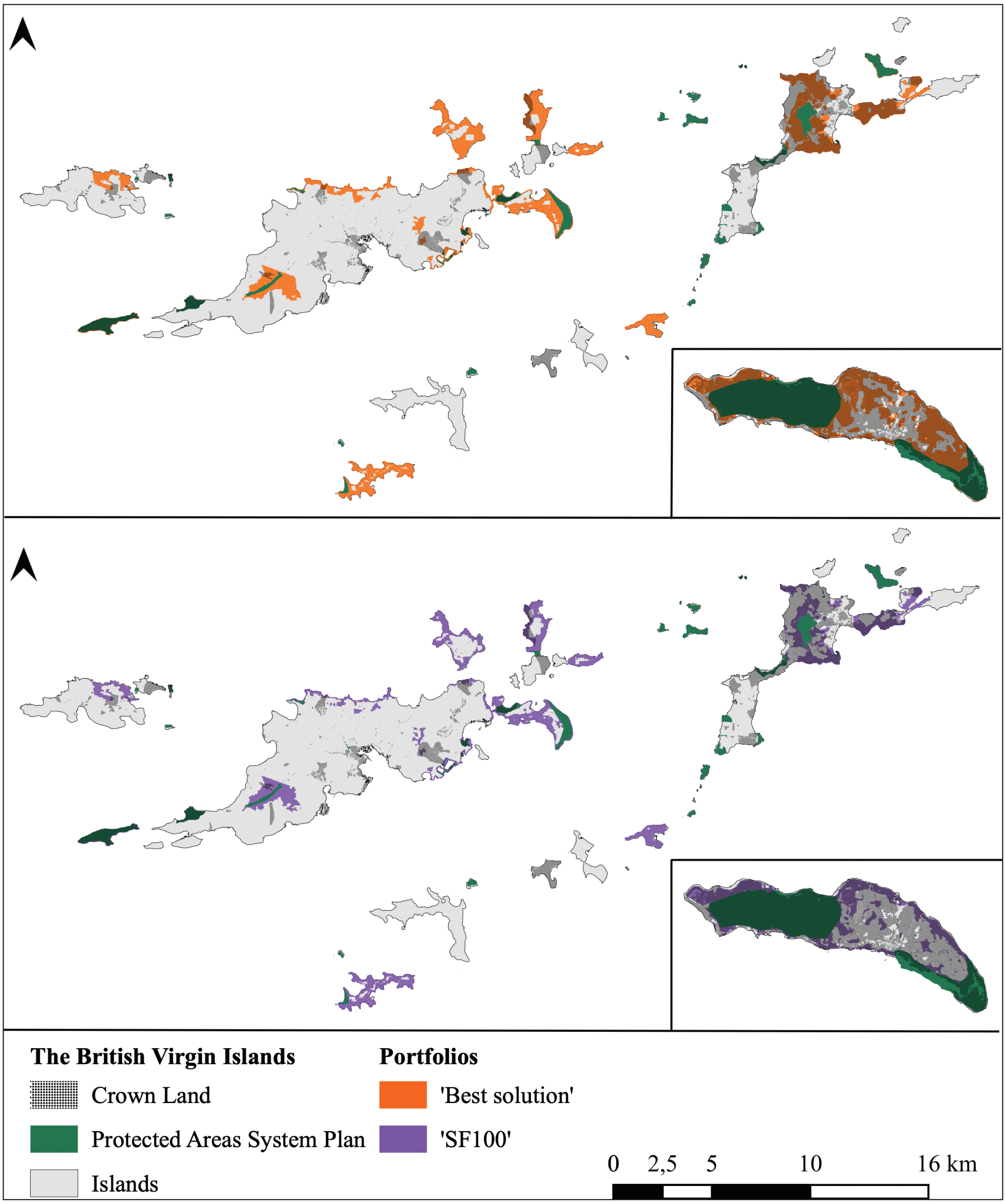

Figure 2. MARXAN portfolios. Maps showing portfolio results for the 'Best Solution' (top) and the planning units with a Selection Frequency of a 100; 'SF100'(bottom). The 'Best Solution' cover almost 10 km^2 more than 'SF100', adding up to 39% and 32% of the BVI, making both portfolios successful in reaching the 2030 Global Biodiversity Framework targets of protecting 30% of the land surface (CBD 2022). See also Suppl. materials 1–3 for a higher resolution overlap between the two portfolios portrayed for each of the three strata of Anegada, Tortola and Virgin Gorda, respectively.

Table 1. Portfolio analysis. The targets met within the four portfolios compared to the current Protected Areas System Plan. The four portfolios were: (1) 'Best Solution', (2) 'SF100', i.e. the planning units selected in all 100 MARXAN runs, (3) 'Best Solution CL', i.e. the overlapping planning units between the best solution and Crown land and (4) 'SF100 CL', i.e. the overlapping planning units between the 'SF100' portfolio and Crown land. The total area of planning units covering the BVI was 166.3 km^2. One record of the endemic *Zanthoxylum thomasianum* was found outside the TIPAs on Tortola and could, therefore, not be selected by MARXAN. Thus, this species did not reach its target of a 100% coverage within the Tortola stratum. However, as the gap made up by this record is very small and could not be protected within the means of this analysis, we still treated it as a 100% coverage for all endemic species.

Portfolio analysis	Protected Areas System Plan	Main portfolios		Crown land portfolios	
		Best Solution	SF100	Best Solution CL	SF100 CL
Size of portfolios					
Area (km^2)	25.4	64.1	53.3	45.7	37.8
Percentage of BVI	15	39	32	28	23
Conservation features covered by portfolios (%)					
Plant records (n)	26	90	90	70	70
Endemics plant records (n)	30	100	100	95	95
Combined area of threatened habitats	31	62	62	44	44
Number of conservation features where conservation targets were met					
Plant Species of Conservation Concern	12	34	34	27	27
Endemics	0	4	4	1	1
Habitats	2	5	5	4	4

habitats (Table 1; Appendix 3: Tables A4, A5). Notably, the 'Best Solution' and 'SF100' cover 90% of all plant records and 62% of the total area for threatened habitats. Both main portfolios reached the conservation targets for all plant taxa and all threatened habitats. When considering the Crown land portfolios, a total of 27 plant taxa, including the endemic *Vachellia anegadensis* and four threatened habitats met their conservation targets. Conservation feature coverage fell to 70% of all plant records and 44% of the total area for threatened habitats. Coverage for eight plant species did not change (Appendix 3: Table A6).

Many conservation features exceeded their conservation targets. For example, 14 plant species in the main portfolios, 'Best Solution' and 'SF100', exceeded their targets greater than 50%, compared to four plant species within the Crown land portfolios (Appendix 3: Table A6). All habitats reached their 30% targets within the main portfolios. Notably, mangrove and salt-pond habitats exceeded their targets by more than 50 percentage points; however, both were already sufficiently represented within the current Protected Areas System Plan (Appendix 3: Tables A5, A6; for example, salt pond was already protected by 94.7% in the current Protected Areas System Plan). This was also the case for 10 plant species, which increased their representation within the main portfolios, even though these targets were already met within the current Protected Areas System Plan (Appendix 3: Table A6). Table 2 elaborates on 10 conservation features that either did not reach their overall targets or failed to reach their targets within the Crown land portfolios.

One conservation feature (*Zanthoxylum thomasianum*) failed to reach its full targets within the main portfolios (Table 2, Appendix 3: Tables A4–A6). *Z. thomasianum* had different targets depending on its location within the BVI and it failed to cover one plant record, which fell outside the TIPAs; however, in the summed results (Table 1), the gap was treated as achieved. Within the

Table 2. Conservation gaps for features that failed to reach their targets within two or four portfolios. The conservation gaps are shown for the current Protected Areas System Plan (current) and the portfolios. Gaps to meet conservation targets are presented once for the main portfolios and once for Crown land portfolios, as they covered the same conservation targets. A negative gap represents the percentage points needed to reach the conservation targets, while a positive gap represents the percentage points that exceed the target. Species with different conservation targets assigned for different strata of the BVI have the relevant stratum indicated in brackets. The IUCN threat status is listed for all plant species (IUCN 2022).

Species (strata of BVI)	IUCN status	Current	Main Portfolios	Crown land portfolios
		Gap (%-point) for portfolios		
Abutilon virginianum Krapov.	EN	-46	4	-36
Guaiacum officinale L. (Anegada)	EN	47	80	80
Guaiacum officinale L. (Tortola)	EN	-70	30	-70
Machaonia woodburyana Acev.-Rodr. (Tortola)	EN	-41	0	-41
Machaonia woodburyana Acev.-Rodr. (Virgin Gorda)	EN	-50	37	18
Metastelma anegadense Britton (Anegada)	EN	-8	60	55
Metastelma anegadense Britton (Virgin Gorda)	EN	-100	0	-75
Miconia thomasiana DC.	NT	-4	70	-4
Pitcairnia jareckii Proctor & Cedeño-Mald.	EN	-10	84	-10
Sabal causiarum (O.F.Cook) Becc. (Anegada)	VU	-90	0	0
Sabal causiarum (O.F.Cook) Becc. (Tortola)	VU	-34	2	-34
Zanthoxylum thomasianum Krug & Urb. (Tortola)	EN	-100	-2	-75
Zanthoxylum thomasianum Krug & Urb. (Virgin Gorda)	EN	-52	38	-25
Upland evergreen forest		-20	13	-10

Crown land portfolios, a total of nine conservation features (eight plant species and one habitat) did not reach their targets. We further note with emphasis on IUCN status, that the two Critically Endangered taxa *Myrcia neokiaerskovii* and *Senna polyphylla* var. *neglecta* (IUCN 2022) reached their conservation targets for all portfolios (Appendices 1, 3: Tables A1, A4).

Discussion

Analysis of the four portfolios

Based on the four resulting portfolios, we found that, by expanding the current Protected Areas System Plan to the main portfolios: 'Best Solution' and the 'SF100', it is possible to reach the conservation targets for all conservation features and meet the 2030 Global Biodiversity Framework commitments for the BVI terrestrial land. The 'SF100' portfolio resulted in a more fragmented and scattered selection of planning units; however, both portfolios covered the same conservation features, suggesting that the additional planning units selected within the 'Best Solution' may only improve connectivity. The additional two Crown land portfolios, which extracted overlapping areas between the main portfolios and Crown land, allowed us to evaluate whether it would be possible to meet conservation targets on areas that do not require land purchase, as approximately 80% of land in the BVI is privately owned. Our results show that we can achieve the 2030 Global Biodiversity Framework targets by solely expanding protected areas into Crown land, although private land would be needed to achieve the conservation targets for all plants, including three of the four BVI endemics (Tables 1, 2). All individual habitat types met their 30% protection targets within the main portfolios; however, this was not the case for the Crown land portfolios as the target was not reached for upland evergreen forest. This further suggests that, to protect 30% of all threatened habitats, protected areas need to expand beyond existing Crown lands. Regardless, the four portfolios offer a significant improvement in protection for all conservation features when compared to the current Protected Areas System Plan and provide evidence and a robust framework for guiding Protected Area expansion.

Many conservation features exceeded their conservation targets (14 plant species and two threatened habitats exceeded their targets by over 50 percentage points) within the main portfolios, as well as four plant species and one habitat within the Crown land portfolios (Appendix 3: Table A6). Although mangrove and salt-pond habitats had already met their targets within the current Protected Areas System Plan, their selected extent also increased within the four portfolios, as well as that for 10 plant species (Appendix 3: Table A6). Given that the primary objective of MARXAN's selection algorithm is efficiency, there is a high likelihood that multiple rare occurrences of conservation features within a planning unit or across neighbouring planning units will be included in the final portfolio. When these conservation features are clustered together in close proximity, the selection of neighbouring planning units can result in targets being exceeded.

Interdisciplinarity between algorithms and experts

If a perfect solution based on perfect input data and variables existed, it might not be the best solution possible due to local politics, land ownership, funding for purchase, stakeholder involvement or current and future land use. MARXAN solutions provide decision support and should be vetted and combined with expert and local stakeholder knowledge to arrive at a solution to implement. This approach is endorsed by Cowling et al. (2003), who suggest the use of systematic conservation planning algorithms should be closely integrated with expert knowledge to combine the strengths of both approaches. The review of MARXAN portfolios by local experts can provide valuable specific insight into landscape context, political environment, biodiversity knowledge and management issues that are not likely captured in a MARXAN portfolio selection (Cowling et al. 2003). Ardron et al. (2010) stated that MARXAN should be used to model a suite of alternative protected area network designs for stakeholders to review and select from, as opposed to giving one definite solution. This is why it is important to model a variety of scenarios whose input parameters originate from stakeholder workshops. Portfolio results should then be presented and reviewed by experts and stakeholders, having the most appropriate portfolio selected or modified.

Prioritising areas for conservation within the TIPAs

TIPAs are often used as a way to highlight protected area gaps and have been used in other areas of the world. Couch et al. (2019) mapped 22 TIPAs in Guinea, West Africa occupying 3.5% of Guinea's land surfaces, highlighting areas of irreplaceable plant diversity and the need to protect against species extinction. Not all 22 areas were protected at the time, although it was stated that if all 22 TIPAs were protected, a vast amount of Guinea's wild plant resources would be safeguarded, including over 60% of threatened plant species and a majority of species and representative areas for nine threatened habitats found across Guinea (Couch et al. 2019). As suggested by The BVI TIPAs National Team (2019a), the integration of the TIPAs into a revised Protected Areas System Plan would be beneficial for the conservation of the 34 Species of Conservation Concern and threatened habitats. Some TIPAs are entire islands or cover large parts of private land and further assessments on these areas within the TIPAs Network are needed to protect a critical percentage of conservation features. Addressing this, the four portfolios presented in this study highlight areas within the TIPAs, where conservation efforts should be prioritised to adequately manage and promote species preservation. Our work provides a science-based framework towards guiding the expansion and subsequent implementation of conservation efforts for the 34 plant species and the five threatened habitats, based on the areas selected for TIPAs (Dani Sanchez et al. 2021) and the Protected Areas System Plan 2007–2017 (Gardner et al. 2008).

Our portfolio results need to be presented as options for experts to review, modify and implement (as per Cowling et al. (2003)). Stakeholders from the National Parks Trust of the Virgin Islands (NPTVI) and Kew need to carefully assess our results and integrate them into a strategic conservation planning design for resource management implementation at relevant scales (Huggins et al. 2007). The Crown land portfolios presented in this work demonstrate how a large number of conservation targets can be achieved without focusing on areas that require land purchase. A recommended first step would be to evaluate areas for conservation within the Crown land portfolios, followed by prioritisation outside Crown lands, paying special attention to conservation features that did not meet their targets, as well as the planning units selected in each MARXAN run; the ‘SF100’ portfolio. Notably, the protection of the BVI endemic plant species would require voluntary partnerships with BVI private landowners. Private protection is possible under the Virgin Islands National Parks Act (Virgin Islands National Parks Act 2006) conservation agreements or under the Virgin Islands Physical Planning Act (Virgin Islands Physical Planning Act 2004) that can protect species within declared Environmental Protection Areas. Moreover, Ball et al. (2009) recommended that planning units identified as having both high selection frequency and a high cost are under immediate threat and should be considered priority areas where closer examination is warranted. Further, in cases where land purchase is not possible, areas could still be recognised within the Protected Areas System Plan as OECMs (other effective area-based conservation measures; IUCN-WCPA Task Force on OECMs (2019)) by engaging with private landowners to promote conservation actions and focused management within their lands.

When expanding protected area networks, we note that management effectiveness guided by a management plan is critical for protected areas to be successful tools for biodiversity conservation (Geldmann et al. 2013). The first target of the 2030 Global Biodiversity Framework focuses on integrating areas in biodiversity-inclusive spatial planning and other effective management activities, to ensure that loss of areas of high biodiversity value is brought close to zero, with respect for local communities and indigenous people (CBD 2022). Watson et al. (2014) stated how a lack of resources, most notably in developing countries, was the primary driver behind weakened performance of protected areas. As protected areas continue to expand, these areas are likely to have increased contact with local communities, which may lead to conflicts (Gooden and ’t Sas-Rolfes 2020). Further, in 2003, CBD found that only 6% of involved countries reported to have adequate resources for carrying out protected area management (Watson et al. 2014). Therefore, we suggest that conservation efforts and any plans to expand protected areas should be followed by an evaluation of adequate resource availability to carry out recommended management actions once the protected areas have been established.

Conclusion

In summary, we demonstrated a science-based and stakeholder-driven framework that identified a representative and efficient protected area network design specific for conserving the unique flora that exists within the British Virgin Islands (BVI). As the BVI is home to 35 plant Species of Conservation Concern (34 with geographical records and, thus, a part of this study), including four which are endemic to the BVI, this work provides a critical foundation to strategically guide decisions on where to expand the current network of protected areas in the BVI. We analysed four portfolios, of which, the two main portfolios fully achieved the conservation targets. It is important to note that the proposed areas within the two portfolios might not be realistically possible to protect due to legal circumstances and resources, such as private ownership. Therefore, we presented two Crown land portfolios, to identify areas within the portfolios that would not require land purchase. However, the number of species and habitats for which conservation targets were met, decreased and most endemics were not well protected under such portfolios. Thus, if all targets are to be met in accordance with the 2030 Global Biodiversity Framework targets (CBD 2022), private areas outside Crown land should be included in a new Protected Areas System Plan or private conservation actions be independently taken if land purchase is not possible. As required by all protected area expansion planning efforts, further assessments and collaboration with stakeholders is recommended to review, approve and implement the necessary conservation efforts. Given the current protected area gaps in the existing Protected Area Systems Plan, our portfolios provide timely insight for conservation decision-makers to act on expanding protection to areas that are urgently needed to safeguard and conserve the rich and unique floral diversity heritage of the BVI. Our approach to evidence-based protected area expansion has wider relevance to the Caribbean Region and the global conservation community, providing a useful template to support nations in their quest to conserve their unique floras and meet international biodiversity commitments.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Each of the authors has fulfilled the following: (1) Contributed significantly to the conception or design of the study; or data acquisition, analysis or interpretation; (2) Participated in drafting the manuscript or provided critical revisions to enhance its content; (3) Given approval for the publication of the final version; and (4) Committed to being responsible for all aspects of the work, ensuring that any enquiries regarding the accuracy or integrity of the work are thoroughly investigated and resolved.

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Data availability

Data were obtained with approval from Virgins Islands Government via the National Parks Trust of the Virgin Islands and Royal Botanic Gardens, Kew. The plant occurrence data used in these analyses were collected over many years by staff of the National Parks Trust of the Virgin Islands and the Royal Botanic Gardens, Kew in collaboration with our regional partners. The complete datasets used are not publicly accessible, in order to safeguard the precise locations of threatened species. Vetted data for the TIPAs can be found on https://tipas.kew.org.

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Appendix 1

Table A1. Conservation features and conservation targets for Plant Species of Conservation Concern.

Species	ID†	IUCN status	Records	Target %
Abutilon virginianum Krapov.	1	EN	83	50
Agave missionum Trel.	2	VU	449	40
Anthurium x selloum K.Koch	3	N/A	17	20
Argythamnia stahlii Urb.	4 (A)	VU	109	20
Argythamnia stahlii Urb.	4 (T)	VU	4	30
Cedrela odorata L.	5	VU	2	50
Croton fishlockii Britton	6	NT	410	20
Erythrina eggersii Krukoff & Moldenke	7	EN	13	50
Galactia eggersii Urb.	8	NT	68	20
Guaiacum officinale L.	9 (A)	EN	18	20
Guaiacum officinale L.	9 (T)	EN	1	70
Ilex urbaniana Loes. ex Urb.	10	VU	23	60
Leptocereus quadricostatus (Bello) Britton & Rose	11	EN	35	100
Machaonia woodburyana Acev.-Rodr.	12 (T)	EN	17	100
Machaonia woodburyana Acev.-Rodr.	12 (VG)	EN	169	50
Malpighia woodburyana Vivaldi	13	VU	304	20
Maytenus cymosa Krug & Urb.	14	EN	186	60
Metastelma anegadense Britton	15 (A)	EN	212	40
Metastelma anegadense Britton	15 (VG)	EN	4	100
Miconia thomasiana DC.	16	NT	53	30
Mitracarpus polycladus Urb.	17	EN	48	70
Myrcia neokiaerskovii E.Lucas & K.Samra	18	CR	59	100
Myrcia neothomasiana A.R.Lourenço & E.Lucas	19	EN	34	60
Peperomia wheeleri Britton	20 (T)	EN	1	100
Peperomia wheeleri Britton	20 (VG)	EN	38	40
Pilea sanctae-crucis Liebm.	21 (T)	EN	21	40
Pilea sanctae-crucis Liebm.	21 (VG)	EN	3	100
Piptocoma antillana Urb.	22	LC	40	10
Pitcairnia jareckii Proctor & Cedeño-Mald.	23	EN	16	10
Psychilis macconnelliae Sauleda	24	NT	78	20
Reynosia guama Urb.	25	NT	207	10
Rondeletia pilosa Sw.	26	NT	106	10
Sabal causiarum (O.F.Cook) Becc.	27 (A)	VU	10	100
Sabal causiarum (O.F.Cook) Becc.	27 (T)	VU	19	50
Senna polyphylla var. neglecta H.S.Irwin & Barneby	28	CR	78	60
Tillandsia x lineatispica Mez	29	N/A	23	20
Tolumnia prionochila (Kraenzl.) Braem	30	NT	66	20
Vachellia anegadensis (Britton) Seigler & Ebinger	31 (A)	EN	651	30
Vachellia anegadensis (Britton) Seigler & Ebinger	31 (VG)	EN	87	100
Varronia rupicola (Urb.) Britton	32	EN	931	30
Zanthoxylum flavum Vahl	33	VU	24	30
Zanthoxylum thomasianum Krug & Urb.	34 (T)	EN	44	100
Zanthoxylum thomasianum Krug & Urb.	34 (VG)	EN	487	60

† For plants which were given different conservation targets depending on the strata they occurred in, location are mentioned in brackets; Anegada (A); Tortola (T); Virgin Gorda (VG).

Table A2. Conservation features and conservation targets for threatened habitats.

Habitat	ID	Extend (km^2)	Target (%)
Coastal evergreen shrubland	35	36.3	30
Mangrove	36	1.9	30
Upland evergreen forest	37	8.2	30
Salt pond	38	7.6	30
Semi deciduous gallery forest	39	0.5	30

Appendix 2

Table A3. Environmental Risk Surface: Intensity scores and influence distances.

Threat	Impact	Impact score	Decay distance (m)	Comments
Agricultural areas (low impact)	Very low	10	100	Significantly low intensity agriculture
Agricultural areas	Low	33	100	Low intensity agriculture in the BVI
Airports (small)	Medium	66	100	Potential future expansions of airports imposed a larger threat level.
Airport (Tortola)	Medium	66	150	Potential future expansions of airports imposed a larger threat level.
Cement plant	High	99	300	Expanding boundaries, air pollution.
Desalination plant	Low	33	100	Unknowns range of impact from outflow pipe, high energy ocean currents assist to dissipate hypersaline water.
Developed land (roads and buildings)	Low	33	0	No impact outside borders.
Electrical plants (small)	Low	33	300	Oil spills, air pollution.
Electrical plants (large)	High	99	300	Oil spills, air pollution.
Garbage dump sites	Medium	66	100	Fire risk, air pollution.
Incinerator	High	99	600	Expanding boundaries, air pollution.
Large Hotels	Medium	66	10	Traffic.
Marina	Low	33	0	No impact outside borders.
Plant nursery	Medium	66	50	Potential introduction of invasive species or pests.
Proposed development in mangroves	High	99	0	Area with proposed development.
Quarries	High	99	150	Expanding boundaries.
Sewage treatment plant	Low	33	0	No impact outside borders.
Solid waste site	High	99	200	Fire risk.
Vehicle dump site	High	99	100	Expanding boundaries.
Water tank	Low	33	30	Small scale due to low population, but risk of future expansion.

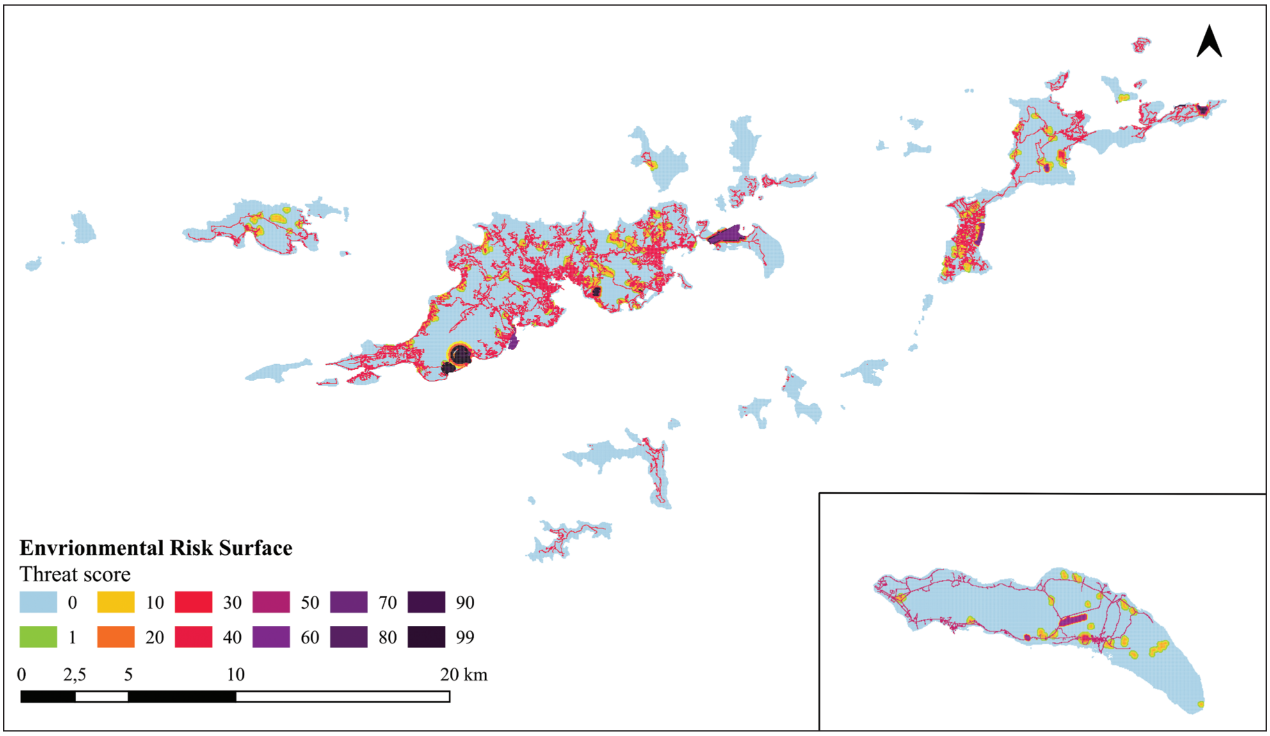

Figure A1. Environmental Risk Surface map over the British Virgin Islands.

Appendix 3

Table A4. Portfolio analysis for Plant Species of Conservation Concern.

	Protected Areas System Plan			Main portfolios‡			Crown land portfolios		
ID†	**Plant records**	**Protected %**	**%-point gap**	**Plant records**	**Protected %**	**%-point gap**	**Plant records**	**Protected %**	**%-point gap**
1	3	4	-46	45	54	4	12	15	-36
2	89	20	-20	305	68	28	193	43	3
3	3	18	-2	6	35	15	4	24	4
4 (A)	27	25	5	109	100	80	107	98	78
4 (T)	2	50	20	3	75	45	2	50	20
5	1	50	0	1	50	0	1	50	0
6	66	16	-4	366	89	69	194	47	27
7	5	39	-12	13	100	50	13	100	50
8	13	19	-1	55	81	61	25	37	17
9 (A)	12	67	47	18	100	80	18	100	80
9 (T)	0	0	-70	1	100	30	0	0	-70
10	23	100	40	23	100	40	23	100	40
11	35	100	0	35	100	0	35	100	0
12 (T)	10	59	-41	17	100	0	10	59	-41
12 (VG)	0	0	-50	147	87	37	115	68	18
13	109	36	16	232	76	56	157	52	32
14	15	8	-52	172	93	33	158	85	25
15 (A)	67	32	-8	212	100	60	201	95	55
15 (VG)	0	0	-100	4	100	0	1	25	-75
16	14	26	-4	53	100	70	14	26	-4
17	1	2	-68	48	100	30	36	75	5
18	57	97	-3	59	100	0	59	100	0
19	31	91	31	34	100	40	34	100	40
20 (T)	0	0	-100	1	100	0	1	100	0
20 (VG)	3	8	-32	32	84	44	32	84	44
21 (T)	4	19	-21	18	86	46	14	67	27
21 (VG)	0	0	-100	3	100	0	3	100	0
22	6	15	5	35	88	78	20	50	40
23	0	0	-10	15	94	84	0	0	-10
24	17	22	2	61	78	58	47	60	40
25	15	7	-3	150	73	63	107	52	42
26	17	16	6	81	76	66	61	58	48
27 (A)	1	10	-90	10	100	0	10	100	0
27 (T)	3	16	-34	10	53	3	3	16	-34
28	6	8	-52	78	100	40	72	92	32
29	4	17	-3	20	87	67	16	70	50
30	20	30	10	54	82	62	48	73	53
31 (A)	151	23	-7	651	100	70	637	98	68
31 (VG)	87	100	0	87	100	0	87	100	0
32	406	44	14	930	100	70	910	98	68
33	9	38	8	24	100	70	24	100	70
34 (T)	0	0	-100	43	98	-2	11	25	-75
34 (VG)	41	8	-52	475	98	38	169	35	-25
All§	1373	26		4736	90		3684	70	

† Strata in brackets; Anegada (A); Tortola (T); Virgin Gorda (VG).
‡ The Best Solution and SF100 portfolios reached the same conservation targets, and have therefore been grouped.
§ Number of plant records protected and their percentage fraction of all records within the analysis.

Table A5. Portfolio analysis for threatened habitats.

ID	Protected Areas System Plan			Main portfolios†			Crown land portfolios		
	Area km^2	Protected %	%-point gap	Area km^2	Protected %	%-point gap	Area	Protected %	%-point gap
35	7.8	22	-9	20.8	57	27	13.6	38	8
36	0.9	50	20	1.6	83	53	1.1	61	31
37	0.8	10	-20	3.5	43	13	1.6	20	-10
38	7.2	95	65	7.4	98	68	7.4	97	67
39	0.0	0	-30	0.3	55	25	0.2	30	0
All‡	16.7	31		33.6	62		23.9	44	

† The Best Solution and SF100 portfolios reached the same conservation targets, and have therefore been grouped.
‡ Area of habitats protected and their area percentage fraction of all threatened habitats within the analysis.

Table A6. Analysis of target achievement for all conservation features.

Conservation features met (0/1)				Exceeded targets with ≥ 50%-point (0/1)		Target achievement (0/1)	
ID†	Protected Areas System Plan	Main portfolios	Crown land portfolios	Main portfolios	Crown land portfolios	Equal for all portfolios	Redundant coverage ‡
1	0	1	1	0	0	0	0
2	0	1	1	0	0	0	0
3	0	1	1	0	0	0	0
4 (A)	1	1	1	1	1	0	1
4 (T)	1	1	1	0	0	0	1
5	1	1	1	0	0	1	0
6	0	1	1	1	0	0	0
7	0	1	1	1	1	1	0
8	0	1	1	1	0	0	0
9 (A)	1	1	1	1	1	1	1
9 (T)	0	1	0	0	0	0	0
10	1	1	1	0	0	1	0
11	1	1	1	0	0	1	0
12 (T)	0	1	0	0	0	0	0
12 (VG)	0	1	1	0	0	0	0
13	1	1	1	1	0	0	1
14	0	1	1	0	0	0	0
15 (A)	0	1	1	1	1	0	0
15 (VG)	0	1	0	0	0	0	0
16	0	1	0	1	0	0	0
17	0	1	1	0	0	0	0
18	0	1	1	0	0	1	0
19	1	1	1	0	0	1	1
20 (T)	0	1	1	0	0	1	0
20 (VG)	0	1	1	0	0	1	0
21 (T)	0	1	1	0	0	0	0
21 (VG)	0	1	1	0	0	1	0
22	1	1	1	1	0	0	1
23	0	1	0	1	0	0	0
24	1	1	1	1	0	0	1
25	0	1	1	1	0	0	0
26	1	1	1	1	0	0	1
27 (A)	0	1	1	0	0	1	0
27 (T)	0	1	0	0	0	0	0
28	0	1	1	0	0	0	0
29	0	1	1	1	0	0	0
30	1	1	1	1	1	0	1
31 (A)	0	1	1	1	1	0	0
31 (VG)	1	1	1	0	0	1	0

Conservation features met (0/1)				Exceeded targets with ≥ 50%-point (0/1)		Target achievement (0/1)	
ID[†]	Protected Areas System Plan	Main portfolios	Crown land portfolios	Main portfolios	Crown land portfolios	Equal for all portfolios	Redundant coverage [‡]
32	1	1	1	1	1	0	1
33	1	1	1	1	1	1	1
34 (T)	0	1[|]	0	0	0	0	0
34 (VG)	0	1	0	0	0	0	0
35	0	1	1	0	0	0	0
36	1	1	1	1	0	0	1
37	0	1	0	0	0	0	0
38	1	1	1	1	1	0 [§]	1
39	0	1	1	0	0	0	0
Sum Plant Species	12	34	27	14	4	8	10
Sum habitats	2	5	4	2	1	0	2

† Strata in brackets; Anegada (A); Tortola (T); Virgin Gorda (VG).

‡ For conservation features within the main portfolios, that experienced a higher coverage even though conservation targets were already adequately achieved in the current Protected Areas System Plan.

§ Almost equal %-point gap.

| The endemic *Zanthoxylum thomasianum* (ID:34) was one plant record short of reaching its target of a 100% in Tortola. However, it is expressed as if all records on Tortola were covered within the 'Best solution' and 'SF100' portfolios, due to the gap being made up of a single plant record, is considerably small, and it being outside of the TIPAs, and thus not possible to conserve within the means of this analysis.

Supplementary material 1

Portfolios - Anegada

Authors: Michalla Alicja Dolata, Nancy Woodfield-Pascoe, Thomas Heller, Michele Dani Sanchez, Sara Bárrios, Steven R. Schill, Patrik Karlsson Nyed, Martin Allen Hamilton, Keith Grant, Colin Clubbe, Bo Dalsgaard

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Link: https://doi.org/10.3897/natureconservation.55.116844.suppl1

Supplementary material 2

Portfolios - Tortola

Authors: Michalla Alicja Dolata, Nancy Woodfield-Pascoe, Thomas Heller, Michele Dani Sanchez, Sara Bárrios, Steven R. Schill, Patrik Karlsson Nyed, Martin Allen Hamilton, Keith Grant, Colin Clubbe, Bo Dalsgaard

Data type: pdf

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Link: https://doi.org/10.3897/natureconservation.55.116844.suppl2

Supplementary material 3

Portfolios - Virgin Gorda

Authors: Michalla Alicja Dolata, Nancy Woodfield-Pascoe, Thomas Heller, Michele Dani Sanchez, Sara Bárrios, Steven R. Schill, Patrik Karlsson Nyed, Martin Allen Hamilton, Keith Grant, Colin Clubbe, Bo Dalsgaard

Data type: pdf

Link: https://doi.org/10.3897/natureconservation.55.116844.suppl3

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Research Article

Recent changes in tropical-dry-forest connectivity within the Balsas Basin Biogeographic Province: potential effects on endemic-bird distributions

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Abstract

Maintaining landscape connectivity is a conservation priority for biodiversity as it may mitigate the adverse effects of forest degradation, fragmentation, and climate change by facilitating species dispersal. Despite their importance for biodiversity conservation, Mexican tropical dry forests (TDFs) face high fragmentation rates due to anthropogenic activities. In this study, we analyzed the connectivity dynamics of TDFs in the Balsas Basin Biogeographic Province (BBBP) between 2013 and 2018, focusing on old-growth and secondary TDF covers, including Protected Areas and Important Bird Areas. We evaluated the effects of connectivity loss and gain on the distribution areas of 30 endemic bird species with ecological associations with TDFs in the BBBP. We found expansion in TDFs accounting for a total increase of 227,905 ha due to secondary forest increase (12%). In contrast, old-growth forests experienced a reduction of 66,576 ha in the study area (8%). We also found a decrease in areas with high and very-high connectivity, coupled with an increase in low connectivity, except for TDFs inside Protected Areas, which increased by 3,000 ha, leading to higher connectivity. There was an increase in total forest cover in 27 species' potential distribution, highlighting the possible role of secondary forests in promoting connectivity between old-growth forest patches. Our results reveal the complex dynamics between forest types, connectivity, and bird-species distributions. Despite an overall increase in forested areas, most TDFs continue to have low connectivity, likely impacting biodiversity, particularly for species that rely on highly conserved ecosystems. This study underscores the importance of integrated conservation strategies considering connectivity, forest recovery, and the dynamics of species-ecosystem interactions.

Key words: conservation priorities, important bird areas, old-grow forests, protected areas, secondary forests

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Introduction

Natural forests face severe threats due to degradation, destruction, and fragmentation, significantly impacting biodiversity worldwide (Wilson 1992; Sala et al. 2000; Betts et al. 2017). Such activities lead to reduced habitat availability, increased isolation of forest patches, and higher vulnerability to alien-species invasion, edge effects, and further degradation (Wilcove et al. 1998; Fahrig 2003; Hulme 2009; Bellard et al. 2022). The primary drivers of forest loss are land-use and land-cover change, with tropical regions accounting for nearly 95% of global deforestation (Newbold et al. 2015; Curtis et al. 2018). The consequences of habitat loss affect all ecosystems, especially regarding reduced landscape connectivity, which has far-reaching implications at both local and regional scales.

Connectivity loss is a critical problem affecting biodiversity, as it hinders species' long-distance movements, making it difficult for them to survive in the face of anthropogenic pressures (Krosby et al. 2010; Damschen et al. 2019). Furthermore, conserving landscape connectivity is crucial for mitigating some of the adverse effects of fragmentation and climate change (Taylor et al. 1993; Correa Ayram et al. 2016; Zanin et al. 2016) by facilitating the colonization of available patches and enhancing gene flow among populations (Whitlock et al. 2000; Haddad et al. 2003, 2015). Changes in spatio-temporal land-use and land-cover patterns can significantly reshape the quantity and quality of available habitats for species, profoundly influencing ecological processes, species movements, and patterns of species richness and abundance (Lindborg and Eriksson 2004; Rocha et al. 2018). This intricate interplay of spatial and temporal connectivity creates a dynamic matrix that shapes ecosystems and influences species distributions (Auffret et al. 2015). Therefore, understanding recent connectivity patterns and their effects on species ranges is essential to comprehend ecological responses to fragmentation caused by climate or land-use and land-cover change, and to implement practical biodiversity conservation actions tailored to species' needs (Metzger et al. 2009; Thompson et al. 2017).

Tropical dry forests (TDFs) rank among the world's most endangered ecosystems, having lost nearly 80% of their original cover (Trejo and Dirzo 2000; Miles et al. 2006; Sánchez-Azofeifa et al. 2014). Recent estimates indicate that up to 85% of the remaining TDFs in the Americas face potential risk (Ferrer-Paris et al. 2019). TDFs occupy approximately 17% of Mexican territory, and their geographic isolation classifies them as ecological islands, nurturing distinct floral and faunal groups, including 24% of México's bird-species richness (Rzedowski 1991; Ceballos and García 1995; Dirzo et al. 2011; Navarro-Sigüenza et al. 2014). The Balsas Basin Biogeographic Province (BBBP) is characterized by a unique combination of climatic, ecological, and biotic factors that have influenced the evolution and distribution of its biota (Morrone 2014). It represents the only interior depression in México with tropical vegetation, making it a remarkable ecosystem (Fernández-Nava et al. 1998). However, human activities in this region have transformed many old-growth forests into secondary ones, leading to a complex landscape of degraded and recovering forests (Sánchez-Colón et al. 2009) that result in a mix of vegetation types that originate from different stages of ecological succession (INEGI

2015, FAO 2020a). Despite the dominance of secondary forests, their proximity to old-growth forests highlights their potential as pathways for species colonization (Gray et al. 2007; Mayhew et al. 2019). Secondary forests, particularly those with a developed tree and shrub canopy, might represent an essential habitat for resident and endemic bird species (Ramírez-Albores 2006), acting as corridors and stepping stones between old-growth forests, which host a wide variety of specialist species (Wright and Muller-Landau 2006; Chazdon et al. 2009; Acevedo et al. 2023).

The BBBP holds eight Protected Areas (PAs; CONANP 2022) and numerous Important Bird Areas (IBAs; Arizmendi and Márquez 2000; Devenish et al. 2009), highlighting its significance for conservation efforts. International agendas, such as the Kunming-Montreal Global Biodiversity Framework, emphasize the urgency of maintaining ecological connectivity (IPBES 2019), and the Convention on Biological Diversity (CBD 2020) urges increases in PA coverage worldwide (Vimal et al. 2021). However, the ongoing changes in land use and cover jeopardize the connectedness of PAs, threatening their ecological integrity and the species they harbor. Therefore, a detailed connectivity analysis is vital to assess conservation efforts and guide effective management strategies to safeguard this biodiverse and ecologically crucial ecosystem (Crooks and Sanjayan 2006; Rodrigues and Cazalis 2020). The goal of this study is to analyze recent connectivity dynamics between 2013 and 2018 across old-growth and secondary TDFs of the BBBP, including PAs and IBAs. This analysis aimed to shed light on the potential effects of these dynamics on the distribution of diverse Mexican endemic bird species with ecological affinity to the TDFs. It provides insights into how these dynamics impact the distribution of endemic bird species, therefore contributing to understanding the conservation needs in an ecologically sensitive region.

Methods

Study area

Located in the states of Guerrero, Jalisco, México, Michoacán, Morelos, Oaxaca, and Puebla, the Balsas Basin Biogeographic Province (BBBP) covers nearly 7,640,000 ha (Fig. 1; Morrone 2014; Morrone et al. 2017). The BBBP is a depression in the interior of México that descends from a plateau that reaches up to 1,000 meters above sea level (m a.s.l.), with some scattered peaks at 2,000 m in the north to 200 m in the extreme west (Dinerstein et al. 2017). The BBBP is delimited by the Trans-Mexican Volcanic Belt and the Sierra Madre del Sur, which places it on the border of two mountain complexes of the Mexican Transition Zone in the Neotropical region (Halffter 1976; Halffter and Morrone 2017).

The BBBP remains as an important area covered by tropical dry forests (TDFs) (Rzedowski 2006; Dirzo et al. 2011). TDFs are primarily dominated by relatively short trees that shed their entire foliage during dry seasons (Miranda and Hernández-X 1963). This is due to highly seasonal characteristics, which include a well-defined rainy season (500 – 1,500 mm/yr), followed by a dry season with five to eight months of continuous drought (Jaramillo et al. 2011).

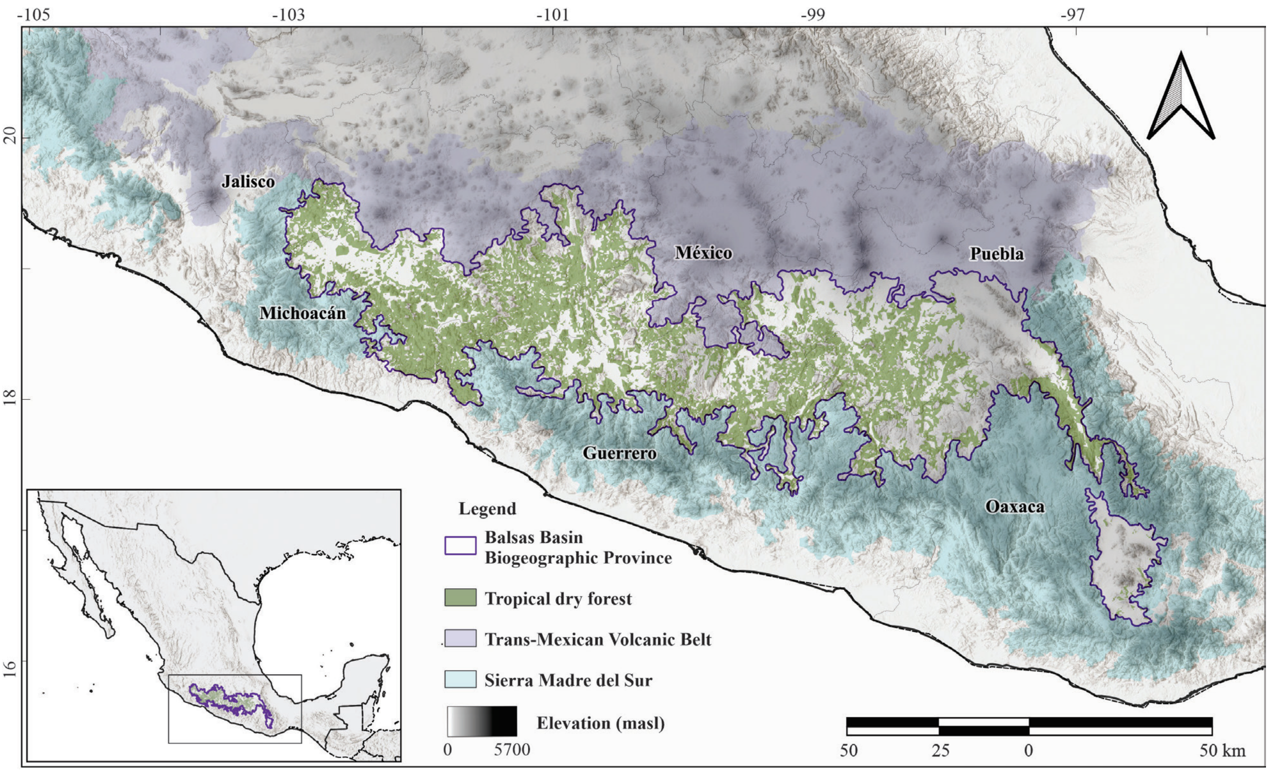


Figure 1. Location of the Balsas Basin Biogeographic Province in México, showing the two adjacent provinces Trans-Mexican Volcanic Belt (light purple), and Sierra Madre del Sur (light blue); the more intense colors (gray scale) represent the higher altitudes.

Species distribution modeling

The selection of avian species for this study was based on the following criteria: 1) species endemic to México (Navarro-Sigüenza and Peterson 2004; Berlanga et al. 2020), 2) species whose distribution overlapped entirely or partially the Balsas Basin Biogeographic Province, 3) species reported as ecologically associated with the TDFs (Stotz et al. 1996; Vázquez-Reyes et al. 2017, 2018) (Suppl. material 1), and 4) species with more than 20 occurrences. Although all selected species occur within TDFs, they are not necessarily exclusive to this vegetation type (Table 1). Their presence in TDFs ranges from preferential to incidental, as they inhabit other vegetation types (Suppl. material 1). From the resulting list, we selected 95% of species (30 species), including a wide range of feeding guilds, forest dependency, and specialization within TDFs, from species that predominantly live in these forests and those that use several other ecosystems (Stotz et al. 1996; Pineda-Diez de Bonilla et al. 2012; Billerman et al. 2022).

To produce the Species Distribution Models (SDMs), we used the Maximum Entropy (MaxEnt) algorithm (Phillips et al. 2006) in the R: KUENM package (Cobos et al. 2019). The KUENM package allows testing many combinations of features with different regularization multipliers to find the best set of parameters, improving the quality of predictions for SDMs (Steele and Werndl 2013; Cobos et al. 2019). Since MaxEnt requires the delimitation of a calibration area (M *sensu* Soberón and Peterson 2005), we defined its extent following the accessible-area approach (Soberón and Peterson 2005; Rojas-Soto et al. 2024) by identifying the intersection between biogeographic provinces (Morrone et al. 2017) and the world's ecoregions (Dinerstein et al. 2017) that contain at least

Table 1. Endemic-bird species considered in this study. Degree of species specialization to Tropical dry forest (**TDF-S**), and forest dependency (**FD**): 3 = high, 2 = medium, 1 = low; Sensitivity to disturbance (**Snts**): h = high, m = medium, l = low; **Feeding guild**: c = carnivores, f = frugivores, g = granivores, i = insectivores, n = nectarivores.

Family	Species	TDF-S	FD	Snts	Feeding Guild
Cracidae	*Ortalis poliocephala*	3	2	l	f
Odontophoridae	*Philortyx fasciatus*	2	1	l	g
Cuculidae	*Piaya mexicana*	2	2	l	i
Apodidae	*Streptoprocne semicollaris*	2	2	l	i
Trochilidae	*Calothorax pulcher*	1	2	m	n
	Phaeoptila sordida	2	2	l	n
	Cynanthus auriceps	2	1	l	n
	Ramosomyia viridifrons	1	1	m	n
	Saucerottia beryllina	2	2	m	n
Strigidae	*Megascops seductus*	2	3	m	i,c
	Glaucidium griscomi	3	2	m	i,c
Trogonidae	*Trogon citreolus*	3	1	l	f,i
Picidae	*Melanerpes chrysogenys*	2	1	l	i
	Melanerpes hypopolius	1	1	l	f
Tityridae	*Pachyramphus uropygialis*	1	2	m	i
Tyrannidae	*Xenotriccus mexicanus*	1	2	m	i
Grallariidae	*Grallaria ochraceiventris*	1	3	h	i
Furnariidae	*Lepidocolaptes leucogaster*	2	3	m	i
Vireonidae	*Vireo hypochryseus*	3	2	m	i
Troglodytidae	*Pheugopedius felix*	2	2	l	i
	Thryophilus sinaloa	3	2	l	i
Mimidae	*Melanotis caerulescens*	2	1	m	i,f
Turdidae	*Catharus occidentalis*	1	2	m	i,f
	Turdus rufopalliatus	2	1	l	f
	Turdus assimilis	2	3	m	f,i
Passerellidae	*Peucaea humeralis*	2	2	m	i
	Peucaea acuminata	1	1	l	g
	Melozone kieneri	2	2	l	g
Cardinalidae	*Passerina leclancherii*	2	1	l	g
Thraupidae	*Sporophila torqueola*	1	1	l	g

one record of the target species. Furthermore, in cases where the intersection of ecoregions and biogeographic provinces extended significantly beyond the last species' record, we incorporated relevant physical factors that could potentially act as barriers to species dispersal (e.g., Tehuantepec Isthmus; Barve et al. 2011). We downloaded all species records from the Global Biodiversity Information Facility (GBIF: Suppl. material 1). The climatic variables used were those published by Cuervo-Robayo et al. (2014), composed of 19 climatic layers at a spatial resolution of 30 seconds (~1 km^2) derived from precipitation and temperature variables. We selected only variables with Pearson correlation (r) value < 0.80 for each species.

We evaluated model performance by calculating the Area Under the Curve (AUC) (Elith et al. 2011), as well as by applying partial Receiver Operating Characteristic Curves (pROC) via AUC ratio, and Akaike's Information Criterion (AIC) calculated as part of the modeling process in the KUENM package. Evaluation parameters were resampled: 50% of random points with 1,000 data iterations. Afterwards, we ranked the observed AUC ratio with pseudo-replicate values, following the proposal of Peterson et al. (2008). Once all SDMs were created, we used the 10-percentile training presence threshold to make binary presence-absence maps. This threshold identifies suitable pixels that are predicted to have similar suitability as those that contain the species occurrence record and rejects 10% of the records with the lowest suitability to minimize over-prediction caused by possible outliers in each species database. We constructed the species richness map by summing the species' model maps to the pixel level with the raster calculator tool in QGIS (QGIS Development Team 2020).

Land use, land cover, and connectivity index

We used vector layers of land use and vegetation cover Series V (INEGI 2013) and Series VII (INEGI 2018) from the National Institute of Statistics and Geography (acronym INEGI in Spanish), both at a scale of 1:250,000. Series V and VII were selected due to their highly comparable methodologies, which reduces potential bias. INEGI's vegetation covers are derived from the interpretation of Geomedian images from the LANDSAT satellite and a field validation carried out by the INEGI before publication. INEGI has developed a methodology to classify vegetation types according to Rzedowski (2006), considering ecological, floristic, and physiognomic characteristics. Vegetation types are also classified into "primary" and "secondary" through an evaluation of the physiognomic characteristics, using a successional framework that considers criteria such as canopy height, leaf cover, compositional diversity, and the prevalence of certain species (using field verifications). "Primary vegetation" is defined by INEGI (2013) as vegetation that remains or appears unaltered, while "Secondary vegetation" refers to a type of primary vegetation that has been removed or altered by various human or natural factors. There are probably no primary forests in the Americas, so we refer to INEGI's "primary" classification as "old-growth". This systematic approach enables a detailed analysis of vegetative patterns, leading to a better understanding of biodiversity and ecosystem stratification. By applying these technical parameters, INEGI's field verifications offer a mechanism for empirical vegetation categorization. To manage the large number of natural vegetation types and land-use classes, we classified them into fewer categories to make them easier to manage. We reclassified all coverage corresponding to Tropical Dry Forest types (e.g., low deciduous, low sub-deciduous, medium sub-deciduous forest; INEGI 2015; Suppl. material 2). These were further labeled into two classes: old-growth and secondary forests, using QGIS (QGIS Development Team, 2020).

To estimate connectivity between TDFs patches in the BBBP, we used the "Conefor Sensinode 2.6" program (Saura and Torné 2009). We calculated the Integral Index of connectivity (IIC) for each of the two time periods of the INEGI coverage (2013 and 2018). The IIC is calculated by considering the size, shape, and spatial arrangement of these patches, providing a numerical value that reflects the overall connectivity and accessibility across the landscape; it ranges from 0 to 1 and increases with improved connectivity (Pascual-Hortal and Saura 2006). Although species respond individually and differently to ecosystem fragmentation (Liu et al. 2018), it is common to adjust and estimate a connectivity index for all study subjects to measure the effects on species richness (Saura 2013). Therefore, the connectivity index was calculated for TDFs, taking a dispersal constant of 1,000 m to the nearest edge of the forest patch, which allows the estimation of the overall connectivity value between vegetation patches (Nathan 2006; Borda-Niño et al. 2017). Then, we defined intervals according to the IIC values, including a) very high, b) high, c) moderate, d) low, and e) very low, determined by the Jenks natural breaks process to estimate the changes that occurred in the IIC scale for the TDFs in the BBBP in the two dates, corresponding to INEGI's Series V (2013) and Series VII (2018). We used the TerrSet environment to estimate the losses, gains, total changes, and exchanges between connectivity classes (Clarklabs 2020).

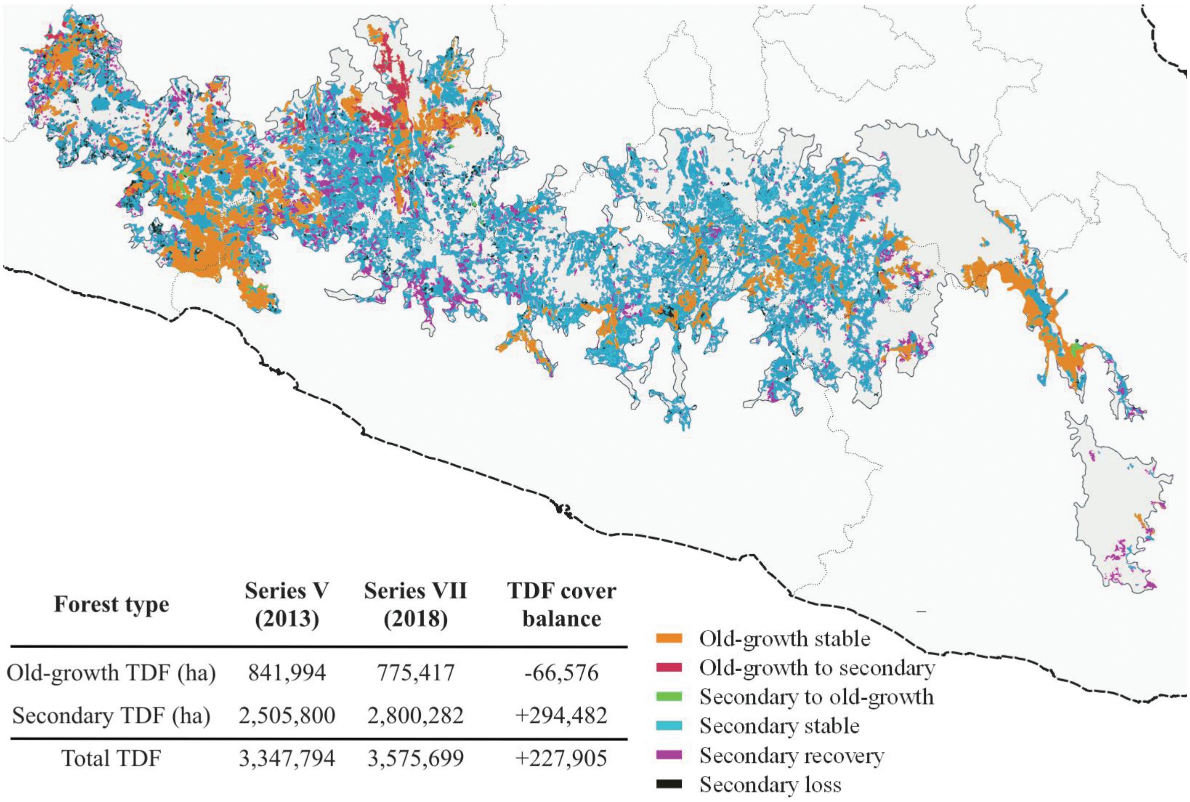
Impact assessment of connectivity change in tropical dry forests for species distributions and priority areas

To identify the impact of connectivity changes over time, we quantified three different features: 1) the connectivity variation between old-growth and secondary forest covers, 2) the connectivity shifts impacting the distribution of each bird species and the overall bird richness, and 3) the connectivity alterations within PAs and IBAs (Arizmendi and Márquez 2000; Devenish et al. 2009, CONANP 2022). Using Boolean Algebra and spatial statistical tools (QGIS), we determined the affected areas for each species and the proportion of their range within each connectivity class. Finally, we assessed the impact on PAs and IBAs by overlaying their polygons on the IIC map to quantify the area affected by connectivity loss.

Results

Within the study area, TDFs experienced an increase from 44% to 47%, which represents a net expansion of 227,905 ha between 2013 and 2018 (Fig. 2). We found a noticeable shift in forest composition, characterized by an increase in secondary forests and a reduction in old-growth TDFs in the BBBP. The west-central and easternmost regions of the BBBP concentrated the main transitions from secondary to forests with characteristics of TDFs old-growth forests.

In 2013, most TDFs within the study area were classified in the lower-connectivity classes, covering a significant portion of the total area (Table 2). In contrast, the areas with the highest connectivity classes, "high" and "very high", accounted for a smaller fraction of the total TDFs. In 2018, there was a decline in the old-growth forest that resulted in the reduction of the TDFs in the "very high" and "high" connectivity classes, equivalent to 7,512 ha, and 103,000 ha, respectively. Nevertheless, when considering both old-growth and secondary forests, there was an overall increase in the "very high" class and a decrease in the "high" class. In contrast, secondary-forest cover gained almost 100,000 ha in its lowest class (very low; Table 2).



Forest type	Series V (2013)	Series VII (2018)	TDF cover balance
Old-growth TDF (ha)	841,994	775,417	-66,576
Secondary TDF (ha)	2,505,800	2,800,282	+294,482
Total TDF	3,347,794	3,575,699	+227,905

Figure 2. Old-growth and secondary tropical dry forests (TDF) dynamics based on INEGI (2013, 2018) in the Balsas Basin Biogeographic Province.

Table 2. Tropical dry forests (TDFs) area per connectivity class. We present the type of forest cover, years, connectivity class in ha, and the percentage shown in parentheses.

Forest type	Year	Connectivity class				
		Very High	High	Moderate	Low	Very Low
Old-growth	2013	194,747 (6)	144,665 (4)	57,099 (2)	113,539 (3)	331,945 (10)
	2018	187,235 (5)	41,529 (1)	153,384 (5)	131,422 (4)	261,848 (7)
Secondary	2013	371,907 (11)	58,758 (2)	215,823 (6)	803,335 (24)	1,055,976 (32)
	2018	452,725 (13)	70,662 (2)	409,067 (11)	713,312 (20)	1,154,516 (32)
Total	2013	566,654 (17)	203,423 (6)	272,922 (8)	916,874 (27)	1,387,921 (42)
	2018	639,960 (17)	112,191 (3)	562,451 (16)	844,734 (24)	1,416,364 (40)

In 2013, forest fragments in the western and central regions of the BBBP had higher connectivity than the rest of the TDFs in the study area, which had “moderate” to “very low” connectivity (Fig. 3). However, by 2018, there was a decrease in connectivity values in the western region of the BBBP, especially in the state of Michoacán where secondary forests declined from “very high” to “moderate”, while "high" connectivity persisted consistently in Guerrero State throughout the study period. The areas in the states of Guerrero and Michoacán with “high” connectivity remained along the borders of the Sierra Madre del Sur Mountain system in the southwestern section of the study area. It is important to note that the increase in connectivity pertained specifically to secondary forests. In contrast, the decrease in connectivity in Michoacán is more generalized and encompasses both old-growth and secondary TDFs.

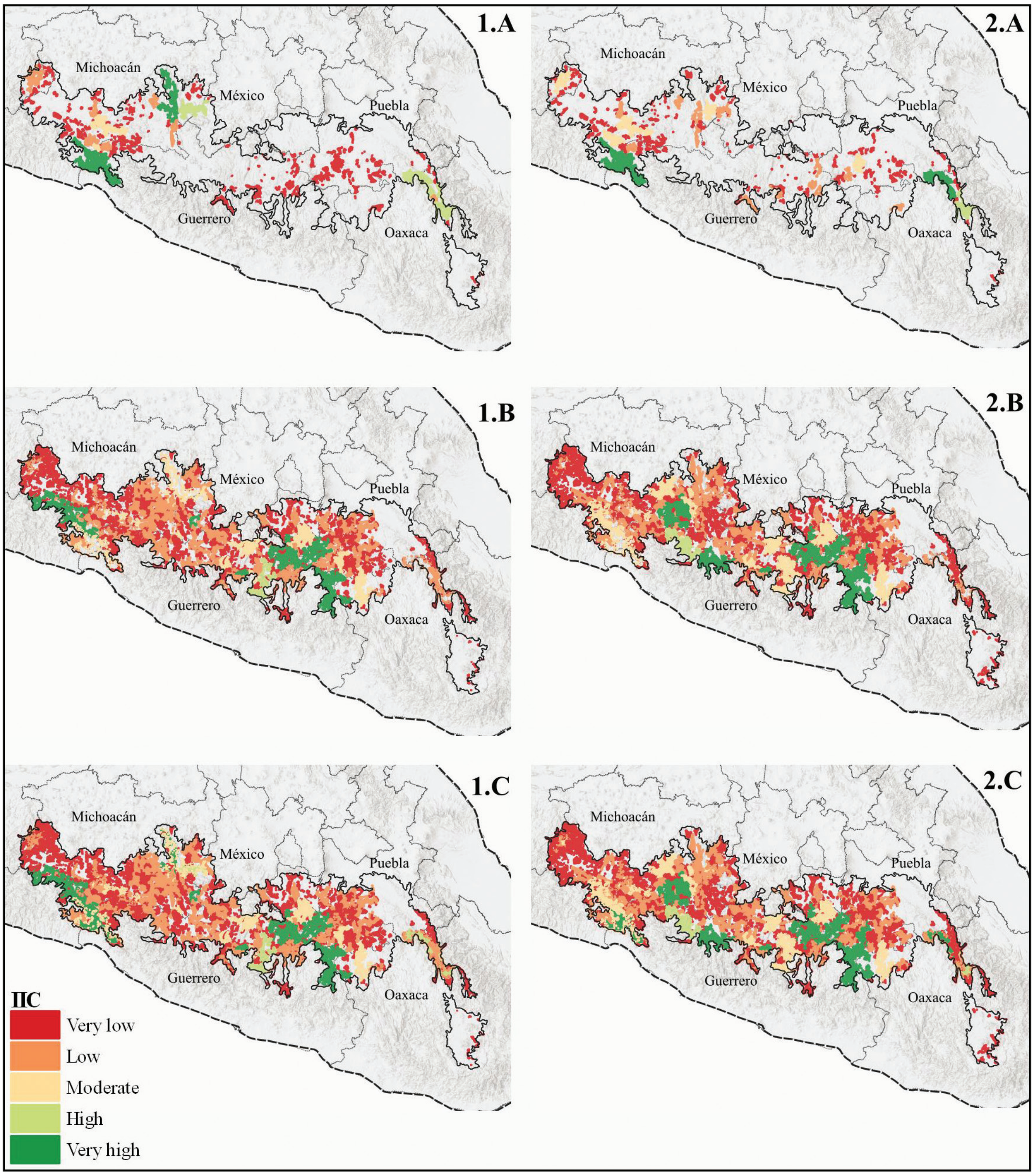


Figure 3. Classes of the Integral Index of Connectivity for 2013 (1) and 2018 (2) **A** old-growth forest cover **B** secondary-forest cover **C** overall TDF cover.

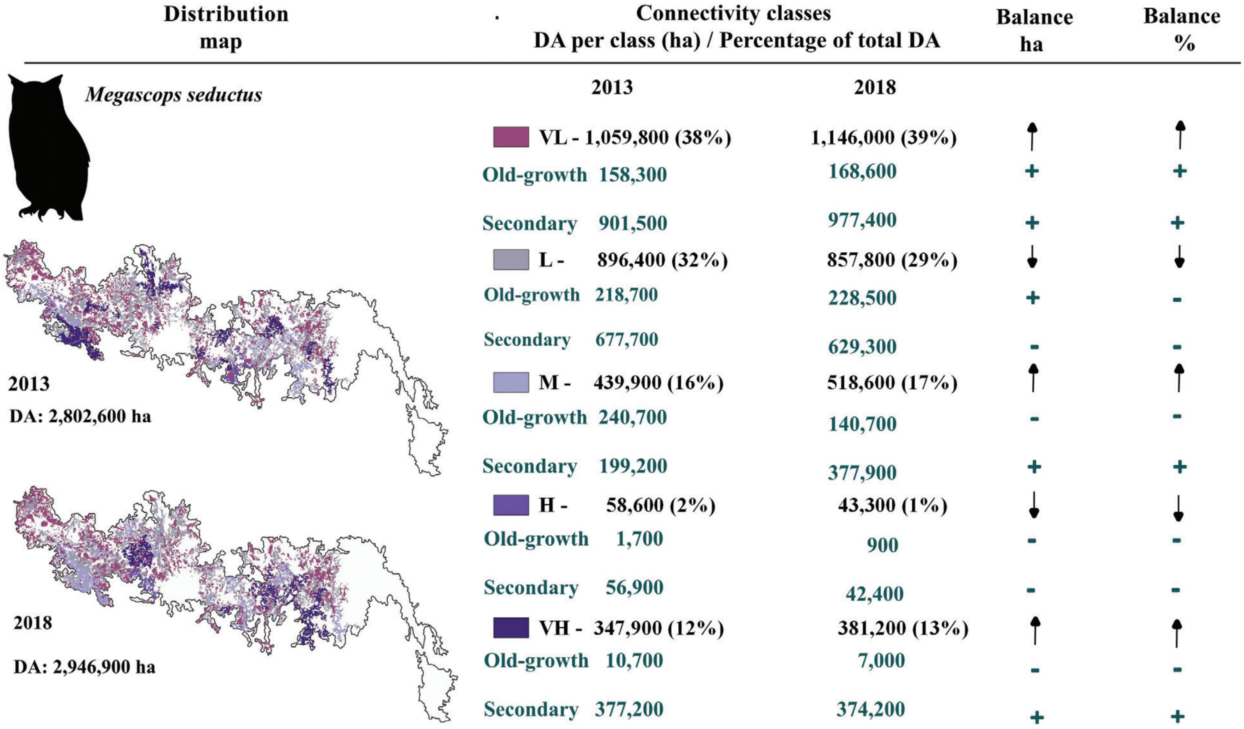
Comparison of the distribution map of the analyzed bird species with the TDF-cover dynamics (including loss, gain, and stable covers), showed that only three of the thirty species in this study (*Sporophila torqueola*, *Turdus assimilis*, *Vireo hypochryseus*) decreased total forest cover within their distribution areas between 2013 and 2018, but, the variation in connectivity change within the range of each species was closely linked to their reliance on old-growth or secondary-forest cover (Suppl. material 3). The expansion of secondary forest occurred predominantly in specific connectivity categories, with 27 species experiencing this growth within connectivity category "very low", 21 species within connectivity category "moderate", and 18 species within connectivity category "very high" (Table 3). Surprisingly, 16 species showed a simultaneous increase in both extreme levels of connectivity (very high and very low), of which 14 species demonstrated a more substantial increase in the "very low" connectivity category than "moderate" (e.g., Fig. 4, for all species, see Suppl. material 3). However, the expansion of secondary forest increases the distribution area for some species. The places where this increase occurred were predominantly associated with connectivity classes "low" and "very low" (see Table 3).

According to the endemic-bird-richness map (Fig. 5), the areas where 26 species are potentially distributed are on the borders of the BBBP alongside the mountain complexes in Michoacán and México States, as well as in the central area of Puebla, Morelos, and Guerrero States. Additionally, some regions with significant levels of endemic-species richness were not included in conservation instruments such as PAs (actually protected spaces) or in suggested areas that are worth preserving, such as IBAs (Fig. 5c). Only 12% of TDF fragments in the BBBP were located in designated protected areas under national conservation categories. In contrast, if all the IBAs were protected, 17% of the TDFs within the study area would be in PAs.

We recorded an increase of 3,000 ha of TDFs inside PAs from 2013 to 2018. However, there are many changes in the forest dynamics inside PAs. We observed an increase of 27,723 ha in areas with "very high" connectivity. The area expanded from 79,098 ha in 2013 to 106,821 ha in 2018. This growth was particularly noticeable in old-growth forests, which experienced an increase of 59,163 ha (Table 4). In contrast, secondary forests in the same connectivity class experienced a substantial decrease of 31,525 ha. Additionally, moderate and very-low connectivity classes tended to increase their area over time in PAs and IBAs (Table 4). On the other hand, areas classified as "high" connectivity experienced a marked decrease inside these priority areas.

Table 3. Number of species that increased, remained stable, or decreased their distribution range within the different connectivity classes in the Tropical Dry Forests (TDFs) of the Balsas Basin Biogeographic Province from 2013 to 2018.

Connectivity class	TDF cover	Increase	Persistence	Decrease
Very high	Old-growth	9	4	17
	Secondary	20	0	10
	Total	18	0	12
High	Old-growth	2	15	13
	Secondary	7	2	21
	Total	7	2	21
Moderate	Old-growth	0	1	29
	Secondary	27	1	2
	Total	21	2	7
Low	Old-growth	23	1	6
	Secondary	5	0	25
	Total	7	0	23
Very low	Old-growth	20	0	10
	Secondary	26	0	4
	Total	27	0	3



Connectivity classes DA per class (ha) / Percentage of total DA 2013	2018	Balance ha	Balance %
VL - 1,059,800 (38%)	1,146,000 (39%)	↑	↑
Old-growth 158,300	168,600	+	+
Secondary 901,500	977,400	+	+
L - 896,400 (32%)	857,800 (29%)	↓	↓
Old-growth 218,700	228,500	+	-
Secondary 677,700	629,300	-	-
M - 439,900 (16%)	518,600 (17%)	↑	↑
Old-growth 240,700	140,700	-	-
Secondary 199,200	377,900	+	+
H - 58,600 (2%)	43,300 (1%)	↓	↓
Old-growth 1,700	900	-	-
Secondary 56,900	42,400	-	-
VH - 347,900 (12%)	381,200 (13%)	↑	↑
Old-growth 10,700	7,000	-	-
Secondary 377,200	374,200	+	+

Figure 4. Example of net change in species distribution area (DA) and percentage of DA for each connectivity class. Connectivity classes are very low (VL), low (L), moderate (M), high (H), and very high (VH). The effect of the change was classified as positive (+), and negative (-) for each forest cover type and overall connectivity.

Table 4. Dynamics of the tropical dry forests (TDFs) cover inside protected areas (PAs), and in the important bird areas (IBAs). We give the connectivity class, TDF cover type: old-growth, secondary, and both (Total), and the extent (ha) for 2013, 2018 and the balance from 2013 – 2018 in the Balsas Basin Biogeographic Province.

Connectivity Class	Forest cover type	PAs (ha)			IBAs (ha)		
		2013	2018	Balance	2013	2018	Balance
Very high	**Total**	**79,098**	**106,821**	**27,723**	**94,447**	**130,307**	**35,860**
	Old-growth	42,026	101,275	59,249	20,214	79,719	59,505
	Secondary	37,072	5,546	-31,525	74,233	50,588	-23,645
High	**Total**	**95,370**	**36,207**	**-59,163**	**102,122**	**39,221**	**-62,901**
	Old-growth	95,370	36,207	-59,163	97,143	39,221	-57,922
	Secondary	–	–	–	4,979	–	-4,979
Moderate	**Total**	**75,808**	**127,601**	**51,793**	**76,417**	**136,556**	**60,139**
	Old-growth	52,510	77,811	25,300	21,560	40,249	18,689
	Secondary	23,298	49,790	26,492	54,857	96,307	41,450
Low	**Total**	**75,949**	**35,530**	**-40,419**	**111,369**	**88,501**	**-22,869**
	Old-growth	30,305	6,583	-23,723	29,061	20,132	-8,930
	Secondary	45,643	28,947	-16,696	82,308	68,369	-13,939
Very low	**Total**	**98,536**	**121,807**	**23,271**	**196,529**	**198,241**	**1,712**
	Old-growth	27,200	31,633	4,433	49,888	46,321	-3,567
	Secondary	71,336	90,175	18,838	146,641	151,920	5,279

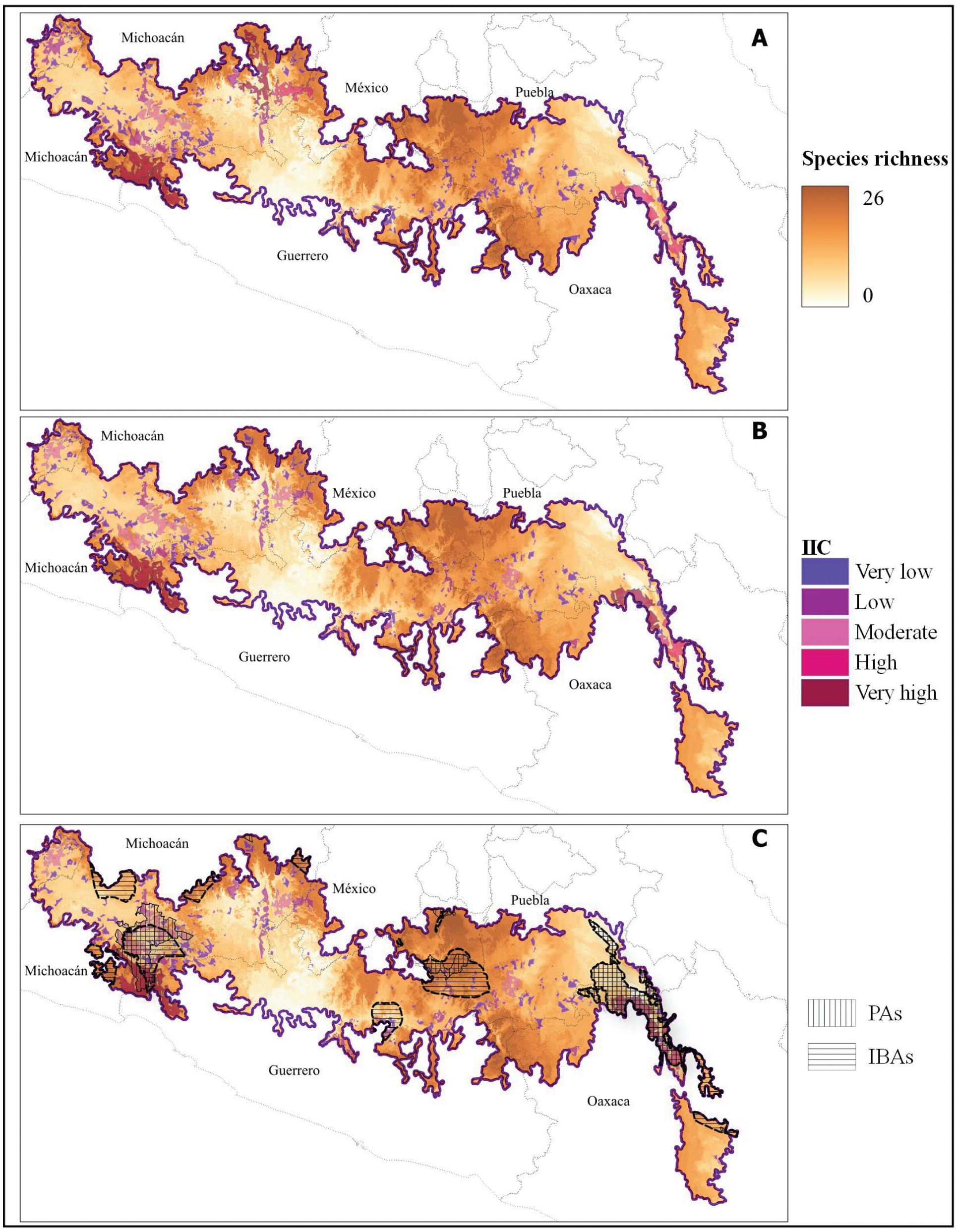


Figure 5. Endemic bird species richness contrasted with the connectivity classes of old-growth forest **A** 2013 **B** 2018 **C.** PAs: Protected Areas and IBAs: Bird Important Areas location within the Balsas Basin Biogeographic Province.

Discussion

In this study, we found evident changes in old-growth and secondary tropical dry forests (TDFs), especially in the north-central BBBP. Concurrently, there was an increase in secondary forests throughout the study area (Fig. 2). Nonetheless, it is important to recognize a possible limitation due to INEGI's definition of primary (old-growth) and secondary forests (2013, 2018). Particularly, within transition zones from primary (old-growth) to secondary TDFs, two scenarios might occur: 1) old-growth forests are changed to other land-use and subsequently are abandoned and initiate a regeneration process, transforming into secondary forests, and 2) areas categorized as secondary forest could be derived from degraded old-growth forests yet retain a structure similar to secondary forest. Additionally, in regions where we detected increases in old-growth TDF cover, the regeneration of old-secondary forests produced changes in the structure and composition of both strata and species that mirror a mature forest, leading to the classification of those covers as primary (old-growth). Our results indicate trends aligning with other national and regional studies. For instance, Velázquez et al. (2010) noted a 17% loss of old-growth vegetation in México, with a slight recovery of 1.5% through revegetation. Rosete-Vergés et al. (2014) recorded a 35% annual decrease in old-growth forests transitioning to secondary forests and a 1.5% secondary forest recovery from other land uses, indicating a widespread shift to secondary forests due to land-use and land-cover changes (Corona et al. 2016, FAO 2020b).

Nevertheless, this growth in secondary forests might be a short-term increase since other researchers, such as Rader and Schneider (2022), found alternating patterns of forest loss and modest regrowth in Quintana Roo. Meanwhile, López-Barrera et al. (2014) reported a net increase in dense and sparse forests in Veracruz, which was attributed to reduced agricultural activities. However, these trends were inconsistent in both studies, fluctuating between gains and losses in varying periods, which could indicate a slow and long-term degradation process. These findings underscore the complex and fluctuating nature of forest dynamics, stressing the need to account for temporal variations in forest-cover analyses.

In the BBBP, poorly connected old-growth TDFs predominate; however, the growth of secondary forests and the regeneration of forests with characteristics similar to old-growth forests from secondary patches led to an increase in the area covered with high connectivity in certain regions. In this sense, promoting the maintenance of remaining old-growth forest patches and encouraging passive forest restoration or natural regeneration can improve the overall connectivity of the landscape and mitigate fragmentation effects (Bennet 1990; Fahrig 2003; Mayhew et al. 2019). Our findings support the thesis that secondary forests play a crucial role in providing connections for diverse species, mainly when found near intact old-growth forests (Gray et al. 2007; Chazdon et al. 2009), highlighting the conservation value of secondary forests in mitigating wildlife decline in fragmented landscapes. It is also important to note that this conservation value increases with secondary forest age, as shown by Rocha et al. (2018). The strategy of floristic enrichment in secondary forests by introducing native species from old-growth forests is a promising strategy to improve successional processes. Integrating these species can favor ecological succession from poorly developed secondary to old-growth forests and facilitate the restoration of biodiversity and ecosystem functionality (Ávila-Lovera et al. 2023). This underscores the need for adaptive-management practices that aim to conserve existing old-growth forests and increase the ecological value of secondary forests. Furthermore, it highlights the importance of enacting robust protective measures through legislation.

Bird communities, including a variety o diverse of guilds and forest dependency levels, are influenced in distinct ways by the age of forest succession, structural characteristics, and landscape variables (Almazán-Núñez et al. 2012; Santamaría-Rivero et al. 2016; Mayhew et al. 2019). Granivorous bird species tend to be much more resilient to changes in vegetation structure than frugivorous species. For instance, bird diversity in the BBBP showed distinct habitat preferences aligned with their Feeding traits (Vázquez-Reyes et al. 2017). The granivorous species *Peucaea humeralis*, *Passerina leclancherii*, and *Philortyx fasciatus* were more frequently observed in secondary TDFs, this may occur because clearing old-growth forests can promote the development of shrubby and herbaceous layers prolific in seed production. The studied bird species exhibited diverse distribution patterns, with some showing potential distribution in poorly connected areas. In contrast, other species were predicted to be more common in zones of higher connectivity, reflecting a complex relationship between the level of connectivity of forest cover and bird distributions. Understanding these dynamics is crucial to identify trends that may compromise the conservation of endemic or priority species in the BBBP and other biodiversity-rich regions. For instance, *Megascops seductus* (Fig. 4), an endemic species to the BBBP, inhabits conserved and disturbed TDFs and agricultural landscapes. However, its population density is significantly greater in old-growth forests than in disturbed regions (Alba-Zúñiga et al. 2009). Consequently, the observed decline in connectivity in old-growth TDFs and the rise in areas with reduced connectivity in secondary TDFs may impact *Megascops seductus* and at least 18 other species that, even if they can occur in secondary TDFs, rely on highly conserved places for food, shelter, and breeding (Thompson et al. 2017). The variability in species prevalence across different connectivity areas underscores the urgency for conservation strategies that recognize the intricate dynamics of species-ecosystem interactions and landscape history in conservation planning (Metzger et al. 2009).

The effectiveness of the Protected areas (PAs) network largely depends on its ability to address the connectivity and dispersal requirements of a diverse range of species (Crooks and Sanjayan 2006). Mexican PAs adhere to political and social frameworks that allow specific human activities and regulate resource extraction with an emphasis on sustainable development and the well-being of local communities (DOF 2022), which, as in other countries, have sparked debates regarding its effectiveness in preserving the ecological integrity of PAs (Miller et al. 2011; Shafer 2015). The increase in old-growth vegetation and the expansion of well-connected TDFs within PAs indicate forest maturation, suggesting that this protection model can contribute to successful conservation efforts in the BBBP. It is necessary to reinforce the management strategies within and around the PA network, allowing the maintenance of successional processes from secondary to old-growth forest (Rocha et al. 2018; Mayhew et al. 2019; Acevedo et al. 2023). This is especially important because of the global conservation priority of TDFs, and their current under-protection and limited representation within existing PAs in the study area (Miles et al. 2006; Portillo-Quintero and Sánchez-Azofeifa 2010; Prieto-Torres et al. 2016). Despite the increases in TDFs within PAs in the BBBP, it is crucial to recognize that TDFs are predominantly in the lower-connectivity classes. This could potentially lead to the isolation of species, particularly those with limited dispersal capabilities (Fahrig 2003; Haddad et al. 2003). Although direct data on the mobility of our studied species is lacking, literature on closely related species suggests that many of them might have large home ranges and greater mobility, which could facilitate connectivity over the 1,000-meter threshold used in this study. For instance, while species with low movement ability, such as quails, tend to have small home ranges, typically ranging between 2–9 hectares (Franco et al. 2006), they tend to be more resilient to disturbed habitats due to their ability to use resources in altered environments, thus enabling them to move between secondary TDFs. In contrast, owl species tend to exhibit larger home ranges that can exceed 1,000 hectares (Carey et al. 1990), but often rely on extensive old-growth forests which can limit movements between poorly connected forests. This observation reinforces the significance of connectivity in conservation strategies and underlines the need for comprehensive management approaches. Such approaches should address these spatial dynamics to prevent the fragmentation and isolation of essential habitats (Crooks and Sanjayan 2006).

Some IBAs may play a crucial role in expanding the PA network and are significant to bird conservation efforts (Arizmendi and Márquez 2000; Devenish et al. 2009). If all IBAs were in the protected area system, the BBBP would have a significantly larger area covered by highly connected TDFs under protection (17% instead of 12%), contributing to international conservation targets (IPBES 2019, CBD 2020). The International Union for Conservation of Nature (IUCN) highlights the importance of incorporating all areas identified as priority areas in terms of ecosystems, species, and resources in PA systems, or to establish initiatives with landowners, government entities, or civil associations to contribute to practical conservation actions (Borrini-Feyerabend et al. 2013; Vimal et al. 2021). Understanding how bird species interact with forest connectivity is critical to develop targeted conservation strategies, allowing the allocation of the limited conservation resources to priority areas (such as IBAs), especially those at lower altitudes which are more susceptible to unsustainable uses, is crucial for effective conservation strategies in the study region.

Conclusions

Tropical dry forests of the BBBP showed changes in their structural connectivity between 2013 and 2018. The old-growth forests in the study area have lost 8% of their original cover; conversely, the coverage of secondary forests has increased by 10%. Also, our results revealed a landscape of changing forest connectivity within the BBBP and its implications for endemic bird species. In some cases, the increase in secondary forests favored connectivity among patches of old-growth forest and thus may reduce the potential adverse effects on bird populations. However, differences in landscape connectivity remain a challenge in maintaining biodiversity. Adaptive management practices may be needed to maintain connectivity and increase the quality of secondary forests for species dependent on tree species typical of old-growth forests.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: ORS, FJSS, AGC. Data curation: AGC. Formal analysis: FJSS, AGC, FLB. Investigation: AGC, FLB, ORS. Methodology: FLB, ORS, AGC, FJSS. Supervision: ORS, FJSS. Validation: FJSS, AGC. Visualization: AGC. Writing - original draft: AGC, ORS, FJSS. Writing - review and editing: FLB.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

Supplementary material 1

Information related to the species used as study case

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Data type: pdf

Link: https://doi.org/10.3897/natureconservation.55.120594.suppl1

Supplementary material 2

INEGI's vegetation covers grouped to be included in the Tropical Dry Forest class

Authors: Alejandra Galindo-Cruz, Francisco Javier Sahagún-Sánchez, Fabiola López-Barrera, Octavio Rojas-Soto

Data type: pdf

Link: https://doi.org/10.3897/natureconservation.55.120594.suppl2

Supplementary material 3

Net change in area and total species range percentage for each connectivity class.

Authors: Alejandra Galindo-Cruz, Francisco Javier Sahagún-Sánchez, Fabiola López-Barrera, Octavio Rojas-Soto

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Research Article

Paleoclimate changes and ecosystem responses of the Bulgarian Black Sea zone during the last 26000 years

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Abstract

Multi-proxy analysis (spore-pollen, dinoflagellate cysts, other non-pollen palynomorphs (NPPs), radiocarbon dating and lithology) was performed on marine sediments from three new cores retrieved during the two cruise expeditions on board the Research Vessel “Akademik” in 2009 and 2011. The Varna transect comprises three cores extending from the outer shelf, continental slope and deep-water zone. The record spans the last 26000 years (all ages obtained in this study are given in calendar years BP (cal. yrs BP)). The pollen record reveals the spreading of steppe vegetation dominated by *Artemisia* and Chenopodiaceae, suggesting cold and dry environments during the Late Pleniglacial – Oldest Dryas (25903–15612 cal. yrs BP). Stands of *Pinus* and *Quercus* reflect warming/humidity increase during the melting pulses (19.2–14.5 cal. ka BP) and the Late Glacial interstadials Bølling and Allerød. The Younger Dryas (13257–11788 cal. yrs BP) coldest and driest environments are clearly demonstrated by the maximum relative abundance of *Artemisia* and Chenopodiaceae. During the Early Holocene (Preboreal and Boreal chronozones, 11788–8004 cal. yrs BP), *Quercus* appeared as a pioneer species and, along with other temperate deciduous arboreal taxa, formed open deciduous forests as a response to the increased temperature. The rapid expansion of these taxa indicates that they survived in Glacial refugia in the coastal mountains. During the Atlantic chronozone (8004–5483 cal. yrs BP), optimal climate conditions (high humidity and increased mean annual temperatures) stimulated the establishment of species-rich mixed temperate deciduous forests. During the Subboreal chronozone (5483–2837 cal. yrs BP), *Carpinus betulus* and *Fagus* expanded simultaneously and became more important components of mixed oak forests and probably also formed separate communities. During the Subatlantic chronozone (2837 cal. yrs BP to pre-industrial time), climate-driven changes (an increase of humidity and a cooling of the climate) appear to be the main drivers of the specific vegetation succession expressed by increased abundance of *Alnus*, *Fraxinus excelsior* and *Salix* along with lianas, suggesting formation of flooded riparian forests (so called ‘Longoz’) lining the river valleys along the Black Sea coast. The first indicators of farming and other human activities have been recorded since 7074 cal. yrs BP. The dinoflagellate cyst (dinocyst) assemblages have been analysed to assess the changes in the Black Sea environment over the last 26000 years in terms of fluctuation in paleoproduction and surface water conditions related to changes in climate, freshwater input and Mediterranean water intrusion. Two major dinocyst assemblages were distinguished: one dominated by stenohaline freshwater/brackish-water species and a successive one dominated by euryhaline marine species.

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The changes in the composition of the assemblages occurred at 7668 cal. yrs BP. The abrupt decrease of stenohaline freshwater/brackish-water species *Pyxidinopsis psilata* and *Spiniferites cruciformis* was followed upwards by a gradual increase in euryhaline marine species, such as *Lingulodinium machaerophorum*, *Spiniferites belerius*, *S. bentorii* and acritarch *Cymatiosphaera globulosa*. The first occurrence of euryhaline marine species took place synchronously with the onset of sapropel deposition. Modern marine conditions were established after 6417 cal yrs BP when an abundance of Mediterranean-related species, such as *Operculodinium centrocarpum* and *Spiniferites mirabilis*, along with other heterotrophic species, occurred. After the stable cold and dry environment during the Last Glacial Maximum, the phytoplankton record of core AKAD 11-17 shows that *Pediastrum boryanum* var. *boryanum* has a cyclical abundance associated with the deposition of four red-brown clay layers between 19.2 and 14.5 cal. ka BP. This event is associated with the major melting phase of European Ice drained by the Danube and Dnieper Rivers in response to climate warming observed after the end of the Last Glacial Maximum. During the Early Holocene, *P. psilata*, characterised by a preference to warmer temperatures, demonstrates its ecological optimum for growth concerning SST reaching maximum relative abundance of 94% between 11072 and 8638 cal. yrs BP. This maximum was interrupted by an abrupt significant short-term decrease in the relative abundance of *P. psilata* centred between 8500 and 8300 cal. yrs BP reflecting cold conditions similar to those of Younger Dryas. This finding, also confirmed by the rapid significant decrease of arboreal pollen, particularly of *Quercus* in the same studied core, is considered a regional expression of the well-known ‘8.2 ka cold event’ which is commonly linked to a meltwater-related perturbation of the Atlantic Meridional Overturning Circulation (AMOC) and associated collapse of oceanic northward heat transport. Our fossil pollen and dinocyst data confirm that the high amplitude temperature anomaly associated with ‘the 8.2 ka cold event’ may have also occurred in south-eastern Europe, at lower latitudes of the western Black Sea coastal area, most probably due to atmospheric transition and/or river discharge.

Key words: Dinoflagellate cyst, non-pollen palynomorphs, radiocarbon dating, spore-pollen analysis

Introduction

The Black Sea, as an almost isolated marginal sea, is particularly sensitive to paleoenvironmental changes and, therefore, Black Sea sediments provide an excellent opportunity for high-resolution studies of past climatic, vegetation, human activity and hydrological changes in the catchment (Bahr et al. 2005). In contrast to shelf records which are affected by erosion during lowstands, pollen and dinocyst records from the continental slope and deep water Black Sea cores are of particular interest as they can provide almost uninterrupted sequences covering the Late Pleistocene and Holocene and can be used to obtain an independent record of regional climate change and land-based interpretation of the reconstructed vegetation and paleohydrological regime. These reconstructions are able to describe the interaction between climate and vegetation and also to clarify the role of coastline and other geomorphological changes, salinity and impacts of human activities in the Black Sea region (Cordova et al. 2009).

The Black Sea sediments have been intensively investigated by multi-proxy analysis during the last five decades. The biostratigraphic investigations of

Quaternary marine sediments taken by the Scientific-Research vessels ``Atlantis-2” and “Glomar-Challenger” established a baseline chronostratigraphy. Palynological investigations of sediments from the deep-water zone allowed Traverse (1974, 1978a, 1978b), Koreneva and Kartashova (1978) and Koreneva (1980) to outline the stratigraphy of sediments, vegetation dynamics and climate changes along the Black Sea coast from the end of the Pliocene through the Pleistocene at a very low resolution of tens of thousands of years. The first detailed marinopalynological investigations of the western Black Sea shelf are those of Roman (1974), Bozilova et al. (1979) and Komarov et al. (1979). Based on marinopalynological data of the western Black Sea sediments, the climatic changes during the Late Glacial and Holocene were estimated and considered to be the main drivers for vegetation changes along the Bulgarian Black Sea coast (Shimkus et al. 1977; Komarov et al. 1978; Bozilova et al. 1979; Chernyishova 1980; Filipova and Dimitrov 1987; Atanassova 1990, 1995, 1999, 2005; Atanassova and Bozilova 1992; Mudie et al. 2007). Palaeoecological changes during the Quaternary and possible sea-level fluctuations during the Holocene were traced by Filipova-Marinova (2003a, 2003b, 2007); Filipova-Marinova and Christova (2004), Filipova-Marinova et al. (2004) and Hiscott et al. (2007). Mudie et al. (2007) showed that organic-walled microfossils including pollen, spores and dinocysts are well-preserved and abundant in deep-water Pleistocene and Holocene sediments. These authors presented pollen assemblages for the last 33000 years and reported the first high resolution Holocene pollen influx data for the SW Black Sea shelf. Filipova-Marinova et al. (2013) reported the first high-resolution palynostratigraphy of Late Quaternary sediments and the chronologically defined vegetation stages with their specific features, particularly a short-term cooling of the Holocene climate, associated with the ‘8.2 ka cold event’ identified for the first time in marine records from the central Bulgarian Black Sea area. Vegetation and environmental dynamics in northern Anatolia during the last 18000 years are reconstructed using multi-proxy records from the southern Black Sea shelf (Shumilovskikh et al. 2012). Mudie et al. (2002) showed the close correlation between marine pollen assemblage zones in the Marmara and southern Black Sea with lakes in northern Turkey and Bulgaria. Quantitative paleovegetation reconstruction and correlation of pollen data of 99 sequences from the Black, Marmara and Azov Seas are presented by Cordova et al. (2009). First palynostratigraphy of western Black Sea sediments was proposed by Filipova-Marinova (2006a), based on 12 representative cores.

The pioneer work of Wall et al. (1973) enabled the use of dinocysts as potential paleoceanographic proxies. Since the 1980s, numerous studies from the Black Sea permitted the definition of distribution patterns in regional levels and have led to determination of the relationship between dinocyst assemblages and sea surface water conditions (De Vernal et al. 1994; Marret 1994; Matthiessen 1995; Mudie and Harland 1996; Mudie et al. 2001; Mudie et al. 2002; Marret and Zonneveld 2003; Marret et al. 2009; Mertens et al. 2012). The studies of van Geel (2001), Brenner (2001), Mudie et al. (2002) and Marret et al. (2009) describe non-pollen palynomorphs (fungal spores and remains of hyphae) as important markers of salinity, nutrient loading and human activity, including ballast discharge, farming and soil erosion. Mudie et al. (2002) and Marret et al. (2009) consider fungal remains as an index of terrigenous sediment influx and transport by large rivers. The Late Quaternary history of connection of the

Black Sea to the eastern Mediterranean, especially the timing and conditions during the Holocene transition of the Black Sea from freshwater/brackish-water to marine has been intensively debated: Ryan et al. (1997, 2003), Aksu et al. (2002), Major et al. (2002), Bahr et al. (2005, 2006), Hiscott et al. (2007), Yanko-Hombach (2007); Londeix et al. (2009), Marret et al. (2009), Verleye et al. (2009) and Herrle et al. (2018). In addition to the Holocene connection with the Marmara Sea, the Black Sea experienced a period of connection to the Caspian Sea when meltwater from Scandinavian ice sheets raised the water level of the Caspian Sea over the Manych depression. This period is documented in the Black Sea by the deposition of a series reddish-brown clay layers (Major et al. 2002; Bahr et al. 2005, 2008).

Recent studies in the western Black Sea are focused mainly on palaeoecological changes during the Late Glacial and Holocene. There is a lack of uninterrupted Late Quaternary sediments from the northern Bulgarian Black Sea area adjacent to Varna. Therefore, in order to obtain appropriate records which would allow a better more detailed description of the pollen and dinocyst stratigraphy and more precise palaeoecological reconstructions of the Bulgarian sector of the Black Sea, two expeditions by the Research Vessel “Akademik” in 2009 and 2011 were carried out. A total of 105 new samples were taken for multi-proxy analysis of sediments from three representative cores: AKAD 11-17 (deep-water zone, water depth: 1805 m, core length: 228.5 cm), AKAD 09-10 (zone of the continental slope, water depth: 1000 m, core length: 240 cm) and AKAD 09-15 (outer shelf zone, water depth 164 m, core length 377 cm).

The aim of this study is to establish the palaeoclimatic, palaeohydrological and environmental dynamics of the Bulgarian Black Sea coastal area during the Late Pleistocene and Holocene, as well as to evaluate the timing and extent of the passage between various events previously described in other studies, based on multi-proxy analysis.

Regional setting

Present-day Oceanography of the Black Sea

The Black Sea (Fig. 1) is located between south-eastern Europe and Asia. It has an area of approximately 432,000 km^2 and a maximum water depth of 2258 m. It is the largest semi-enclosed inland basin in the world, where the deep-waters and surface waters are not mixed (Nikishin et al. 2003; Murray et al. 2007). The oxygenated surface layer overlies an anoxic deeper layer with elevated H_2S concentrations up to 380 µM (Murray et al. 2007). The Black Sea has an extremely large drainage basin of more than two million km^2, collecting the water from almost all the European countries, except the westernmost ones. The hydrologic configuration of the Black Sea is controlled by basin bathymetry and also by fluvial inputs from the discharge of the largest European rivers including the Danube, Dnieper, Dniester and the Southern Bug. Ukrainian rivers Dnieper, Southern Bug and Dniester contribute about 65 km^3/yr and the Danube River is with a mean water discharge of about 200 km^3/yr (Panin 2008). These inputs, plus those from smaller rivers from the Bulgarian and north Turkish margin, create a surface low salinity layer across the whole of the Black Sea. The net freshwater input to the Black Sea is about 300 km^3/yr and derives from the riv-

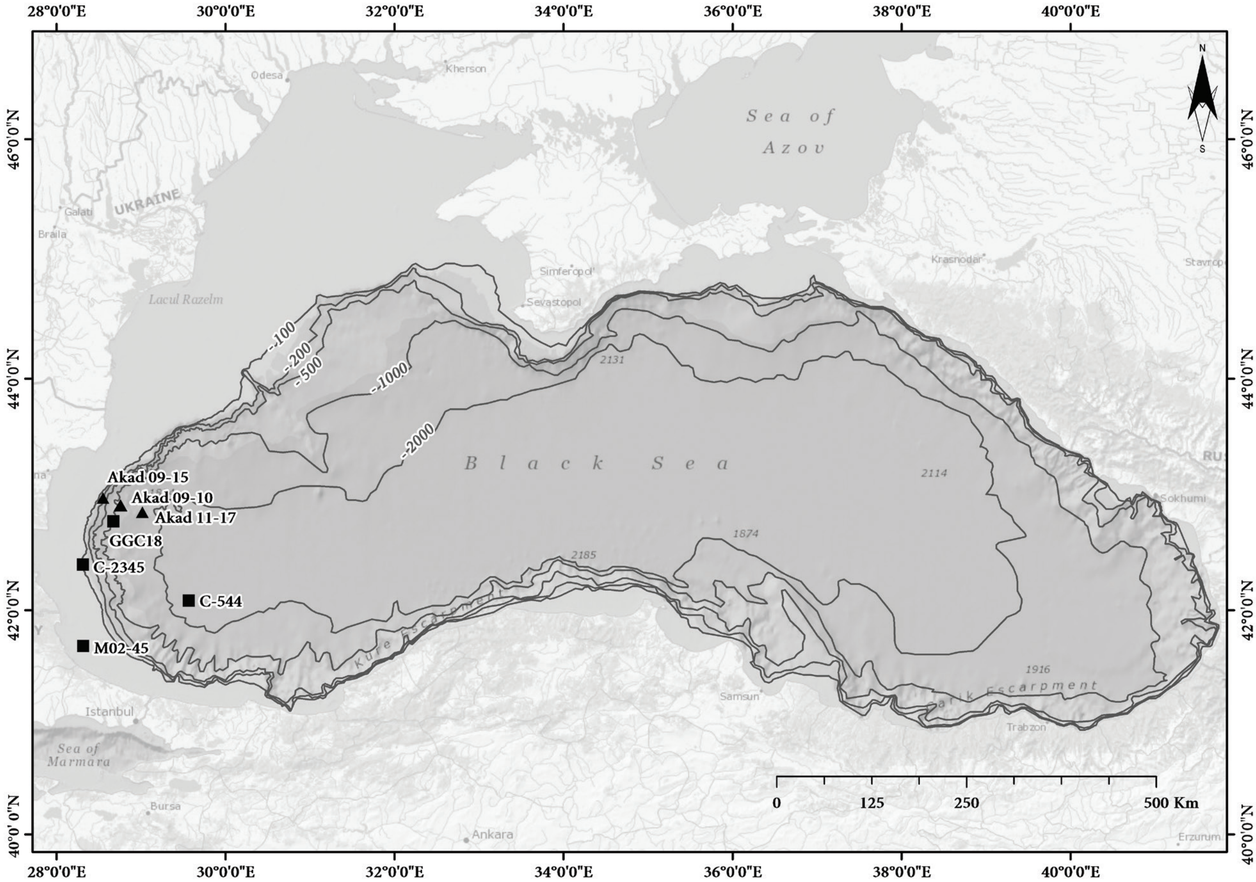

Figure 1. Location of the studied cores (black triangle) AKAD 11-17, AKAD 09-10 and AKAD 09-15 and four reference cores (black square) discussed.

er input plus precipitation. The Black Sea is presently connected to the Global Ocean through the narrow and shallow Bosphorus and the Dardanelles Straits that limit the salinity and oxygen provided to the Black Sea (Özsoy et al. 1994; Kerey et al. 2004). In addition to this marine connection, the Black Sea currently also has connection with the freshwater Sea of Azov through the narrow Strait of Kerch that has a constant outflow of waters to the Black Sea (Kosarev et al. 2007). The water exchange occurs between the Black and Marmara Seas via the Bosphorus Strait as a two-layer water flow (Özsoy et al. 1994). About 600 km^3/yr cooler (5–15 °C) and less saline (18–20‰) water mass flows out of the Black Sea through the Bosphorus Strait and, from there, through the Sea of Marmara into the Dardanelles Strait as a surface outflow into the Mediterranean (Özsoy et al. 1995). This surface outflow is compensated by about 300 km^3/yr of warmer (15–20 °C) and more saline (38–39‰) mixed Marmara and Aegean Sea waters that flow into the Black Sea through the Bosphorus as a deep inflow. This produces a density-stratified water column due to the largely varying salinities entering the region. The surface layer 0–50 m is well oxygenated, while the deeper water layer 100–2243 m is highly anoxic and rich in sulphides. The approximate permanent location of the halocline is 50 to 200 m (Murray et al. 2007). Today, the surface water circulation in the Black Sea consists of two large cyclonic (counterclockwise) central gyres that define the eastern and western basins. The gyres are bounded by the wind-driven 'Rim current' that flows along the edge of the continental shelf and above the continental slope around the whole basin (Oguz et al. 1993). Data from autonomous profiling floats have shown currents typically have a velocity of 15 cm/s at 200 m depth along the 'Rim Current' jet around the basin. At depths of 750 m and 1500 m, current velocities of 5 cm/s have been recorded with the deeper current closely following the topography along the southern margin of the Black Sea. Outside the Rim Current, numerous quasi-permanent coastal eddies are formed as a result of upwelling around the coastal apron and "wind curl" mechanisms.

Sedimentology of the Black Sea

The most recent pelagic sediment layers in the Black Sea can be divided into three units:

- Unit I is represented by micro-laminated sediments, rich in plankton-derived carbonates finely laminated coccolith-bearing ooze. The calcareous material is derived from marine coccolithophorid *Emiliania huxleyi*, which have formed part of the Black Sea plankton since 2700 years BP (Jones and Gagnon 1994). Blooms of *E. huxleyi* still occur in the Black Sea each year during late spring and summer. This unit was deposited in oxygen-depleted bottom waters.
- Unit II sediments contained micro-laminated sapropels, deposited under anoxic marine conditions between 2720 ± 160 ^{14} C yrs BP and 7540 ± 130 ^{14}C yrs BP. The base of the sapropel has been dated to 7540 ± 130 ^{14}Cyrs BP in cores running in depth from 400 to 2200 m suggesting that anoxia developed at the same time throughout the interior Black Sea (Jones and Gagnon 1994). The onset of Unit II is characterised by the occurrence of finely-laminated layers rich in aragonite crystals (Ross and Degens 1974; Jones and Gagnon 1994; Soulet et al. 2011) and by a sharp increase in Total Organic Carbon (Bahr et al. 2008).
- Unit III sediments are older than 7540 ± 130 ^{14}Cyrs BP and were deposited when the Black Sea was a freshwater to brackish lake and are characterised by a mix of organic-poor clays and silts (Izdar and Ergün 1991; Hay et al. 1991). Unit III sediments have organic contents < 1%. Sediments consist of homogenous mostly centimetre-scale laminated muddy clay deposited under lacustrine conditions.

Geomorphology of the Bulgarian sector of the Black Sea

The large continental shelf in the north-western Black Sea narrows in the southerly direction. On the basis of the relief, shape, time of formation, character and speed of sedimentological processes, three geomorphological zones can be outlined in the western Black Sea shelf: littoral or inner, central and peripheral or outer (Dimitrov 1979). The littoral zone is considered to be of Holocene age. It extends from the coast to a depth of 20 to 50 m of the central part of the Bulgarian Black Sea coastal area. Active wave impact, erosion and accumulation are characteristic processes for this zone (Khrischev 1984). The littoral zone is separated from the central one by a depression 17–20 m deep on the northern Black Sea shelf and 65–70 m deep on the southern Bulgarian Black Sea shelf.

The central zone lies between 50 and 72 m in depth. Within it, three subzones run parallel to the coast: an inner depression, an area of depositional bars and a depositional plain. This zone experiences a high sedimentation rate, typically about 2.5 m/kyr (Khrischev 1984). The peripheral zone extends to depths between 90 m and 120 m. It is subdivided into an outer depression and an area of barrier bars and its low sedimentation rate is due to sediment removal by strong bottom currents (Dimitrov 1979). The north-western Black Sea shelf is covered only by a very thin blanket of Holocene sediments and, in some parts, the Holocene layers are completely absent due to the strong (> 50 $cm.s^{-1}$) cyclonic 'Rim current' (Oguz and Besiktepe 1999) which transports most of the suspended sediments alongside the coast to the south (Panin et al. 1999).

According to the general morphostructural plan of the Black Sea deep-water basin, the continental slope covers 25% of its surface. The transition of the shelf to the continental slope is gentle and has a convex-up profile. The continental slope of the Bulgarian Black Sea zone is characterised by deeply-indented relief including land-sliding complexes, fault slopes, ledges and submerged valleys and canyons. Nine systems of submerged valleys are established in the area (Alexiev 2002). The transition from the continental slope to the deep basin increases in the western Black Sea from 1100 m in the north to 2000 m in the south.

The continental foot is formed by the confluence of the sedimentation materials of the submerged delta valleys. The gentle transition of the steep continental slope to the abyssal plain is accomplished by its slightly undulating plain surface. The formation of the modern shape of the continental slope took place mainly during the Pleistocene. The large input of terrestrial sediments that are the main constructive material for the continental foot was disrupted by the breaking-off access of the coastal rivers to the outer zone of the continental slope during the Holocene. The limit between the continental foot and the abyssal plain is difficult to be located. However, the isobaths 2000–2100 m localise a typical abyssal plain which declines slightly towards the deepest part of the Black Sea Basin. The abyssal plain is the earliest formed morphological element of the Black Sea (Alexiev 2002).

Climate

According to Velev (2002), the Bulgarian Black Sea coast belongs to the Continental-Mediterranean climatic area and is influenced by three different climatic regimes. Climate in the northern part and the Eastern Danube lowland is affected by strong continental influences. Prevailing winds are northeasterly and mean annual precipitation is about 450–500 mm, with a maximum in June and a minimum in February. Mean January temperature is around 0 °C, dropping to -2 °C inland. In the south, the climate is transitional Mediterranean. Mean annual precipitation is estimated at about 500–600 mm, with rainfall mostly in the autumn-winter seasons. Mean January temperature is 2–3 °C and, in July, it is 22 °C. The dry summer period lasts from July to September. Winds blow mostly from the southeast and rarely from the northeast. Mean annual precipitation over the mountain areas of the coast (500 m a.s.l.) is about 600–1000 mm (Velev 2002). The western part of the coast is under the influence of the humid conditions of central Europe with precipitation more than 1000 mm/year.

Recent vegetation

The Bulgarian Black Sea coast covers a narrow strip of land located to the west of the Black Sea coastline. It is 375 km long and 30 to 50 km wide and includes Southern Dobrudzha, the Eastern Stara Planina Mountains (Balkan Range), Burgas Plain and the Eastern Strandzha Mountains. Climate controls and local topography play a dominant role in determining the pattern of highly-varied natural vegetation. This area is considered as a major pollen source area for the investigated core sediments with consideration of wind pattern, river input and gyre systems in the Black Sea. In addition to the vegetation distribution map, simplified characteristics of vegetation types are presented by Bondev (1991). According to this author, the study area falls within the Black Sea region of the Euxinian province of the European deciduous forest. The vegetation is represented by steppe vegetation, different types of temperate deciduous forests, reed, psammophytic and halophytic vegetation. Steppe vegetation of natural origin dominated by *Stipa capillata* L., *Agropyron brandzae* Pantu et Solac., *Koeleria brevis* Stev., *Stipa lessingiana* Trin. et Rupr., *Artemisia lerchiana* Weber, *Adonis vernalis* L., *Adonis volgensis* Steven ex DC and *Paeonia tenuifolia* L., amongst others, is preserved only in the northern Bulgarian Black Sea coastal area, in the South Dobrudzha region (Cape Shabla and Cape Kaliakra areas). Xerothermic forest communities dominated by *Quercus cerris* L. and *Q. frainetto* Ten. are widespread in the eastern Balkan Range area (Eastern Stara Planina Mts.) (Cape Emine area). Restricted areas on northern slopes and lower moisture ravines are occupied by stands of southeuxinian taxa such as *Quercus polycarpa* Schur. and *Fagus orientalis* Lipsky along with *Carpinus betulus* L., *Acer campestre* L., *Q. cerris* and *Tilia tomentosa* Moench. Xeromesophytic communities comprised of *Q. polycarpa* and *Carpinus betulus* are spread in lowland sites and on hilltops, as well as in the Strandzha Mountains. Mediterranean elements, such as *Quercus pubescens* Willd., *Carpinus orientalis* Mill., *Fraxinus ornus* L., *Phillyrea latifolia* L., *Celtis australis* L. and *Colutea arborescens* L. are distributed along the southern Black Sea coast. Relic southeuxinian forests of *Fagus orientalis*, with an undergrowth of evergreen shrubs (*Rhododendron ponticum* L., *Ilex aquifolium* L. and *Daphne pontica* L.) cluster the more humid ravines of the Strandzha Mts. (Veleka River, Rezovska River and Sozopol area). Riparian forests (so called Longoz) line rivers flowing into the Black Sea and coastal lakes. The main components of these forests are *Fraxinus oxycarpa* Willd., *Ulmus minor* Mill., *Carpinus betulus*, *Quercus pedunculiflora* C. Koch and *Alnus glutinosa* (L.) Gaerth. The most characteristic for these periodically flooded forests is the presence of lianas such as: *Hedera helix* L., *Periploca graeca* L., *Clematis vitalba* L., *Vitis vinifera* L. and *Smilax excelsa* L. Reed vegetation represented by communities dominated by *Phragmites australis* (Cav.) Trin. ex Steud., *Typha angustifolia* L., *T. latifolia* L. and *Schoenoplectus lacustris* (L.) Palla are spread along the rivers and the periphery of coastal lakes (Kochev and Jordanov 1981). Psammophytic vegetation includes communities mainly of *Leymus racemosus* (Lam.) Tzvel. ssp. *sabulosus* (Bieb.) Tzvel., *Ammophilla arenaria* (L.) Link, *Centaurea arenaria* Bieb. ex Willd., *Galilea mucronata* (L.) Parl and shrub communities with *Cionura erecta* (L.) Grsb. growing on the sandy beaches and dunes. Halophytic vegetation presented by communities of *Salicornia europaea* has limited distribution in habitats with high salinity.

Material and methods

Coring and sampling

Sediments pertinent to this study were collected during expeditions in 2009 and 2011 onboard the Research Vessel *Akademik* owned by the Institute of Oceanology of the Bulgarian Academy of Sciences. The two cruise expeditions recovered a series of sediment cores on a number of shallow-to-deep transects from the Bulgarian Black Sea area. The Varna transect consists of three cores taken from the shelf, continental slope and deep-water zone of the Black Sea (Fig. 1). Sediment core Akad 09-10 was collected with a Vibracore device, because of the feature of the Black Sea consolidated shelf sediments and cores Akad 09-15 and Akad 11-17 were collected with Gravity Corer device. The both devices have the same dimensions of the diameter of a metal tube and a length of metal tube, respectively Ø12 cm and 4 m. The recovered sediment cores were cut in 1-m sections on board and carried to the lab. The sediment was pulled from the steel tube in its plastic steeve, split lengthwise in two half cylinders, photographed, visually lithologically described and sampled. Samples of 1 cm^3 were removed from the centre of the split section to avoid contamination with any younger sediment smeared downwards during the insertion of the sediment into the plastic steeve. The cores were lithologically described (Fig. 2) and sampled at every 10 cm on the board of the Research Vessel “Akademik” for further analyses.

Core Akad 11-17 (42°51'13.50"N, 29°01'08.50"E) was recovered from a water depth of 1805 m in the Black Sea deep-water zone (Fig. 1). The investigated length of the core is 229 cm (Fig. 2). From the core base up to the depth of 59 cm, the core sediments are typical lacustrine clay sediments with alternately light and dark layers, deposited under freshwater to brackish conditions (Unit III). From 229 to 133 cm, red-brown clay with grey clay is deposited in several pulses. These red-brown clay layers can be found in the sediments from the whole western Black Sea Basin. In the interval 155–153 cm were described six sand silt lamination layers. Above the red-brown clay was described an alternation of light grey clay with dark grey clay with a band of sulphides. At 69, 72, 72.5, 76, 80, 84, 90, 109–110 cm was described 1 mm thin sand silt lamination. The marine units II and I contain organic-rich microlaminated sapropel sediments and carbonate-rich finely laminated coccolith ooze and are ascertained in the core intervals from 59–27 cm and 27–0 cm to the top, respectively (Fig. 2). The base of the sapropel deposition in the core Akad 11-17 was described at 59 cm. The base of the coccolith ooze was described at 27 cm.

Core Akad 09-10 (42°54.8'N, 28°45.6'E) was recovered from a water depth of 1000 m on the Bulgarian Black Sea continental slope (Fig. 1). The investigated length of the core is 242 cm (Fig. 2). From 242 to 122 cm, sediments are typically lacustrine (Unit III) with homogeneous light to dark grey clay deposited under freshwater to brackish conditions. An important lithological feature of Unit III is the deposition of red-brown clay layers within the range from 236 to 216 cm. The onset of marine sapropel sediment deposition is lithologically determined at 126 cm (Unit II). The sapropel mud is finely laminated, more firm, dark green-grey with three white laminae in the interval from 122 to 123 cm in the lower part of marine Unit II. The boundary between Unit II and Unit I is delimited by the presence of coccolith ooze as well as the stable appearance of *Emiliania huxleyi* at 50 cm.

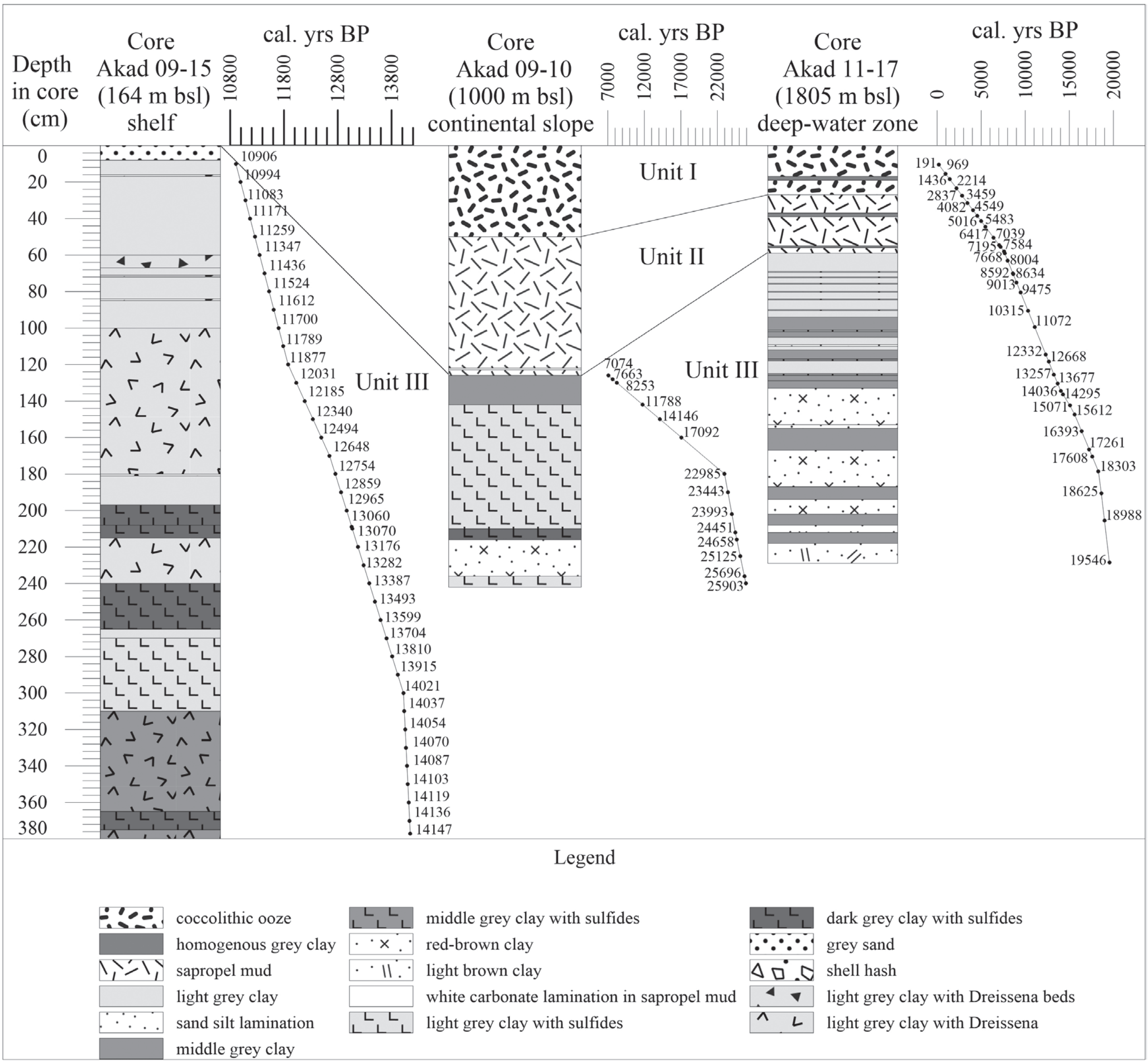

Figure 2. Lithological and geochronological correlations of the studied cores AKAD 11-17, AKAD 09-10 and AKAD 09-15.

Core Akad 09-15 (42°58.628'N, 28°33.147'E) was recovered from a water depth of 164 m on the Bulgarian Black Sea shelf (Fig. 1). The investigated length of the core is 380 cm. In this core, only lacustrine sediments (Unit III, lutite) were established (Fig. 2). From 380 to 8 cm, sediments are light to dark grey clay with rare whole disarticulated small freshwater *Dreissena* sp. and more fragmented shells. At 180 cm is described a layer of shell hash. In the interval from 8 to 0 cm, sediments are of grey sand.

Pollen analysis and zonation

A total of 105 samples were selected for palynological, dinoflagellate cyst and other non-pollen palynomorph analyses. Each sample consists of 1 cm^3 of wet sediment. The sampling interval was 10 cm. Sediments characterised by much higher sedimentation rates were sampled with varying resolution (Figs 2–8). All samples were processed according to the standard procedure of Faegri and

Iversen (1989). The laboratory technique includes treatment with hot 10% hydrochloric acid (HCl), cold 40% hydrofluoric acid (HF), solution of zinc chloride ($ZnCl_2$), 10% potassium hydroxide (KOH), glacial acetic acid) CH_3COOH), 2 min acetolysis and glacial CH_3COOH and ethanol (C_2H_5OH). The removal of mineral components was performed using sodium pyrophosphate and hydrofluoric acid (Birks and Birks 1980). The obtained suspension is stored in glycerine and then used for microscope analysis. A minimum of 500 up to 1000 identifiable pollen grains from terrestrial plants and a minimum of 100 up to 300 dinoflagellate cysts and non-pollen palynomorphs were counted in each sample to ensure statistical significance.

Pollen types were identified using the reference collection of modern pollen types of the Museum of Natural History of Varna, keys in Erdtman et al. (1961), Beug (1961, 2004), Moore and Webb (1978), Faegri and Iversen (1989) and photographs of Reille (1992, 1995). Dinoflagellate cysts taxonomy is based on Wall et al. (1973) and Marret et al. (2004). Other non-pollen palynomorphs (NPPs) were identified by the keys in van Geel et al. (1981), van Geel (2001) and Jankovska and Komarek (2000). The total pollen sum (PS) used for percentage calculations of the individual percentages includes arboreal pollen (AP) and non-arboreal pollen (NAP). Pollen of aquatics, spores, dinoflagellate cysts and other NPPs are excluded from the PS. Their presence was expressed as percentage of the total PS (van Geel et al. 1981). The software TILIA v.1.17.16 (Grimm 2011) was used for pollen and dinocyst percentage calculations and construction of spore-pollen and dinocyst diagrams. A 10× exaggeration of the horizontal scale was used to show changes of low-percentage taxa. The spore-pollen diagrams were subdivided into fifteen local pollen assemblage zones (LPAZ) (Figs 3, 5, 7) and two local dinoflagellate assemblage zones (LDAZ) (Figs 4, 6, 8) using visual inspection of the main changes in taxa composition (described in details in Table 2). The cluster analysis programme CONISS (Grimm 1987) is applied for more precise zonation (Figs 3, 5, 7). These LPAZ are correlated with the established regional pollen assemblage zones (RPAZ) IV to IX after Filipova-Marinova (2006a) (Table 3). In the spore-pollen diagrams, taxa are ordered by their plant functional type (Prentice et al. 1996).

Correlations and interpretation of data from spore-pollen analysis

The stratigraphic subdivision of sediments from the western Black Sea area is based on qualitative interpretation of the pollen and spore assemblages and the vertical and spatial distribution of selected indicator taxa. The pollen assemblage zones distinguished are based entirely on the percentage abundances of the predominant and indicator pollen and spores in the assemblages. Pollen spectra delimited for each assemblage zone were obtained from several samples in each sediment core and provide a picture of vegetation changes for the period represented by sediments. According to Birks (1973), the assemblage zones for an individual core have to be considered as local pollen assemblage zones (LPAZ). As these zones are present in two to several sediment cores in adjacent areas, they are further delimited as regional pollen assemblage zones (RPAZ). These RPAZ can be proposed as a regional biostratigraphy and they can be correlated in time and space with concurrent chronostratigraphic scales (Berglund 1983). These LPAZ and RPAZ (Table 3) are tentatively correlated to

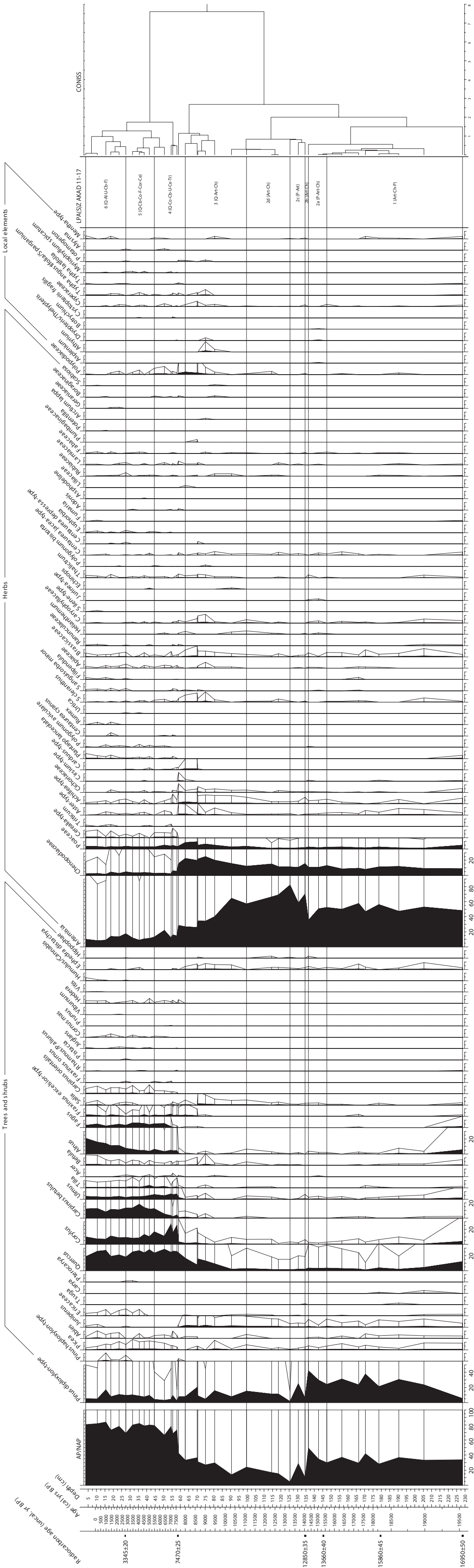

Figure 3. Percentage spore-pollen diagram of Core AKAD 11-17 (Black Sea deep-water zone).

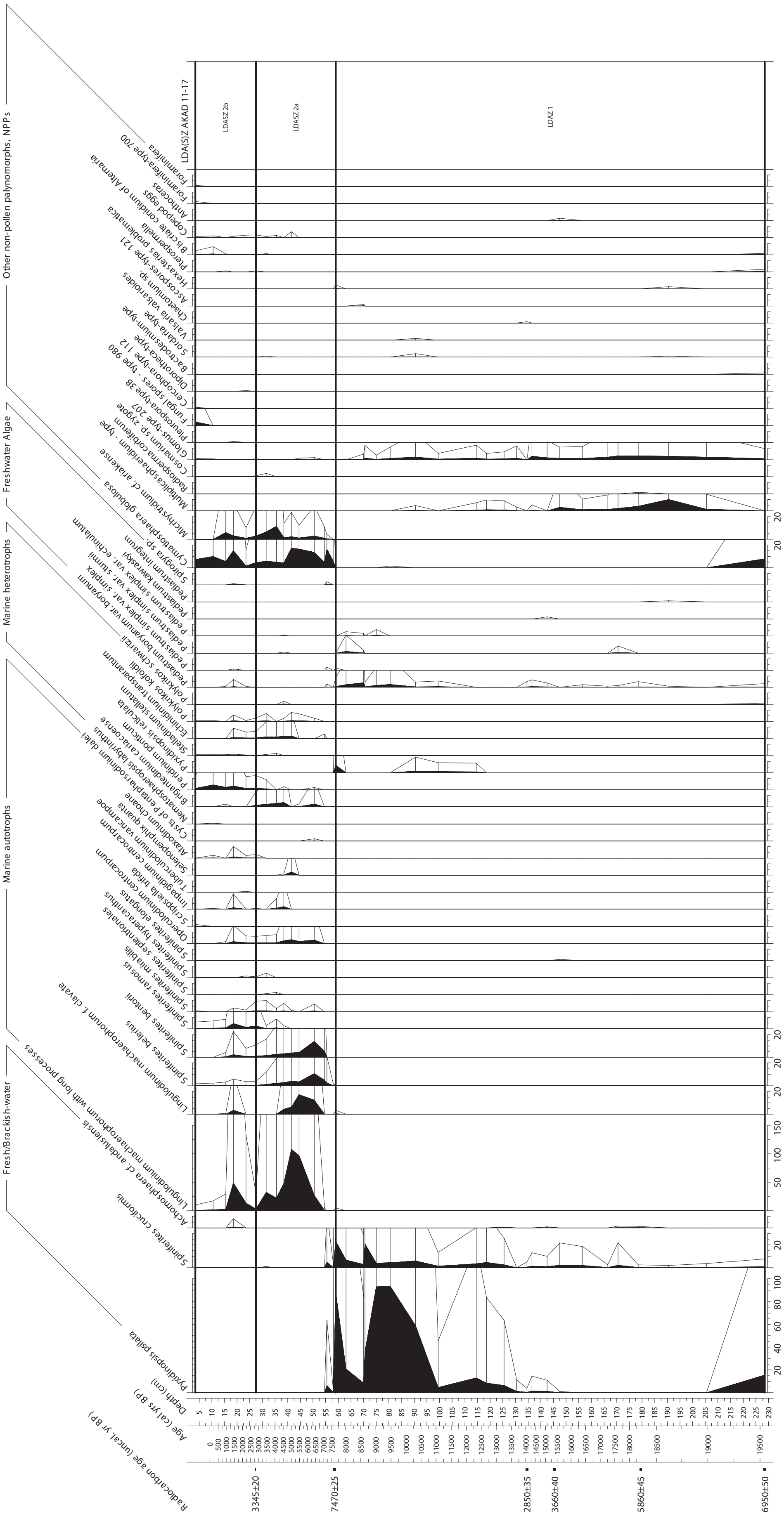

Figure 4. Percentage diagram of dinocysts, other algae and non-pollen palynomorphs (NPPs) in Core AKAD 11-17 (Black Sea deep-water zone).

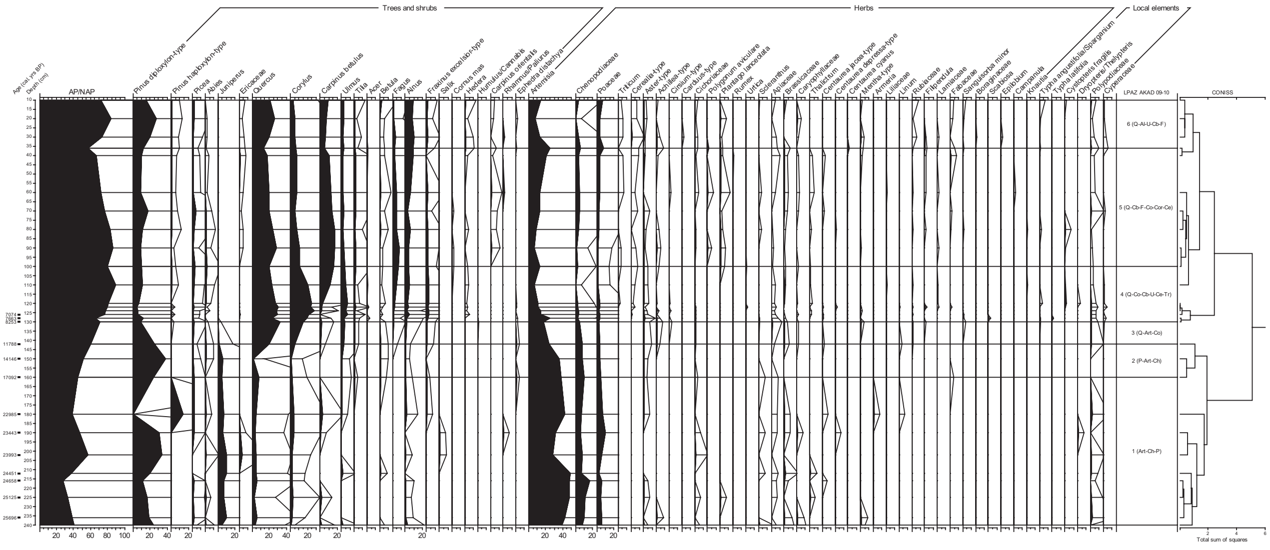

Figure 5. Percentage spore-pollen diagram of Core AKAD 09-10 (Black Sea continental slope).

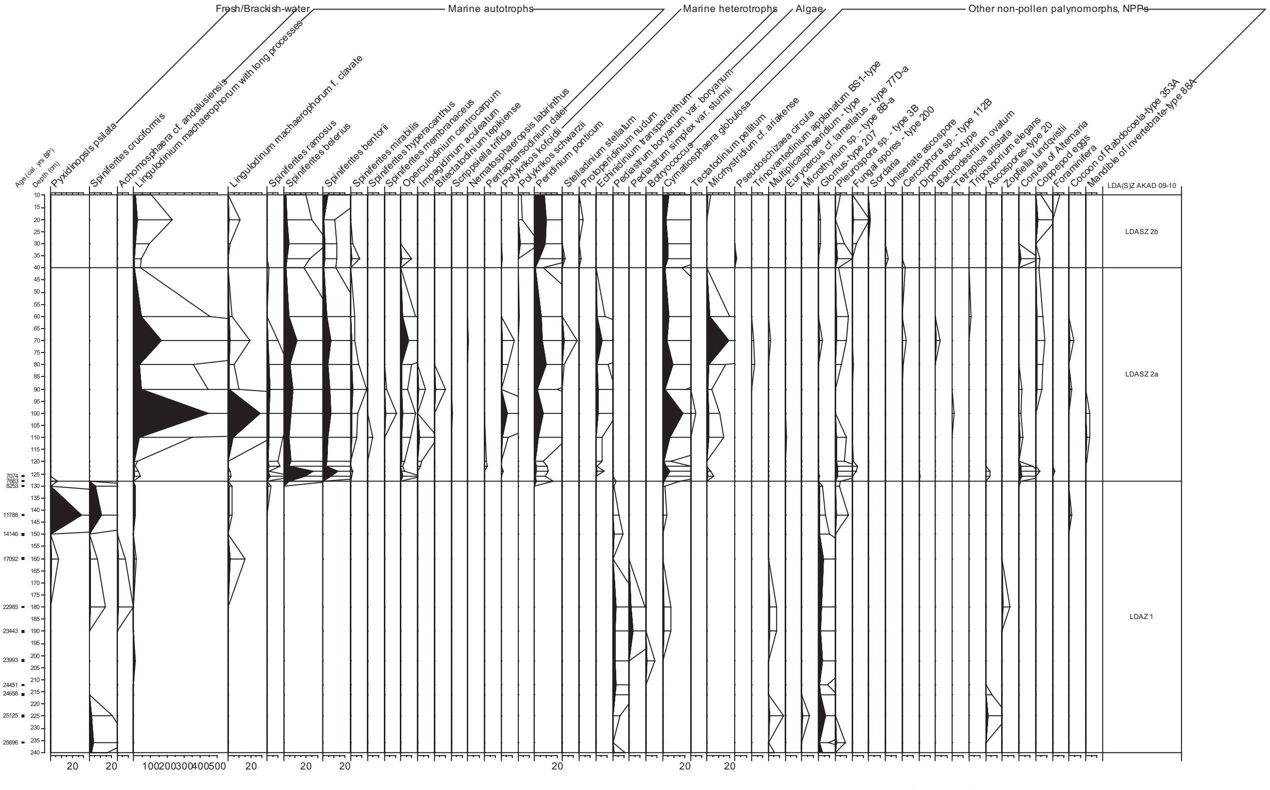

Figure 6. Percentage diagram of dinocysts, other algae and non-pollen palynomorphs (NPPs) in Core AKAD 09-10 (Black Sea continental slope).

the Regional Black Sea stratigraphic scale of Shopov (1991), Archaeological Chronology of Todorova (1986) and to the traditional northern European climatostratigraphy of Blytt–Sernander (1876–1908).

Radiocarbon dating and age modelling

Radiocarbon (^{14}C) dating was performed on 15 selected sediment layers (Table 1) spanning all three lithological units and an “Age vs. Depth” model was estab-

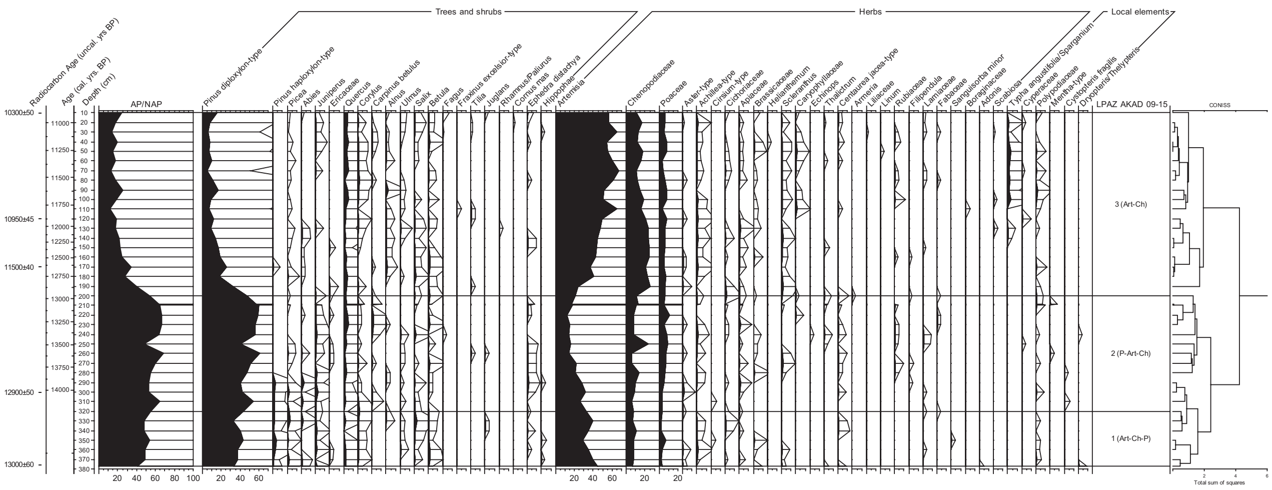

Figure 7. Percentage spore-pollen diagram of Core AKAD 09-15 (Black Sea shelf).

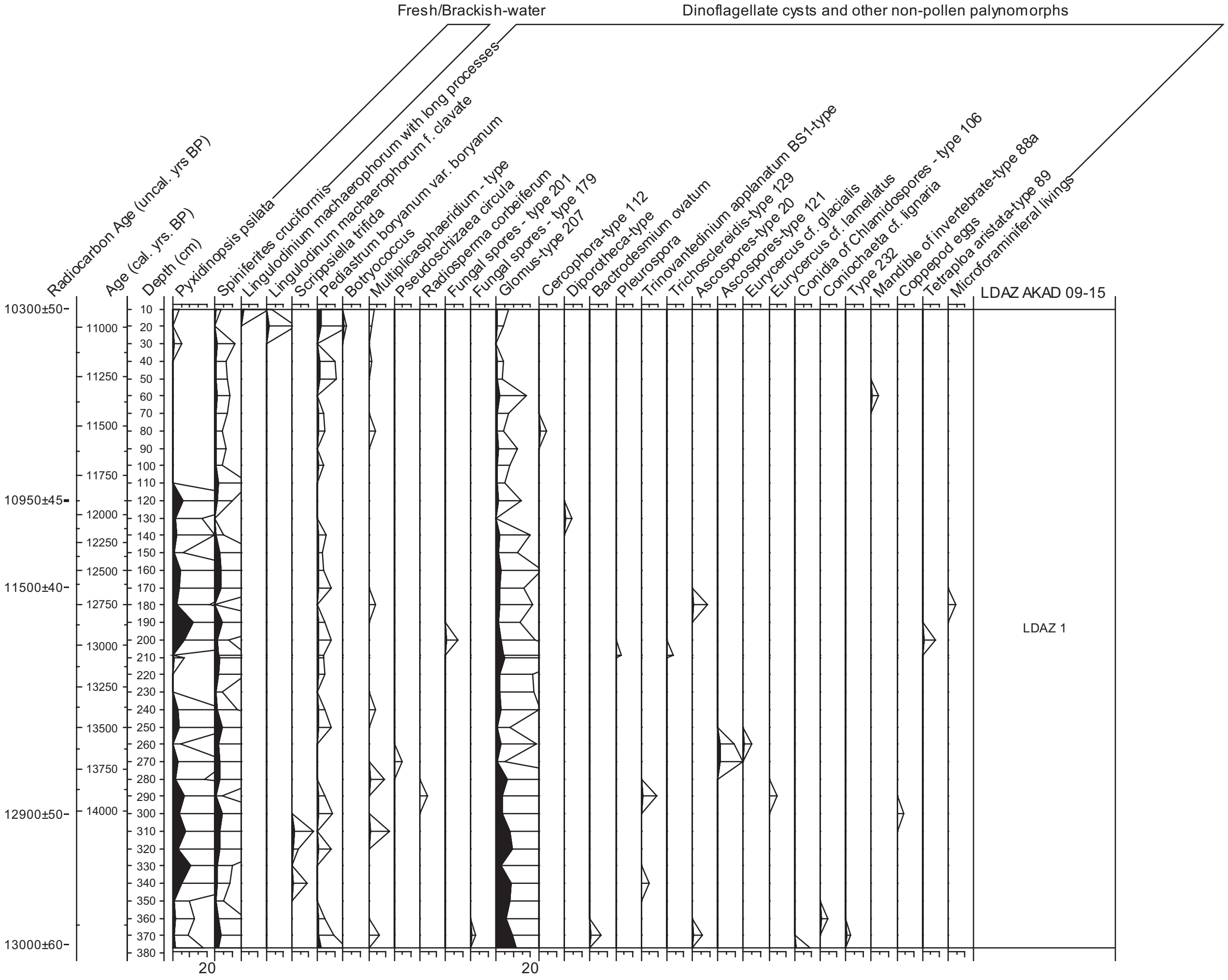

Figure 8. Percentage diagram of dinocysts, other algae and non-pollen palynomorphs (NPPs) in Core AKAD 09-15 (Black Sea shelf).

lished for all three cores (Fig. 2). Nine sediment samples from Core Akad 09-10 and Akad 09-15 were selected for ^{14}C AMS dating of bulk organic carbon at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) Facility,

Table 1. Chronology of the studied cores AKAD 11-17, AKAD 09-10 and AKAD 09-15.

Core AKAD	Depth (cm)	Lab. No	Material dated	Uncalibrated yrs BP	Calibrated yrs BC (2σ range)	Calendar yrs BP*
11-17	27	GdA-2598	Bulk	3345±20	909–732	2759
11-17	58.5	GdA-2599	Bulk	7470±25	5722–5537	7584
11-17	134.5	GdA-2601	Bulk	12850±35	12170–11866	13971
11-17	145.5	GdA-2602	Bulk	13660±40	13687–13233	15395
11-17	179.5	GdA-2603	Bulk	15860±45	16587–16175	18346
11-17	228.5	GdA-2604	Bulk	16950±50	17798–17343	19534
09-10	126	OS-79014	Bulk	6920±40	5271–4978	7074
09-10	180	OS-79016	Bulk	19850±170	21500–20592	22985
09-10	212	OS-79017	Bulk	21100±170	23073–22054	24451
09-10	240	OS-79018	Bulk	22400±230	24444–23510	25903
09-15	10	OS-79753	Mollusk	10300±50	9151–8757	10906
09-15	120	OS-79754	Mollusk	10950±45	10181–9582	11877
09-15	170	OS-90808	Mollusk	11500±40	10807–10603	12648
09-15	300	OS-79756	Mollusk	12900±50	12234–11892	14021
09-15	375	OS-74855	Mollusk	13000±60	12562–11954	14144

* detrital carbon correction (reservoir effect) of 363 ± 41 years is applied.

WHOI, USA. Six more sediment samples from Core Akad 11-17 were selected for ^{14}C dating at the Gliwice Radiocarbon Laboratory of the Silesian Institute of Technology, Poland. An "Age vs. Depth" model (Fig. 2) was developed for all three cores by 2σ-range calibration of the available radiocarbon dates (Table 1, Fig. 2) with the online calibration programme CALIB 7.1. (Stuiver et al. 2017), using the MARINE13 curve (Reimer et al. 2013) with application of a Reservoir effect (ΔR) of 363 ± 41 yrs (Siani et al. 2000) in order to correlate the obtained results with the available geochronological and archaeological data. The sedimentation rate for this model was calculated by Tilia software (Grimm 2011). This method allows each pollen sample as well as the LPAZ boundaries to be assigned to the relevant calendar age in years BP. All the dates in the pollen diagrams and the text are given as calendar years BP (cal. yrs BP).

Results

On the pollen diagrams, six LPAZ from Core AKAD 11-17 (LPAZ AKAD 11-17 1–6) and four LPASZ (LPASZ AKAD 11-17 2 a, b, c, d) (Fig. 3); Six LPAZ from Core AKAD 09-10 (LPAZ AKAD 09-10 1–6) (Fig. 5); Three LPAZ from Core AKAD 09-15 (LPAZ AKAD 09-15 1–3) (Fig. 7) were recognised. They reflect successive changes in vegetation development in the study area. Two local dinoflagellate cyst (dinocyst) assemblage zones (LDAZ 1 and 2) were distinguished (Figs 4, 6, 8). The second dinocyst zone is subdivided into two subzones (LDASZ-2a and LDAZ-2b). All zones are described in detail in Table 2.

Discussion

Pollen assemblages

Regional Pollen Assemblage Zone IV (*Artemisia* – Chenopodiaceae – *Pinus*) 25903–15612 cal. yrs BP

RPAZ IV has a Late Pleniglacial-Oldest Dryas/Upper Neoeuxinian age (25903–15612 cal. yrs BP). It is represented in cores AKAD 09-10 (LPAZ AKAD 09-10 1 and 2) (Fig. 5, Tables 2, 3) and AKAD 11-17 (LPAZ AKAD 11-17 1) (Fig. 3, Tables 2, 3). Sediments with Late Pleniglacial and Oldest Dryas age cannot be separated by pollen analysis in cores studied, because of the similarity in pollen spectra.

Table 2. Description of the local pollen assemblage zones and subzones (LPA(S)Z) and local dinoflagellate cyst assemblage zones and subzones (LDA(S)Z) from cores AKAD 11-17, AKAD 09-10 and AKAD 09-15.

LPAZ AKAD11-17-1 (228.5–147.5 cm) 19546-15612 cal. yrs BP **_Artemisia_-Chenopodiaceae-_Pinus_** Dominant non-arboreal pollen (NAP) (up to 72%), mainly *Artemisia* (around 50%) and Chenopodiaceae (10%). Continuous Poaceae (ca. 2%), *Aster*-t. (1%) and Brassicaceae (1%). Sporadic *Achillea*-t., Caryophyllaceae and *Scleranthus*. Arboreal pollen (AP) dominated by *Pinus diploxylon*-t. (up to 25%). Regular presence with low values (up to 1%) of *Picea*, *Abies*, *Juniperus* and *Ephedra distachya*. Gradual decrease of *Quercus* from 12% up to 3% and of *Corylus* (3.8-0.5%) at zone top. Continuous low *Betula* and *Ulmus* (up to 0.8%). Sporadic *Alnus*, *Carpinus betulus*, *Fagus* and *Tilia*.	LPASZ AKAD11-17-2a (147.5–136.5 cm) 15612-14295 cal. yrs BP **_Pinus_-_Artemisia_-Chenopodiaceae** Maximum of *Pinus diploxylon*-t. (up to 42%), coincident with decrease of *Artemisia* (50 to 35%), constant Chenopodiaceae (to 12.8%). Constant presence of *Quercus* (ca. 4%), *Corylus*, *Ulmus* and *Salix* (ca. 1%). Sporadic *Hippophae*. Poaceae, Cichoriaceae, *Aster*-t., *Achillea*-t., Brassicaceae and *Centaurea jacea*-t. are continuously presented.	LPASZ AKAD11-17-2b (136.5–134.5 cm) 14295-14036 cal. yrs BP **_Artemisia_-Chenopodiaceae** One spectrum: Rapid rise of *Artemisia* (35-70%) and Chenopodiaceae (up to 16%). Presence of Poaceae (2%) and *Achillea*-t. (2%). Rapid decrease in *Pinus diploxylon*-t. (from 42% to 6%). Slight increase in *Juniperus* and *Ephedra distachya*. Decline of *Quercus* (from 4% to 1.5%).
LPASZ AKAD11-17-2c (134.5–125.5 cm) 14036-13257 cal. yrs BP **_Pinus_-_Artemisia_** Two spectra: Rapid rise of *Pinus diploxylon*-t. (from 6% to 25%), decrease of *Artemisia* (from 70% to 58%) and Chenopodiaceae (from 15% to 10%). Continuous Poaceae (around 2%). Presence of *Picea*, *Quercus*, *Corylus*, *Juniperus* and *Ephedra distachya*.	LPASZ AKAD11-17-2d (125.5–99.5 cm) 13257-11072 cal. yrs BP **_Artemisia_-Chenopodiaceae** Sharp rise of NAP (up to 86%), including highest values of *Artemisia* (around 67%), max. at the zone bottom. (up to 82%). Gradual increase of Chenopodiaceae (11-16%) and Poaceae (1-3%) at the zone top. *Pinus diploxylon*-t. after sharp decrease is stabilised below 11%. Constant *Quercus* (around 2-3%), increased *Corylus* (up to 1%) at the zone top. Regular low presence of *Picea*, *Juniperus*, Ericaceae, *Ephedra distachya*, *Betula*, *Salix* and *Hippophae*. Presence of numerous heliophytes including *Helianthemum*, *Scleranthus*, Caryophyllaceae, *Centaurea jacea*-t.	LPASZ AKAD11-17-3 (99.5–63 cm) 11072-8004 cal. yrs BP **_Quercus_-_Artemisia_-Chenopodiaceae** Gradual rise of *Quercus* (1-16%), coincidently with decrease of *Artemisia* (from 64% to 34%). Abrupt decrease in *Pinus diploxylon*-t. (up to 5%) at the zone top. Trend to rise of *Picea*, *Betula*, *Corylus* and *Salix* (up to 2%) at the zone top. First significant presence of *Carpinus betulus* (6%). Presence of *Ephedra distachya* (0.8%) and *Hippophae* (0.2%). *Typha angustifolia/Sparganium*-t., *Athyrium* and *Dryopteris/Thelypteris*-t. appear.
LPAZ AKAD11-17-4 (63–44.5 cm) 8004-5483 cal. yrs BP **_Quercus_-_Corylus_-_Carpinus betulus_-_Ulmus_-_Cerealia_-_Triticum_** Dominant *Quercus* (max. 29%) and *Corylus* (max. 27%). Consistent increase in *Ulmus* (0.5-7 %) and *Tilia* (up to 1.2%). Abundance of *Salix* and frequent *Betula* (about 1.1%). Rise of *Alnus* (up to 3.1%) and *Fagus* (up to 5.4%) at the zone top. Sporadic *Fraxinus excelsior*-t., *Acer*, *Pistacia* and *Hippophae*. Constant presence of *Hedera*. *Carpinus orientalis* and *Fraxinus ornus* appear. Decrease in *Pinus haploxylon*-t. (from 20% to 2.4%). Increase in *Picea* (up to 1.1%). *Abies*, *Juniperus* and Ericaceae are sporadic. Decrease in *Artemisia* (from 64.4 to 13%) and Poaceae (from 8.5 to 2.6%). Decline of Chenopodiaceae (from max. 24.9% to 2.4%). Appearance of *Cerealia*-t. (1.3%) and *Triticum*. *Achillea*-t., *Aster*-t., Cichoriaceae, Brassicaceae, *Scleranthus*, *Plantago lanceolata*, *Polygonum aviculare*, Caryophyllaceae and *Centaurea jacea*-t. present. Polypodiaceae (around 0.8%), *Typha angustifolia/Sparganium*-t., *Typha latifolia* and Cyperaceae are continuously present.	LPAZ AKAD11-17-5 (44.5–27.5 cm) 5483-2837 cal. yrs BP **_Quercus_-_Carpinus betulus_-_Corylus_-_Fagus_-_Carpinus orientalis_-_Cerealia_** Dominant *Quercus* (about 28.4%). Gradual rise and maximal presence of *Carpinus betulus* (12.2-18.4%). Decrease in *Corylus* within the zone (16.8 to 8.1%). Constant presence of *Alnus* (around 6.1%), *Tilia* (1.8%), *Carpinus orientalis* (0.7%), *Fagus* (6%), *Betula* (0.1%), *Pinus diploxylon*-t. (9.5%). Decrease inof *Ulmus* (6-2.3%) at the zone top. Rise of Ericaceae (up to 0.9%). Sporadic *Salix*, *Acer* and *Ephedra distachya*. Continuous *Hedera*; *Humulus/Cannabis* appear. Reduced *Artemisia* (34% to 8%), Chenopodiaceae (21% to 3.6%) and Poaceae (6.6% to 3.6%). *Cerealia*-t. and *Triticum* are continuously presented (around 0.6%). Presence of *Aster*-t., *Achillea*-t., Cichoriaceae, *Carduus*-t., *Plantago lanceolata*, *Polygonum aviculare*, *Scleranthus*, *Filipendula* and Apiaceae. Polypodiaceae, Cyperaceae, *Typha angustifolia/Sparganium*-t., *Myriophyllum spicatum* and *Alisma* are sporadic.	LPAZ AKAD11-17-6 (27.5–3.5 cm) 2837 cal. yrs BP-preindustrial time **_Quercus_-_Alnus_-_Ulmus_-_Carpinus betulus_-_Fagus_** Gradual rise of AP (69 to 84%). Dominant *Quercus* (around 20%). Rise of *Alnus* (max. 20%) at the zone top. Continuous *Ulmus* (around 3.7%) and *Fagus* (around 5%). Increase in *Carpinus betulus* (up to 8%) and *Corylus* (up to 7%). Constant *Carpinus orientalis* and *Tilia* (around 1%). Constant *Hedera* (0.3%). *Vitis* and *Humulus/Cannabis* appear. Reduced *Artemisia* (to 8.4%) at the zone top. Chenopodiaceae and Poaceae are low (2-3%). Continuous *Cerealia*-t. (1%) and *Triticum* (0.2%). *Plantago lanceolata*, *Polygonum aviculare*, *Centaurea cyanus* and *Rumex* are also presented.

LDAZ AKAD11-17-1 (228.5–59 cm) 19546-7668 cal. yrs BP **_Pyxidinopsis psilata-Spiniferites cruciformis_** Dominant dynoflagelate cysts of *Pyxidinopsis psilata* (two max. 93.7 and 80.5%), then disappearing. Continuous *Spiniferites cruciformis* (two max. 20.9% and 23.2% at the zone top), then disappearing. Rise of *Pediastrum boryanum* var. *boryanum* (up to 5.1%), *Pediastrum simplex* var. *simplex* (around 3.7%), *Pediastrum simplex* var. *sturmii* and *Pyxidinopsis reticulata* are also presented. Other NPPs such as acritarchs *Cymatiosphaera globulosa* and *Hexasteria problematica* and fungal spores of *Bactrodesmium*-type, *Sordaria*-type, *Valsaria valsaroides*, *Ascospore*-type, Biscriate conidium of *Alternaria* and animal remains of *Anthoceras* are sporadic.	LDASZ AKAD11-17-2a (59–27.5 cm) 7668-2837 cal. yrs. BP **_Lingulodinium machaerophorum-Spiniferites belerius-Spiniferites bentorii_** Increase of *Spiniferites bentorii* (max. 14%) and *S. belerius* (max. 10.5%) at the zone bottom, followed by rapid increase in *Lingulodinium machaerophorum* with long processes (max. 107.7%). Substantial presence of *S. ramosus*, *S. mirabilis* and *S. hyperacanthus*. *Operculodinium centrocarpum* (3.4%), *Brigantedinium cariacoense* (3.8%), *Impagidinium centrocarpum* (1.4%) and *Echinidinium transparantum* (1.5%) appear at the zone bottom and then decrease to 0.1%. *Peridinium ponticum*, *Spiniferites mirabilis*, *Protoperidinum stellatum* and *Spiniferites hyperacanthus* are sporadic. Increase in acritarchs *Cymatiosphaera globulosa* (up to 17.2%), then decreasing. First appearance of *Micrhystridium* cf. *ariakense* (max. 11.5%), *Polykrikos kofoidii* (0.6%) and Copepod eggs (0.5%). Sporadic *Polykrikos schwartzii*, *Pleurospora*-t.3B, *Spirogyra* sp. and *Selenopemphix quanta*. Other NPPs such as Biscriate conidium of *Alternaria*, *Pentaphrasodinium dalei* and Glomus-type are rare	LDASZ AKAD11-17-2b (27.5–3.5 cm) 2837 cal. yrs. BP – present **_Lingulodinium machaerophorum-Spiniferites ramosus_** Decrease of *Lingulodinium machaerophorum* (48.9% to 1.7%), *Spiniferites bentorii* (to 0.4%) and *S. belerius* (to 0.2%). Increase in *S. ramosus* (up to 4.7%) and *Peridium ponticum* (to 4.5%). Sporadic presence of *Ataxodinium choane*, *Achomosphaera* cf. *andalousiense*, Fungal spores-type 980, Biscriate conidium of *Alternaria*, *Pediastrum boryanum* var. *boryanum*, *Pediastrum simplex* var. *sturmii*. Constant values of acritarchs *Cymatiosphaera globulosa* (15-5.7%). Presence of *Micrhystridium* cf. *ariakense* (max. 5.9%)
LPAZ AKAD09-10-1 (240–160 cm) 25903-17092 cal. yrs BP **_Artemisia_-Chenopodiaceae-_Pinus_** Dominant *Artemisia* (28-49%), constant Chenopodiaceae (6-16%), high *Pinus diploxylon*-t. (max. 33%). Sporadic *Picea* and *Abies*. Maximum of *Juniperus* (5-10%). Low *Quercus* (2.7%) and *Corylus* (1.6-4.3%). Sporadic Ericaceae, *Betula*, *Carpinus betulus*, *Salix*, *Ulmus* and *Alnus*. Continuous Polypodiaceae (2%). Many Late-Glacial heliophytes are presented.	LPAZ AKAD09-10-2 (160–142 cm) 17092-11788 cal. yrs BP **_Pinus-Artemisia_-Chenopodiaceae** Dominant *Pinus diploxylon*-t., decreasing *Juniperus* (from 7.1% to 1.7%). Appearance of *Pinus haploxylon*-t. and *Ephedra distachya*. Decrease in *Artemisia* (from 42% to 24.2%) and Poaceae (from 7.1% to 3.8%). Constant Chenopodiaceae (around 10%). Rise of *Quercus* (from 4.3% to 19.8%), constant *Corylus* (around 2%).	LPAZ AKAD09-10-3 (142–130 cm) 11788-8253 cal. yrs BP **_Quercus-Artemisia-Corylus_** Two spectra: *Pinus diploxylon*-t. strongly reduced to ca. 8% at the zone top. High *Quercus* (max. 32.5%), increased *Corylus* (from 5.5% to 15%), *Ulmus* (up to 4%) and Fagus (to 1.6%). Frequent *Carpinus betulus* and *Betula*. Sporadic *Humulus/Cannabis* and *Ephedra distachya*. Decreased *Artemisia* (up to 17.3%), Chenopodiaceae (up to 4.8%) and Poaceae (up to 2.5%).
LPAZ AKAD09-10-4 (130–100 cm) 8253-ca.5500 cal. yrs BP **_Quercus-Corylus-Carpinus betulus-Ulmus-Cerealia-Triticum_** Dominant *Quercus* (around 30%), *Corylus* at max. (28.3%) in the middle of the zone. Increase of *Carpinus betulus* (from 1.2% to 16.3%), *Fagus* (from 0.9% to 7.1%) and *Alnus* (from 1.2% to 4.6%) at the zone top. Continuous *Ulmus* (around 7%), *Fraxinus excelsior*-t. (2.8%) ant *Tilia* (1.9%). Constant *Hedera* (ca. 0.5%). Appearance of *Cornus mas* and *Acer*. *Ephedra distachya* disappear. Reduced *Artemisia* (from 24% to 0.6%), Chenopodiaceae (from 3.8% to 0.7%) and Poaceae (from 2.5% to 1.4%). Substantial *Cerealia*-t. (0.8%) and *Triticum* (0.8%). Frequent *Aster*-t. and *Achillea*-t. (up to 1%). Appearance of *Plantago lanceolata* and *Urtica*. Sporadic *Filipendula* and *Scleranthus*.	LPAZ AKAD09-10-5 (100–36 cm) ca.5500-ca.2800 cal. yrs BP **_Quercus -Carpinus betulus-Fagus-Corylus-Carpinus orientalis-Cerealia_** Dominant *Quercus* (from 20% to 28%), *Carpinus betulus* at max. (17.5%), followed by a decrease to 1.5%. Maximum of *Fagus* (7.5%), followed by decrease to 2.8%. Decline of *Corylus* (from 20% to 5.8%) and *Ulmus* (from 3.7% to 1.6%). First appearance of *Carpinus orientalis* (1.3%). Continuous presence of *Pinus diploxylon*-t. (10%). Increase in-*Artemisia* (from 6.5% to 24.6%). Chenopodiaceae and Poaceae are low (ca. 3-4%). Substantial *Cerealia*-t. (1%) and *Triticum* (1%). Presence of *Plantago lanceolata* (1.2%), *Polygonum aviculare*, *Centaurea jacea* and *Filipendula*.	LPAZ AKAD09-10-6 (36–10 cm) ca.2800 cal. yrs BP – present **_Quercus-Alnus-Ulmus-Carpinus betulus-Fagus_** Dominant *Quercus* (26%); increasing of *Alnus* (up to 10%) and *Ulmus* (up to 3%). Constant *Carpinus betulus* (17%), *Fagus* (3%), *Fraxinus excelsior* (1.6%) and *Hedera* (1.3%). Decrease in *Artemisia* (from 24.6% to 7.7%), Chenopodiaceae (from 5.5% to 1.1%). Increase in Poaceae (ca. 7.6%). Significant *Cerealia*-t. (1.6%) and *Triticum* (0.6%). Appearance of *Plantago lanceolata*, *Scleranthus* and *Carduus*-t.
LDAZ AKAD09-10-1 (240–128 cm) 25903-7663 cal. yrs BP **_Pyxidinopsis psilata-Spiniferites cruciformis_** Dominant dinoflagelate cysts of *Pyxidinopsis psilata* (max. 28.2%) and *Spiniferites cruciformis* (1.4 to 10.7%). Constant presence of green algal species *Pediastrum boryanum* var. *boryanum* (2.2% to 3.4%), *Pediastrum simplex* var. *sturmii* (around 3.7%), *Botryococcus* sporadic. High *Glomus-t.207* (1.5% to 6.3%). *Multiplicasphaeridium*-t., *Pleurospora*-t.3B, *Ascospores*-t.20, *Achomosphaera* cf. *andalousiense* and *Cymatiosphaera globulosa* are sporadic. First appearance of *Spiniferites ramosus* at the zone top. *Lingulodinium machaerophorum* appear sporadically at the zone top.	LDASZ AKAD09-10-2a (128–40 cm) 7663-ca.2800 cal. yrs. BP **_Lingulodinium machaerophorum-Spiniferites belerius-Spiniferites bentorii_** Rapidly increasing dominant *Lingulodinium machaerophorum* with long processes (two max. 446% and 166%). *L. machaerophorum* f. *clavate* at max 28.7% at the zone bottom, followed by sharp decrease. *Spiniferites belerius* (max. 26.2%) and *S. bentorii* (max. 12.9%) form peaks at the zone bottom, then decreasing up to around 6%. High *Brigantedinium cariacoense* (around 10%). Substantial S. *mirabilis*, *S. membranaceus* and *S. hyperacanthus*. Constant *Polykrikos kofoidii* (0.7-5%), *S. ramosus* (2.2%), *Operculodinium centrocarpum* (0.9-7.2%), *Echinidinium transparantum* (0.6-5.4%) and *Impagidinium aculeatum* (0.5-1.8%). Sporadic *Tectatodinium pellitum*, *Polykrikos schwartzii*, *Protoperidinum stellatum* and *Bitectatodinium tepikiense*. Rising of acritarch *Cymatiosphaera globulosa* (18.3%), *Micrhystridium* cf. *ariakense* (max. 20%). *Pleurospora*-t.3B and Copepod eggs are also presented.	LDASZ AKAD09-10-2b (40–10 cm) ca.2800 cal. yrs. BP – present **_Lingulodinium machaerophorum-Peridinium ponticum_** Dominant *Peridinium ponticum* (10%). Decrease in *Lingulodinium machaerophorum* (3.7%), *Spiniferites bentorii* and *S. belerius*. *Polykrikos schwartzii* increase (up to 2%). *Protoperidinum nudum* and *Operculodinium centrocarpum* are sporadic. Decrease in acritarchs *Cymatiosphaera globulosa* (1.8%). *Pleurospora* sp.-t.3B, Fungal spores-t.200, *Sordaria* and Copepod eggs are also presented.

LPAZ AKAD09-15-1 (377–320 cm) 14147-14054 cal. yrs BP ***Artemisia*-Chenopodiaceae-*Pinus*** Dominant *Artemisia* (max. 44%), constant Chenopodiaceae (11%), high *Pinus* diploxylon-t. (39-42%). Continuously low *Quercus* (2.7%), *Corylus* (1.5%), *Salix* (1%), *Betula* (1%), *Ulmus* (0.6%), *Juniperus* (2%), *Hippophae* (0.5%). Presence of *Ephedra distachya* (up to 0.8%). Continuous Poaceae (around 7%). Numerous Late-Glacial heliophytes are presented.	LPAZ AKAD09-15-2 (320–200 cm) 14054-12965 cal. yrs BP ***Pinus*-*Artemisia*-Chenopodiaceae** Dominant *Pinus* diploxylon-t. (max. 61%) decreasing *Artemisia* (from 31.5% to 12%); Chenopodiaceae peak in the middle of the zone (23.6%). Increase in Poaceae to 10%. Many Late-Glacial heliophytes are presented. Constant *Quercus* (4%), *Salix* (2%), *Betula* (2%), *Corylus* (1%).	LPAZ AKAD09-15-3 (200–10 cm) 12965-10906 cal. yrs BP ***Artemisia*-Chenopodiaceae** Dominant *Artemisia* (max. 67%) and Chenopodiaceae (24.6%); constant Poaceae (6-8%). Late-Glacial heliophytes are abundant. *Typha angustifolia/Sparganium* and Cyperaceae appear. Increase of Polypodiaceae (1-2%).
LDAZ AKAD09-15-1 (377–10 cm) 14147-10906 cal. yrs BP ***Pyxidinopsis psilata-Spiniferites cruciformis*** Dominant dynoflagelate cysts of *Pyxidinopsis psilata* (10%) and *Spiniferites cruciformis* (4%). Constant presence of green algal species *Pediastrum boryanum* var. *boryanum* (2%). Sporadic acritarchs of *Pseudoschizaea circula*. Constant presence of *Glomus*-type 207 (max. 11% at the zone bottom).		

Paleovegetation reconstruction, based on typical high values of non-arboreal taxa and the presence of cold-resistant and heliophilous taxa such as *Artemisia* and Chenopodiaceae, suggest spreading of cold and dry steppes. Different taxa of Poaceae were also important elements in the steppe communities along the coast together with other heliophilous taxa from Asteraceae, Cichoriaceae, Apiaceae, Brassicaceae, Caryophyllaceae and *Helianthemum*. According to Prentice et al. (1993), the growing season soil moisture deficit and low winter temperature would have maintained an open vegetation. However, climate aridity along the Bulgarian Black Sea coast was not so extreme because the presence of the desert shrub *Ephedra distachya*, that is an indicator of dry continental climate and an important constituent of the glacial flora, is constant but low. Patches of eurythermic conifers such as *Pinus* grow along the coast in valleys where favourable microclimatic conditions may have prevailed. The high percentage values of *Pinus diploxylon*-type pollen could also be an effect of long distance transport which became significantly more pronounced in an environment with sparse vegetation (Pardoe et al. 2010). Warming/humidity increase during the melting pulses (19.2 to 14.5 cal. ka BP) after the Last Glacial Maximum is expressed by the open coniferous forests development. The constant maximum presence of *Glomus* spores suggests significant erosion processes.

Temperate deciduous arboreal taxa such as *Quercus*, *Corylus*, *Ulmus*, *Betula* and *Alnus* show constant presence in the pollen diagrams. The presence of single pollen grains of *Tilia*, *Carpinus betulus*, *Abies*, *Ulmus*, *Fraxinus excelsior* and fern spores of Polypodiaceae suggests an increase of temperature that is seen in the paleotemperature record of Greenland Ice sheet-2 (GISP-2) (Blunier and Brook 2001). Probably, warming/humidity increase during these melting pulses is expressed by the open coniferous forest development. Isolated patches of these taxa must have survived in micro-environmentally favourable pockets. These data suggest that these small pockets in the Black Sea coastal mountains play an important refugial role in the survival of temperate arboreal taxa along south-eastern Europe during the Last Full Glacial (Beug 1975; Bottema 1980; Bennett et al. 1990; Tzedakis 1993; Willis 1994). Our results corroborate

Table 3. Correlation between local and regional pollen assemblage zones and subzones (modified after Filipova-Marinova (2006a)).

											2345		09-15		GGC18		09-10		544		11-17	
cal. kyrs. BP (Fig. 2)	**Northerneuropean climatostratigraphy (Blytt 1876–Sernander 1908)**				**Regional stages and substages (Shopov 1991)**		**Archaeological Chronology (Todorova 1986)**	**Regional PAZ**	**subzones**	**Pollen assemblages (Filipova-Marinova 2006)**	**Local PAZ**	**Pollen assemblages (Filipova-Marinova 2003b)**	**Local PAZ**	**Pollen assemblages (Table 2)**	**Local PAZ**	**Pollen assemblages (Filipova-Marinova et al. 2013)**	**Local PAZ**	**Pollen assemblages (Table 2)**	**Local PAZ**	**Pollen assemblages (Filipova et al. 1989)**	**Local PAZ**	**Pollen assemblages (Table 2)**
2.8	**HOLOCENE**	Subatlantic			BLACKSEA	New Black Sea	Iron Epoch	**IX**		*Q-U-Al-Cb-Sa-F*	**8**	*Q-U-Al-F*			**6**	*Q-Al-Cb-U-F*	**6**	*Q-Al-U-Cb-F*	**6**	*Q-Al-Cb-U-F*	**6**	*Q-Al-U-Cb-F*
5.5		Subboreal				Old Black Sea	Early Bronze Age	**VIII**		*Q-Cb-Co-F-Cor-Ce*	**7**	*Q-Cb-Co-F*			**5**	*Q-Cb-F*	**5**	*Q-Cb-F-Co-Cor-Ce*	**5**	*Q-Cb-Co-F*	**5**	*Q-Cb-Co-F*
		Atlantic					Transitional Period	**VII**		*Q-Co-Cb-U-Tr-Ce*	**6**	*Q-Co-U-Cb-Ti*			**4**	*Q-Co-Cb-U-F-*	**4**	*Q-Co-Cb-U-Ce-Tr*	**4**	*Q-Co-U-F-Cb*	**4**	*Q-Co-Cb-U-Ce-Tr*
8.2							Late Eneolithic															
		Boreal					Neolithic	**VI**	**b**	*Q-U-Co-Art*	stratigraphic hiatus				**3**	*Q-U-Art*	**3**	*Q-Art-Co*	**3**	*Q-Co-U-Cb-Art*	**3**	*Q-Art-Ch*
11.7		Preboreal							**a**	*Q-P-Art*					**2**	*Q-P-Art*						
13	**PLEISTOCENE**	Late Würm	Late Glacial	Younger Dryas	Upper-New-euxinian			**V**	**d**	*Art-Ch*	**5**	*Art-Ch*	**3**	*Art-Ch*	**1**	*Art-Ch-P*	**2**	*P-Art-Ch*	**2**	*Art-Ch*	**2d**	*Art-Ch*
14				Allerød					**c**	*P-Art-Ch*	**4**	*P-Art-Ch*	**2**	*P-Art-Ch*					**1**	*P-Art-Ch*	**2c**	*P-Art*
14.3				Older Dryas					**b**	*Art-Ch-Po*	**3**	*Art-Ch-Po*	**1**	*Art-Ch-P*							**2b**	*Art-Ch*
15.6				Bølling					**a**	*P-Art*	**2**	*P-Art*									**2a**	*P-Art-Ch*
				Oldest Dryas				**IV**		*Art-Ch-P*	**1**	*Art-Ch-P*									**1**	*Art-Ch-P*
25.9			Late Pleniglacial														**1**	*Art-Ch-P*				

Legend: Al = *Alnus*, Art = *Artemisia*, Be = *Betula*, Cb = *Carpinus betulus*, Ce = *Cerealia*-type, Ch = Chenopodiaceae, Co = *Corylus*, Cor = *Carpinus orientalis*, F = *Fagus*, P = *Pinus diploxylon*-type, Po = Poaceae, Q = *Quercus*, Sa = *Salix*, Ti = *Tilia*, Tr = *Triticum*, U = *Ulmus*; --- missing stratigraphic units.

with data of core 22-GC3 from the southern Black Sea region (Shumilovskikh et al. 2012), Ioanina (north-western Greece) (Tzedakis 1993) and Thenagi Phillipon (Bottema 1980). Similar pollen spectra were found at ca. 27990 ± 300 ^{14}C yrs BP by Frenzel (1964) at Stilfied in Austria and were related to the Paudorf Interstadial. This interstadial is also established, although less clearly and dated at 27295 ± 1120 ^{14}C yrs BP for the Black Sea by Komarov et al. (1979). Mudie et al. (2002, 2007) recognised a Pleniglacial pollen assemblage zone with relatively low AP values suggesting a steppe-forest vegetation and moderately high precipitation in winter for Marmara Sea core MAR 95-04, with age of 29540 ± 1540 to 21950 ± 310 ^{14}C yrs BP and in the south-western Black Sea Core MAR 98-04 which has a radiocarbon age of 33550 ± 330 ^{14}C yrs BP (Aksu et al. 2002).

The Paleoclimate model of Peyron et al. (1998) for the Last Glacial maximum (LGM) shows that the climate in southern Europe during the Pleniglacial was characterised by extremely low temperatures and humidity throughout the year. Reconstructed temperatures for the area north of the Mediterranean Sea were lower than today: -15 ± 5 °C for the coldest month and -10 ± 5 °C for the annual mean temperature. In Greece, the available moisture was 20% lower with a precipitation anomaly of ca. 600 ± 200 mm. Kutzbach and Webb (1993) also suggested the Full Glacial conditions in eastern Europe with extremely cold and arid climate and predicted January temperatures as low as -20 °C.

These marine deposits are found below the 30 m isobath in almost all investigated cores of the western Black Sea shelf. In the peripheral (outer) shelf zone, they form clearly defined depositional bodies of coastal or barrier type sediments at the depth of 100 to 120 m (Khrischev and Shopov 1978). Chepalyga (2002) points out that the eustatic regression of the World oceans had led to loss of the two-way connection with the Mediterranean Sea and a subsequent evaporative drop in the level of the Black Sea to a strand-line of -90 m or lower.

Regional Pollen Assemblage Zone V
15612–11788 cal. yrs BP

This zone is distinguished in all cores studied and could be correlated with the Late Glacial/Neoeuxinian age. All stadials and interstadials were determined palynologically and four subzones were presented.

Regional Pollen Assemblage Subzone Va (*Pinus – Artemisia*)
15612–14295 cal. yrs BP

Subzone Va corresponds to the Bølling Interstadial of the European Late Glacial and can be referred to as the Upper Neoeuxinian. It is clearly separated only in core AKAD 11-17 (LPASZ AKAD 11-17 2a) (Fig. 3, Tables 2, 3), but cannot be separated in core AKAD 09-10 (LPAZ AKAD 09-10 2) (Fig. 5, Tables 2, 3) because the pollen analysis in this core section is not of high resolution. The basic characteristic of the vegetation succession during this period of moderate warming is the restriction of areas occupied by steppes dominated by *Artemisia* and the existence of open forests and scattered patches with *Pinus* and *Quercus* along the coast. The contribution of the thermophilous broad-leaf arboreal taxa and the decrease of spread of the desert shrub *Ephedra distachya* suggest climate amelioration. Although some of the *Pinus diploxylon*-type pollen could be of long distance origin, these high percentages (more than 50%) suggest a local and regional expansion of *Pinus* at the expense of shrubs and herbs, as is clearly recorded. This rapid interstadial expansion testifies that *Pinus* survived along the coast during the Late Glacial period. Detached stands of some temperate arboreal taxa, such as *Quercus*, *Ulmus*, *Corylus* and *Salix*, may also have been preserved in suitable localities. Increase of *Pinus diploxylon*-type and some thermophilous pollen taxa was also reported for the Black Sea sediments by other authors (Shimkus et. al. 1977; Traverse 1978b; Komarov et al. 1979) and for the Marmara Sea (Mudie et al. 2002).

Regional Pollen Assemblage Subzone Vb (*Artemisia* – Chenopodiaceae – Poaceae)
14295 – 14036 cal. yrs BP

Subzone Vb suggests stadial environmental conditions. The available Age vs. Depth model (Fig. 2) allows this subzone to be correlated to the Older Dryas Stadial of the Würm Late Glacial and to the Upper Neoeuxinian. It is represented in cores AKAD 09-15 (LPAZ AKAD09-151) (Fig. 7, Tables 2, 3) and AKAD 11-17 (LPASZ AKAD11-17 2b) (Fig. 3, Tables 2, 3). The Older Dryas was very short-lasting and cool. It is always hard to recognise it in sediments on the basis of paleoflora. This is the reason many authors accept Bølling and Allerød as an interstadial complex in the terrestrial pollen records from central and eastern Europe (Lang 2003). The precise distinction and correlation between cores is difficult and obscured due to the differences in both the processes of sediment formation and the succession of local vegetation between particular parts of the coast. Pollen spectra are dominated by *Artemisia* and Chenopodiaceae, suggesting the wide spread of xerophytic and halophytic herb communities along the coast. The percentage presence of *Pinus diploxylon*-type is slightly higher in LPAZ AKAD 09-15 1 from the Black Sea shelf for the time span 14174 to 14059 cal. yrs BP compared to other cores studied from the deep-water zone. High values of non-arboreal pollen suggest a cold and dry climate which is also confirmed by the presence of *Juniperus* and *Ephedra distachya* pollen.

Regional Pollen Assemblage Subzone Vc (*Pinus* – *Artemisia* – Chenopodiaceae) 14036 – 12965 cal. yrs BP

Subzone Vc is considered as an interstadial sequence analogous with the Allerød Interstadial of the Late Glacial and correlated to the Upper Neoeuxinian. It is represented in cores AKAD 09-15 (LPAZ AKAD 09-15 2) (Fig. 7, Tables 2, 3) and AKAD 11-17 (LPASZ AKAD11-17 2c) (Fig. 3, Tables 2, 3). The steppe vegetation is still rich in plant taxa, but more restricted in area, particularly the vegetation of dry habitats. The pollen record shows AP increase, particularly *Pinus diploxylon*-type pollen and presence of temperate arboreal taxa, such as *Quercus*, *Corylus*, *Ulmus*, *Salix* and *Betula*. The maximum of *Pinus diploxylon*-type pollen occurring in Core AKAD 09-15 might signal climate changes (increase of temperature and humidity). The increase in temperate arboreal pollen indicates the limited migration of some trees from the south-eastern European refugia. van der Hammen et al. (1971) and Beug (1982) also proposed the existence of refugia in southern Europe at elevations from 500 to 800 m a.s.l.

Regional Pollen Assemblage Subzone Vd (*Artemisia* – Chenopodiaceae) 12965 – 11788 cal. yr BP

Subzone Vd is associated with the last most significant rapid climate deterioration of the last Late Glacial Stage, i.e. the Younger Dryas Stadial and has an Upper Neoeuxinian age. This cold period is of global importance and is recognised everywhere in Europe as an episode of pronounced cooling (Berglund et al. 1994). The subzone Vd is represented in cores AKAD 09-15 (LPAZ AKAD 09-15 3) (Fig. 7, Tables 2, 3) and AKAD 11-17 (LPASZ AKAD 11-17 2d) (Fig. 3, Tables 2, 3). The reversal from interstadial towards stadial conditions occurred after 12965 cal. yrs BP, corresponding to the termination of the Allerød phase in western Europe and to the GISP-2 ^{18}O climate record (Stuiver et al. 1995). This phase is also clearly recognised in the Marmara Sea (Mudie et al. 2002, 2007) and in Lake Van (Wick et al. 2003).

This succession reflects the expansion of xerophytic herb (steppe) vegetation. Palynological data show that, in addition to the predominant light-demanding xerophytic and halophytic taxa such as *Artemisia* and Chenopodiaceae, many other taxa, such as Poaceae, *Aster*-type, *Achillea*-type, *Centaurea*, *Thalictrum*, Apiaceae and Caryophyllaceae have also participated in these steppe communities. The extremely high percentages of *Artemisia* suggest that the Younger Dryas climate of the Bulgarian Black Sea coast is analogous to that of the Last Glacial maximum. *Chenopodiaceae* is always subdominant to *Artemisia* during the Pleniglacial and the Late Glacial Stadials (Atanassova 2005; Mudie et al. 2007; Shumilovskikh et al. 2012; Filipova-Marinova et al. 2013). This confirms that Younger Dryas is the coldest and driest period along the northern Bulgarian Black Sea coast during the whole Late Glacial. Strong evidence of this climatic deterioration can be seen in the increase and continuous presence of *Juniperus* and the indicator of the cold and dry climate *Ephedra distachya*. High percentage values of *Artemisia* and Chenopodiaceae may have also been a result of the wide extent of halophytic species such as *Salsola ruthenica*, *Suaeda maritima*, *Salicornia europaea* and *Artemisia maritima* growing on the beach area and on the part of the modern shelf after the withdrawal of the sea to -100 m during the Neoeuxinian regression (Chepalyga 2002). There is a steep decline in AP, reflected by a decrease of pollen of *Pinus diploxylon*-type from 13257 cal. yrs BP onwards and restricted occurrence of almost all deciduous arboreal taxa. Stands of *Pinus*, *Quercus*, *Corylus*, *Carpinus betulus*, *Ulmus*, *Tilia* and *Betula* were sparsely distributed in favourable moisture localities in the Eastern Balkan Range (Stara Planina Mts.). This supports the hypotheses of Beug (1975) that refugia of deciduous trees would have been located at mid-altitude sites where the precipitation would have been higher than on the plains during this arid glacial period. This information is of great importance in tracing the main migration routes of these deciduous trees along the Bulgarian Black Sea coast after the last glaciation. Probably, stands of *Pinus* occupied the higher parts of the coastal plateaux, where conditions were more favorable for the growth of trees because of higher atmospheric humidity.

Multiple factors may have been important in determining the vegetation changes in this region, including climate oscillations (Cordova et al. 2009). Such a vegetation type can be attributed to the reduced pollen production under the influence of cold and dry glacial conditions (Willis 1994). Independent evidence provided by isotope analyses makes the distinction that timing and duration of the Late Glacial and Early Holocene aridity along the Black Sea and eastern Mediterranean is strongly linked with the summer insolation (Wright et al. 2003; Tzedakis 2007). Palynological records from the Black Sea region also confirm the results from climate modelling experiments of Kutzbach and Webb (1993) that summer insolation maxima override the effects of the North Atlantic circulation in the continental lowlands of the Balkans. Similar pollen assemblages appear also in other marine cores from the western Black Sea shelf and deep-water zone and show that this climate oscillation has an ambiguous signal in palynological records and is clearly apparent along the whole Bulgarian Black Sea coast (Atanassova 2005; Shumilovskikh et al. 2012; Filipova-Marinova et al. 2013). The Younger Dryas event in the western Black Sea area is also dated by the *Artemisia* maximum at ~ 10660 cal. yrs BP (Mudie at al. 2007). A large increase in non-arboreal pollen (NAP) with peaks in *Artemisia* and *Ephedra* and increased Chenopodiaceae is found in Core MAR 98-12 of the nearby Marmara Sea during the Younger Dryas (Mudie et al. 2002; Caner and Algan 2002). According to Niklewski and van Zeist (1970) and Connor et al.

(2013), aridity, rather than temperature during the glacials, seems to have been the key factor limiting lowland forest development during this steppe phase.

Regional Pollen Assemblage Zone VI (*Quercus – Pinus – Artemisia*) 11788 – 8004 cal. yrs BP

This zone could be correlated with the Preboreal-Boreal chronozone/Old Black Sea Substage and is represented in cores AKAD 09-10 (LPAZ AKAD 09-10 3) (Fig. 5, Tables 2, 3) and AKAD 11-17 (LPAZ AKAD 11-17 3) (Fig. 3, Tables 2, 3). The studied sediments with Preboreal age cannot be separated from that of the Boreal age in Cores AKAD 09-10 and AKAD 11-17, because the pollen analysis in this section is not of high resolution. This zone is marked by the abrupt change from *Artemisia* – Chenopodiaceae to a *Quercus* – *Pinus* – *Artemisia* assemblage. The increased values of total AP and deciduous arboreal taxa suggest an establishment of pioneer formations mainly of *Quercus* and *Pinus*, most probably in a mosaic structure. This change would be in line with the notion of Giesecke et al. (2011) that the beginning of the Holocene is a period of pronounced and frequent change in the vegetation composition. The pollen records from the Black Sea continental slope and deep-water zone suggest a forest-steppe vegetation succession. The vegetation was relatively open, based on the diversity of xerophytic and mesophytic herbs in the pollen records. Abundance of non-arboreal taxa, such as *Artemisia*, Chenopodiaceae, Poaceae and Asteraceae was still high and may indicate that humidity was a major limiting factor in the widespread distribution of forests along the coast or that there were still large areas of disturbed open soil. The first step in the afforestation along the western Black Sea coast began after 11788 cal. yrs BP with the rapid increase of some arboreal taxa.

The most characteristic feature for the Early Holocene vegetation palaeosuccession is the early appearance of *Quercus* as a pioneer element in open pine forests, while, in central and northern Europe, the light-demanding species *Corylus avellana* started to spread in open forests dominated by *Pinus* and *Betula* where interspecies competition was probably of little importance (Birks and Line 1993; Tallantire 2002; Finsinger et al. 2006). The example of *Corylus avellana* shows that the same species can show different behaviour with respect to its regional expansion. *Betula* does not play a prominent role along the Bulgarian Black Sea coast in contrast to high mountain areas (Bozilova and Tonkov 2000; Stefanova et al. 2006). The pollen diagrams from the southern Black Sea region (north-western Turkey) suggest greater occurrence of *Betula* during the Early Holocene than in the modern vegetation cover (Bottema 1990).

The characteristic expansion of *Quercus* is due to the increase in temperatures and humidity. Probably, different oak species, such as *Q. cerris*, *Q. frainetto*, *Q. pubescens* and *Q. polycarpa*, took part in the composition of these forests. In addition to *Quercus*, several temperate taxa, such as *Ulmus* and *Tilia*, were also present in these forests. The presence of *Ulmus* supports the assumption of Stojanov (1950) that, in the past, *Ulmus* forests were the most important components of the vegetation of the lowlands. *Carpinus betulus* is still an insignificant component of the oak forests, as well as *Corylus*, *Tilia* and *Fagus*.

The first increase of deciduous arboreal pollen, mainly of *Quercus* is dated at about 9630 ± 520 ^{14}C yrs BP at Core-544 of the deep-water zone (Filipova et al. 1989) and about 9945 ± 160 ^{14}C yrs BP at Core-149 of the nearby estuary of the

Veleka River (Filipova-Marinova 2003a). Mudie et al. (2002) also reported that *Quercus*, *Carpinus betulus* and *Ulmus* were present in the Early Holocene and began to expand after 8500 ^{14}C yrs BP. Similar changes in vegetation composition along the coast during that time have also been documented by Atanassova (2005) and Shumilovskikh et al. (2012). Such vegetation change is classically observed for the beginning of the Holocene at the Mediterranean area (Tzedakis 2007). In Romanian Carpathians, the spread of oak forests occurred after that of *Ulmus* at about 10800 cal. yrs. BP (Feurdean et al. 2010), while, at higher elevations of Bulgarian mountains, it started together with the expansion of *Betula* forests after 11200 cal. yrs BP (Tonkov et al. 2002). Early pollen increase in *Quercus*, *Ulmus*, *Tilia* and *Acer* in marine records indicates rapid expansion of these temperate taxa during the Early Holocene, likely from local sources and suggests that they probably had Glacial refugia in the Strandzha Mountains and the Stara Planina Mountains (the Balkan Range). According to Lang (1985), it is not certain if *Quercus*, *Ulmus*, *Tilia*, *Fraxinus* and *Acer* occurred together in the mixed oak forests of Europe, because the distribution of these taxa depends on edaphic conditions and topography. Most probably, in the Bulgarian coastal region, *Quercus*, *Ulmus* and *Corylus* were distributed on the richest soils of the southern slopes and the hills of the Strandzha Mountains and the Stara Planina Mountains (the Balkan Range), while *Fagus* and *Carpinus betulus* were spread on northern slopes and along the humid ravines. The early expansion of temperate arboreal taxa and the presence of pollen grains of *Fagus*, *Rhododendron*, Ericaceae and *Juglans* support the notion of van der Hammen et al. (1971), Huntley and Birks (1983) and Bennett et al. (1990) that there were refugia of these taxa in the Strandzha Mountains during the glaciations.

The Sofular Cave (Zonguldak Province, north-western Turkey) record also suggests a fast re-vegetation with trees and shrubs at the onset of the Early Holocene (Fleitmann et al. 2009). These results are in accordance with the presumption of Leroy and Arpe (2007), that parts of the Black Sea mountains were Glacial refugia for temperate trees, which facilitated their rapid re-advance at the onset of the Holocene (11600 cal. yrs BP).

Brewer et al. (2002) consider that the climate of the Early Holocene acted as the strongest controlling factor on the spread of the oak. According to Davis et al. (2003), the Early Holocene warming and later equilibrium has been mainly modulated by increased winter temperatures. Shimkus et al. (1977) and Komarov et al. (1979) also suggest climate warming and increase of arboreal taxa along the Black Sea coast from 10737 ± 315 ^{14}C yrs BP. Around the Marmara Sea, the amelioration of climate occurred around 10200 ^{14}C yrs BP (Mudie et al. 2002).

Warming at the onset of the Holocene also allowed a rapid spread of oak along the Atlantic coast of Europe (Brewer et al. 2002). According to Berglund et al. (1984), such vegetation change is probably a biotic reaction of the climate improvement before about 10000 years including the rise of temperature. The delay of spreading of other arboreal species, such as *Carpinus betulus*, *Corylus*, *Fagus* and *Alnus*, along the coast was probably due to the low humidity of the Early Holocene climate.

Pinus diploxylon is noted as the major contributor to the Early Holocene pollen assemblages (Mudie et al. 2002). However, along the Bulgarian Black Sea coast, percentage values of *Pinus diploxylon*-type declined, while the deciduous arboreal taxa expanded their presence in the pollen spectra. Taking into account the over-representation of *Pinus* pollen in modern surface samples from the western and central Balkan Range (Stara Planina Mts) (Filipovitch et al. 1997) and from the Black Sea coast (Pardoe et al. 2010), the high values of *Pinus* pollen could be partly due to the long distance transport. The formation of isolated stands of *Pinus*on higher localities on the Bulgarian Black Sea coast could not be excluded.

A rapid and very short-term steep decline of arboreals is established in marine core AKAD 11-17 from the deep-water zone at 8500 to 8300 cal. yrs BP (70–65 cm) and in core AKAD 09-10 from the continental slope at 8253 cal. yrs BP at the transition of Boreal and Atlantic chronozones (130 cm) (Figs 3, 5). This event could be caused by the changes in climate parameters or short climate extrusion that would trigger synchronous shifts in vegetation composition. For the first time, evidence for such an abrupt cooling event was identified in the pollen diagram from Core GGC-18 from the south-western Black Sea continental slope (Filipova-Marinova et al. 2013). This short-term arid phase in Black Sea coastal palaeoclimate is confirmed by a decrease in arboreal pollen, particularly of *Quercus* and *Ulmus* simultaneously with the increase of *Artemisia*, presence of the indicator of cold climate *Ephedra distachya* and low sedimentation rate between 8500 and 8253 cal. yrs BP. This event should be considered as a vegetation response to the "8200 yrs BP cold event" (Magny et al. 2003; Alley and Ágústsdóttir 2005; Bahr et al. 2005; Le Grande et al. 2006) as other pollen diagrams from Germany and Finland also show such synchronous pronounced short decline of AP between 8500 and 8300 cal. yrs BP (Giesecke et al. 2011). According to the pollen-based temperature reconstructions, a significant drop in average winter temperatures in the order of 4 °C has been estimated during this rapid climate event, while summer temperatures appear to have been comparable to those currently prevailing in the north-western Carpathians (Feurdean et al. 2008). In Thenagi Philippon (northern Greece) during the "8200 yrs BP event", a significant reduction in tree pollen is observed, that is representative of a decline in winter temperatures of more than 4 °C caused by perturbation of North Atlantic circulation (Pross et al. 2009). Tinner and Lotter (2001) consider this event as a very rapid synchronous response of southern European vegetation to suborbital climate change. In the Balkan Region, this rapid climate change was also identified in the Holocene sequences from the Carpathians (north-western Romania) (Feurdean et al. 2010), Thenagi Philippon (north Greece) (Müller et al. 2011) and Lake Prespa (Macedonia) (Panagiotopoulos et al. 2012). In the Bulgarian high altitudes in the Rila Mts., this event is manifested by the decline of arboreal pollen accumulation rate to ca. 500 grains cm^{-2}/yr^{-1} at 8230 cal. yrs BP, particularly by the decline in *Pinus diploxylon*-type and less of *Betula* (Tonkov et al. 2016). The decrease in temperate forests most probably reflects a decrease in humidity (Combourieu Nebout et al. 2009; Dormoy et al. 2009). A shift in moisture regime led to a shift in the vegetation composition (Tinner and Lotter 2006). According to Roberts et al. (2011), deciduous oak species require high soil moisture during the summer, probably a decrease in soil moisture level at summer contributing to the *Quercus* declining trend observed in the cores studied.

Regional Pollen Assemblage Zone VII
(*Quercus – Corylus – Carpinus betulus – Ulmus – Triticum – Cerealia*)
8004 – 5483 cal. yrs BP

This RPAZ can be correlated to the Atlantic chronozone of the Middle Holocene that corresponds to the Old Black Sea Substage. It is represented in cores

AKAD 09-10 (LPAZ AKAD 09-10 4) (Fig. 5, Tables 2, 3) and AKAD 11-17 (LPAZ AKAD 11-17 4) (Fig. 3, Tables 2, 3). The temperate deciduous arboreal pollen taxa reach their maximum, indicating rapid spread of stable mixed oak forests. Their maximal distribution is recorded at 6417 cal. yrs BP in LPAZ AKAD 11-17 4 and at 5745 cal. yrs BP in LPAZ GGC18 4. The high values of AP suggest that forests become denser. *Quercus* appears to be the major arboreal taxon in the mixed oak forests with abundant *Corylus*, *Carpinus betulus*, *Ulmus*, *Fraxinus excelsior*, *Tilia* and *Acer*. The optimal climate conditions as well as moisture balance of the Black Sea Region were determining factors that stimulated the extensive spreading of these forests. This expansion of mixed oak forests was also attributed to the rise of the Black Sea level during this period resulting in an increase in atmospheric moisture content. According to Tonkov and Bozilova (1995), high humidity on the Balkan Peninsula was reached after 8000 ^{14}C yrs BP and the climate was determined by the transport of air masses from the Atlantic Ocean. The presence of the indicator taxa, such as *Hedera* and *Humulus*/*Cannabis*, confirms an increase in humidity and rise in mean annual temperatures.

Corylus expanded in the local stand mainly at the expense of *Ulmus* and became widespread from 7584 to 5483 cal. yrs BP. This taxon has high pollen productivity in open areas. Pollen data suggest the great extent of monodominant communities of *Corylus* in open areas, but probably also as an undergrowth of the oak forests. The maximum percentage values of *Corylus* could be associated with a short-term fluctuation of climate parameters, but also with a clearance of mixed oak forests for enlargement of cultivated areas along the coast as is seen by the synchronous maximum values of *Cerealia*-type pollen (Filipova-Marinova 2006a). Interspecies competition could not be excluded, because the rapid reduction of *Corylus* values corresponds to the beginning of the continuous curves of *Carpinus betulus* and *Fagus* in all pollen diagrams from the Bulgarian Black Sea coast from the end of Atlantic around 5500 cal. yrs BP. The increase of *Carpinus betulus* started after 6417 cal. yrs BP in LPAZ AKAD 11-17 4 (Fig. 3). The first regular occurrence of *Fagus* suggests the presence of this taxon at about 9000 cal. yrs BP. The expansion of *Fagus* started as early as 8283 cal. yrs BP (8355 ± 75 ^{14}C yrs BP) (Filipova-Marinova 2003b) at the southern part of the Bulgarian Black Sea coast, while at the northern Bulgarian Black Sea coast, the areas occupied by *Fagus* expanded after 6500 cal. yrs BP (LPAZ AKAD11-17 4) reflecting favourable conditions for its spreading and it started to be better represented in suitable localities at lower altitudes. The maximum of *Fagus* occurred around 3260 cal. yrs BP (3070 ± 100 yrs BP) (Filipova 1985). Nowadays, communities of this species could be found in the Eastern Balkan Range at altitudes between 170 and 450 m a.s.l. (Bondev 1991). Another broadleaf tree pollen type in the diagrams is *Acer*. A regular occurrence of *Acer* pollen from 7750 cal. yrs BP is indicative of a local presence of this taxon.

The presence of *Alnus*, together with several occasional pollen grains of *Hedera*, confirms the increase in humidity and temperature along the coast. Submediterranean elements such as *Carpinus orientalis* and *Fraxinus ornus* also occurred near the coastline. *Carpinus orientalis* appeared and probably occupied some areas after the degradation of mixed oak forests due to a human impact that influenced the natural vegetation. The first occurrence of *Juglans* is registered at 7584 cal. yrs BP in LPAZ AKAD 11-17 4. The earliest appearance of several occasional pollen grains of *Juglans* along the southern Bulgarian

Black Sea coast during the Holocene is registered for Preboreal, ca. 10000 cal. yrs BP (Filipova-Marinova 2003a), confirming the possible relic origin of this taxon in the Balkan Peninsula (Bottema 1980).

The first appearance of pollen of anthropophytes, such as *Cerealia*-type, *Triticum*, *Plantago lanceolata* and *Polygonum aviculare*, coincides with the decline of *Corylus* and *Ulmus*, marking human impact during the Late Eneolithic period, 6790–6320 cal. yrs BP (Bozilova and Beug 1992, 1994; Filipova-Marinova and Bozilova 2002, 2003). Human impact was significant along the western Black Sea coast as reflected in pollen diagrams from the Bulgarian Black Sea coastal lakes (Todorova 1986; Bozilova and Filipova 1991; Bozilova and Beug 1994). Terrestrial pollen records from the eastern Black Sea coast reveal anthropogenic impact starting at 4000 ^{14}C yrs BP (Connor et al. 2007).

Regional Pollen Assemblage Zone VIII
(*Quercus – Carpinus betulus – Corylus – Fagus – Carpinus orientalis – Cerealia*)
5483 – 2837 cal yrs BP

The characteristic vegetation succession and the available Age vs. Depth model allow correlation of this RPAZ to the Subboreal chronozone of the Middle Holocene that also corresponds to the Old Black Sea Substage. It is represented in cores AKAD 09-10 (LPAZ AKAD 09-10 5) (Fig. 5, Tables 2, 3) and AKAD 11-17 (LPAZ AKAD 11-17 5) (Fig. 3, Tables 2, 3). The pollen record shows that mixed oak forests still dominate, but a change in the forest composition is registered after 5483 to 2857 cal. yrs BP. The most prominent feature for this zone is the increase of *Carpinus betulus*. The maximum of *Carpinus betulus* pollen is also dated at 5680 ± 65 ^{14}C yrs BP in the Arkutino Lake (Bozilova and Beug 1992) and at 5650 ± 100 ^{14}C yrs BP in the Shabla-Ezeretz Lake (Filipova 1985). According to Filipovitch et al. (1998), this species formed a separate belt on higher areas in the Balkan Range at about the same time. The hornbeam belt was also common on eastern Carpathians during the Subboreal and dated to around 4210 ± 35 ^{14}C yrs BP (Tantau et al. 2011). The presence of *Corylus*, *Ulmus*, *Fraxinus excelsior*, *Tilia* and *Acer* suggest that these taxa also appeared in the mixed oak forests. The decline of *Corylus* started at the same time as *Fagus* and *Carpinus betulus* became established around 5500 cal. yrs BP. The expansion of *Fagus* during the Subboreal must have been triggered by an external factor. This factor may have been climatic change or human influence which started to increase at least in lowland areas. Forests of *Fagus orientalis* expanded in the moist ravines of the Strandzha Mountains (Filipova-Marinova 2003a), that is also reflected in marine sediments from cores studied. The local restriction of *Ulmus* is probably connected to the *Fagus* expansion which may have out-competed *Ulmus* within the local forest stand. The decrease of *Ulmus* and the increase of *Carpinus orientalis* is due to the destructive human activities during the Early Bronze Age (3200–2600 yr BP) (Todorova 1986) that is confirmed by the increase of anthropogenic indicators, such as *Cerealia*-type, *Triticum*, *Plantago lanceolata* and *Polygonum aviculare*.

Regional Pollen Assemblage Zone IX
(*Quercus – Ulmus – Alnus – Carpinus betulus – Salix – Fagus*)
2837 cal. yrs BP – pre-industrial time

This RPAZ can be correlated with the Subatlantic chronozone of the Late Holocene and coincides with the New Black Sea Substage. It is represented in cores AKAD 09-10 (LPAZ AKAD 09-10 6) (Fig. 5, Tables 2, 3) and AKAD 11-17 (LPAZ AKAD 11-17 6) (Fig. 3, Tables 2, 3). There is a slight reduction of pollen of deciduous arboreal taxa although they are still dominant. Modern plant communities began to form after 2837 cal. yrs BP. The decrease in mixed oak and hornbeam forests at the beginning of Subatlantic is probably due to the human impact during the Iron Epoch, that is also confirmed by the persistent presence of *Cerealia*-type and *Triticum*, as well as anthropophytes *Plantago lanceolata* and *Polygonum aviculare*. In areas with erosion, mixed oak forests were replaced by communities of *Carpinus orientalis* and *Quercus pubescens*. The increase of *Corylus* may also reflect deforestation. However, climate-driven changes cannot be excluded. Prominent cooling and increase in humidity are recorded in northern Europe from 2800 to 2500 cal. yrs BP that broadly coincides with the transition of Subboreal to Subatlantic. According to van Geel and Berglund (2000), the cooling during the last 3000 years can be related to the abrupt decrease in solar activity around 2900–2800 cal. yrs BP. Probably the increase of humidity and cooling of the climate along the Black Sea coast were the main reasons for the specific succession and increased abundance of *Alnus*, *Fraxinus excelsior*-type and *Salix* along with lianas and formation of flooded riparian forests (‘Longoz’) lining the river valleys along the Black Sea coast, dominated nowadays by *Alnus glutinosa*, *Fraxinus oxycarpa*, *Ulmus minor*, *Carpinus betulus* and *Quercus pedunculiflora*.

Dinocyst assemblages

For a more correct and detailed reconstruction of the natural environment of the Black Sea, the changes in the relative abundance of dinoflagellate species and their assemblages recorded in three cores from the NW Black Sea were studied (Fig. 1) since marine cores are very sensitive and indicative of changes in environmental parameters, such as sea surface temperature (SST), sea surface salinity (SSS) and nutrient variability (Popescu et al. 2009). Two of the cores studied AKAD 09-10 (Fig. 6, Table 2) from the continental slope and AKAD 11-17 (Fig. 4, Table 2) from the deep-water zone, provide long and well-dated continuous Late Quaternary records from the Black Sea spanning the Pleniglacial, Late Glacial and Holocene, while Core AKAD 09-15 (Fig. 8, Table 2) from the peripheral shelf zone represents only a Late Glacial record due to removal of Holocene sediments by strong bottom currents (Dimitrov 1979). Two distinct local dinoflagellate cyst (dinocyst) assemblage zones (LDAZ) were recorded: one dominated by endemic stenohaline freshwater/brackish-water species *Pyxidinopsis psilata* and *Spiniferites cruciformis* (LDAZ 1) and a successive one composed of euryhaline brackish to hypersaline species (LDAZ 2). The second zone is subdivided into two subzones (LDASZ 2a and LDASZ 2b). Variations in dinocyst assemblages from the studied cores are very similar and comparable to those studied previously from the western Black Sea for the same period (Wall et al. 1973; Wall and Dale 1974; Mudie et al. 2001, 2002, 2004, 2011; Filipova-Marinova 2006b; Marret et al. 2009; Verleye et al. 2009; Shumilovskikh et al. 2013).

Regional dinocyst assemblage zone 1 (RDAZ 1)
(*Pyxidinopsis psilata – Spiniferites cruciformis*)
25903 – 7668 cal. yrs BP

This zone comprises sediments deposited between 25903 and 7668 cal. yrs BP and could be correlated to the Late Pleniglacial, Late Glacial and Early Holocene (Neweuxinian stage). This assemblage is represented in all three cores studied: AKAD 11-17 from 19546 to 7668 cal. yrs. BP (230–59 cm) (Fig. 4, Table 2); AKAD 09-10 from 25903 to 7663 cal. yrs BP (240–128 cm) (Fig. 6, Table 2); AKAD 09-15 from 14147 to 10906 cal. yrs BP (377–10 cm) (Fig. 8, Table 2). The zone is characterised by a very low number of species and the dominance of two stenohaline freshwater/brackish-water dinoflagellate species *Pyxidinopsis psilata* and *Spiniferites cruciformis* accompanied by fresh-water algal taxa, represented by coenobia of the colonial chlorococcalean algae *Pediastrum* and rarely *Botryococcus* and *Achomosphaera* cf. *andalusiensis*. The spherical *Pyxidinopsis psilata* is a well-known brackish-water species and an important indicator of cooler SST during glacial periods (Matthiessen and Brenner 1996). However, Brenner (2001) found that occurrence and abundance of *P. psilata* of annual varves of Baltic Sea sediments is controlled by additional as yet unknown factors other than salinity. Based on modern distribution of *P. psilata*, Zonneveld and Pospelova (2015) considered this species as a euryhaline temperate one with wide environmental parameter range considering SST and SSS. The ecological affinities and the significance of *Spiniferites cruciformis* as a crucial indicator of sea surface salinity (SSS) has been an ongoing debate not only recently, but since its discovery. It has been defined classically as a fresh-water/brackish-water species, whose salinity appears not to have exceeded 7‰ (Wall and Dale 1974). Kouli et al (2001) identified *S. cruciformis* in lacustrine Late Glacial sediments and suggested that *S. cruciformis* is essentially a freshwater taxon with morphological variations of the cysts that only partly may be linked to salinity variations. Eaton (1996) suggests that the cruciform shape of *S. cruciformis* must be a stress effect of low salinity waters of less than 12‰. In modern surface sediments, *S. cruciformis* and *P. psilata* have been recorded only in the Caspian and Aral Seas at SSS of around 12–13‰ (Marret et al. 2004) and *P. psilata* in the Baltic Sea at salinity of 12‰ (Yu and Berglund 2007). Based on analogous correlations, most of the authors suggest a maximum SSS of around 12‰ during the lacustrine stage of the Black Sea (Wall et al. 1973; Wall and Dale 1974; Mudie et al. 2001, 2002, 2004; Filipova-Marinova 2006b; Marret et al. 2009; Verleye et al. 2009; Shumilovskikh et al. 2013). The data from our cores revealed that, in addition to the dominant stenohaline brackish-water dinoflagellate species *P. psilata* and *S. cruciformis*, the well-known freshwater indicators, such as *Pediastrum boryanum* var. *boryanum*, *P. simplex* var. *sturmii*, *P. kawraskyi*, *P. integrum* and rare *Botryococcus*, *A.* cf. *andalusiensis* and acritarch *Multiplicasphaeridium*-type, are also present even in modest percentages (note that dinocysts and algae percentages were calculated based on the pollen sum). The investigation of ecological affinities of *Pediastrum* indicates that this taxon is common to rare at a salinity of about 5‰ (Hiscott et al. 2007) although records from brackish habitats are also documented (Brenner 2001). Detailed studies of surface sediment samples from the Baltic Sea show that the dominant species *P. boryanum* var. *boryanum* and *P. kawraskyi* are stenohaline species associated with a wide range of temperature and trophic conditions, dominant in salinity from 6–8‰ (a full range is 5–9‰), *P. simplex* occurring in sediments of less than 3–5‰ salinity while *Botryococcus* is a more south-tolerant taxon sometimes living in brackish waters (Matthiessen and Brenner 1996). *Achomosphaera* cf. *andalusiensis* is a species of cold north-temperate climate and low salinity melt-water fluvial conditions (Harland 1983). All these data support our estimation that the limit of SSS for the Neweuxinian stage of the Black Sea falls in the range of 5–12‰.

The dinocyst record shows the low abundance or absence of stenohaline brackish-water species *P. psilata* from 19 to 15.5 ka yrs BP in core AKAD 11-17 and to 17.1 ka yrs BP in core AKAD 09-10 probably connected with the lack of a favourable environment for the growth of this species due to the strong melt-water and terrigenous input during that time. *Pediastrum boryanum* var. *boryanum* (considered a good indicator of freshwater input) has cyclical abundance associated with the deposition of four red-brown clay layers between 19 and 14 ka BP. The red-brown layers have been previously distinguished and dated from the north-western Black Sea shelf from 18.3 to 15.5 ka BP, in several pulses (Major et al. 2002; Bahr et al. 2006). Four red-brown layer intervals were identified and dated by Soulet et al (2016) from 17.2 to 14.8 ka BP. From isotopic and geochemical proxies, Denton et al. (2010) argue that the deposition of the red-brown layers was linked to major melting phases of European ice, drained by the north-western Danube and Dnieper Rivers in response to the climate warming observed after the end of the Last Glacial Maximum (LGM) from 21 to 18 ka BP. Bahr et al. (2005) also indicate four major episodes equivalent to four layers of Caspian water spilling into the Black Sea. According to Major et al. (2002), it is difficult to support a direct northern source for the red-brown layers associated with the European melt-water event. During the time of the red-brown layers' deposition, the Scandinavian Ice sheet dammed large lakes and diverted rivers and melt-water drainage southwards towards the River Volga and into the Caspian Sea. These melt-waters caused the Caspian Sea to overflow over the Manych Depression (+20 m a.s.l.) into the Black Sea (Mangerud et al. 2004). Bahr et al. (2005) assume that these sediments have been transported to the shelf and the slope by strong surface currents such as the present-day 'Rim Current' gyre. The occurrence of acritarch *Glomus*-type 207 in the sediments provides evidence for terrigenous input and erosional rate increase during the deposition of these red-brown layers. The decrease in the freshwater algae *Pediastrum* is marked between 14259 and 14036 cal. yrs BP. According to its stratigraphic position at the Older Dryas sediments, it may reflect a drier with reduced river input and/or cooler climate at that time. The increase of *P. psilata* and *S. cruciformis* during the interstadials Bølling and Allerød, identified in dinocyst record of core AKAD 11-17, indicates increased phytoplankton activity and rather brackish Black Sea waters during that time, characterised by a marked increase in temperate arboreal pollen suggesting warmer and humid conditions along the Black Sea coast. The upward increase of *P. psilata*, *S. cruciformis* and *P. boryanum* var. *boryanum* between 13257 and 11072 cal. yrs BP indicates Younger Dryas SST colder than during the Bølling/Allerød in agreement with the pollen record of this core and other cores from the surrounding regions (Bottema et al. 1995; Mudie et al. 2002; Filipova-Marinova, 2003b), as well as with the return to almost LGM values of $\delta^{18}O_{prec}$ established by Bahr et al. (2006). The maximum relative abundance of *P. psilata* up to

94% in the Early Holocene record from core AKAD 11-17 is of considerable interest. Similar abundance of *P. psilata* was also found in most records from the north-western Black Sea, for example, 93–97% in core MO2-45 (Marret et al. 2009); 91–97% in core 22-GCC3 (Shumilovskikh et al. 2013); 92% in core GeoB7625-2 (Verleye et al. 2009). The explanation of why *P. psilata* considered a cool brackish-water species shows maximum relative abundance during the Early Holocene is complicated. Preboreal and Boreal chronozones of the Early Holocene are characterised by warming and humidity increase, that is previously stated in pollen records (Komarov et al. 1979; Mudie et al. 2007; Filipova-Marinova et al. 2013) and in the varved $\delta^{18}O$ paleo-term records from the Sofular Cave that tracks the isotope signature of Black Sea surface water (Göktürk et al. 2011). During the Early Holocene, characterised by a warmer climate, the stenohaline freshwater/brackish-water species *P. psilata* that have a preference for warmer temperatures and ice-free conditions (Zonneveld and Pospelova 2015), together with *S. cruciformis*, demonstrate their ecological optimum in growth concerning SST reaching maximum relative abundance between 11072 and 8638 cal. yrs BP. This maximum was interrupted by an abrupt significant short-term decrease in the relative abundance of *P. psilata* which reached values close to those characteristic of glacial periods (from 84–20%), as well as *S. cruciformis* (from 21–3%). This shift, centring between 8500 and 8300 cal. yrs BP in the deep-water core AKAD 11-17, is considerable and reflects a strong cooling of climate. This episode could be attributed to a marked short-term (with a duration of about 200 years) climate deterioration against the background of the climate amelioration during the Early Holocene. This event was also revealed in the same core by pollen data, particularly based on ecological requirements of the vegetation, represented by the ratio of relative abundance of thermophilous arboreal taxa and steppe elements including cold-resistant and heterophyllous taxa. The pollen-driven decrease in temperatures correlates with the decrease in phytoplankton productivity. The same cooling phase was previously assigned in sequence of GGC-18 from the south-western Black Sea continental slope indicating the complexity of environmental changes throughout the Basin (Filipova-Marinova et al. 2013) and confirming that the climate of the Early Holocene acts as the strongest controlling factor (Brewer et al. 2002). Bahr et al. (2006) also established that prevailing high temperatures during the Early Holocene led to authigenic calcite precipitation through increased phytoplankton activity, interrupted by the ‘8.2 ka cold event’. The findings reported above represent the regional expression of the well-known in the Northern Hemisphere ‘8.2 ka cold event’ in the Greenland $\delta^{18}O$ ice which is commonly linked to a melt-water-related perturbation of the Atlantic Meridional Overturning Circulation (AMOC) (Magny et al. 2003; Alley and Ágústsdóttir 2005; Bahr et al. 2005; Le Grande et al. 2006) and associated collapse of oceanic northward heat transport (Ellison et al. 2006). This climate anomaly is also confirmed in the Aegean Region by multi-proxy studies. Early to Middle Holocene season-specific SST and $\delta^{12}O$ seawater, based on dinocyst and foraminiferal records established in marine sediments revealed a prominent short-term (approximately 150 yrs) cooling event in the central Aegean Sea centred on 8200 cal. yrs BP, coeval to the 8.2 ka BP cold event (Marino et al. 2009). Pollen record from Thenaghi Philippon (NE Aegean Sea) provides evidence for a massive climate-induced turnover in terrestrial ecosystems of the Aegean Region associated with the 8.2 cal. ka BP cold event as a response to the North-Atlantic thermohaline circulation slowdown (Pross et al. 2009). Using a phytoplankton-based SSS record, based on the *Emiliania huxleyi* transfer function, Herrle et al. (2018) found a reduced outflow of low salinity waters from the Black Sea into the north Aegean Sea corresponding to the 8.2 cal. ka BP cold event that was caused by cooler and drier climatic conditions over Europe. All these data support the statement that high amplitude temperature anomaly linked to the 8.2 cal. ka cold event may have also occurred in lower latitudes much further in the southerly direction than previously assigned. The Black Sea like the Aegean Sea is sufficiently distant and isolated from the North Atlantic not to be directly affected by its oceanic circulation, therefore, signal transmission must have been atmospheric (Marino et al. 2009). The 8.2 cal. ka cold event in many ways mimics the distribution and anomaly type of the Younger Dryas. It is marked, in general, by cooling with summer-time values in the order of 1°C by a strong shift in vegetation (Alley and Ágústsdóttir 2005).

Regional dinocyst assemblage subzone 2a (RDASZ 2a)
(*Lingulodinium machaerophorum – Spiniferites belerius – Spiniferites bentorii*)
7668 – 2837 cal. yrs. BP

A prominent change of the composition of dinocyst assemblages from freshwater/brackish-water is observed at the boundary of LDAZ AKAD 11-17-1 and LDAZ AKAD 11-17-2a. The abrupt decrease of stenohaline freshwater/brackish-water species *S. cruciformis* and *P. psilata* at 7668 cal yrs BP indicating, according to Mudie et al. (2001), the inability of these species to survive the abrupt salinity change to values as high as 10–12‰ (Deuser 1972) or even 18‰ (Wall and Dale 1974) was followed upward by a gradual increase of euryhaline marine species. The dinoflagellate cyst assemblage was dominated by euryhaline marine species, such as *Lingulodinium machaerophorum*, *Spiniferites belerius*, *Spiniferites bentorii* and acritarch *Cymatiosphaera globulosa* and is characterised by high species diversity. The change of assemblage occurs coincidently with the lithological change from banded lutite to sapropel which has been dated also at 7668 cal. yrs BP. Modern marine conditions, influenced by Mediterranean waters, were detected after 6417 cal. yrs BP, when an abundance of Mediterranean-related species *Operculodinium centrocarpum*, *Spiniferites mirabilis* and acritarch *Mychristidium* (occurring in hypersaline waters) along with euryhaline heterotrophic species, such as *Brigantedinium cariacoense*, *Echinidinium transparantum* and *Polykrikos kofoidii*, occurred. The immigration of marine species to the north-western Black Sea zone from 7668 to 6417 cal. yrs BP indicates a progressive increase in salinity due to more open connection with the Mediterranean. All these species are characteristic components of climate amelioration in Quaternary sequences. Such dinoflagellate cyst assemblages tolerate salinity values of 17–9‰ (Deuser 1972). The isotopic salinity estimates are in close agreement with the present day SSS of 18‰ (Mudie et al. 2002). The recorded sporadic occurrence of acritarchs, such as *Hexasteria problematica* and Fungal spores *Sordaria*-type, *Cercophora*-type 207 and *Ascospores*-type 121 in the studied records, appeared to be the best index of terrigenous input from soil erosion and markers of farming practice (Mudie et al. 2002). The most abundant euryhaline species *L. machaerophorum* responds to the amelioration of climate and shows three peaks in the record of core AKAD 11-17: at 5016, 3459 and 1436 cal. yrs BP and in the core AKAD 09-10 at 220, 190 and 140 cm. These peaks could be related to the increased SST, SSS, a stratified water column (for instance, calm periods between upwelling events) and nutrient input due to the increased human activity during the Late Eneolithic, Early Bronze Age and Iron epoch along the Black Sea coast, confirmed also by pollen data. According to Mudie et al. (2011), blooms of *L. machaerophorum* cysts may indicate water-column stratification and nutrient enrichment more strongly than either salinity or sea level. Peaks of another characteristic component of climate amelioration, such as *Cymatiosphaera globulosa*, are synchronous with those of *L. machaerophorum* and also reflect mainly nutrient levels and stratification of the water column. The first appearance of *Peridinium ponticum*, a species related geographically to the Black Sea, is recorded from 6417 cal. yrs BP. This species is considered a good proxy for the reconstruction of Holocene salinity variations since its relative abundance fluctuates asynchronously with *L. machaerophorum* with long processes (Mudie et al. 2011).

Regional dinocyst assemblage subzone 2b (LDASZ 2b) (*Lingulodinium machaerophorum – Spiniferites ramosus – Peridinium ponticum*) 2837 cal. yrs. BP – pre-industrial time

The composition of the Late Holocene LDASZ AKAD 11-172b is identical to that of LDASZ AKAD 11-17-2a, but the decrease in the relative abundance of almost all marine euryhaline species since 2837 cal. yrs BP is noticeable. The increase in abundance of species that tolerate low-salinity water conditions, such as *P. ponticum* and *S. ramosus*, indicate certain freshening. Since *P. ponticum* is a heterotrophic species (Dale 1996), nutrient availability also influences its abundance (Verleye et al. 2009) as recorded by pollen data, particularly by the increase inhuman activity indicators. The sporadic occurrence of freshwater algal species *P. boryanum* var. *boryanum* and *P. simplex* var. *sturmii*, as well as *Spirogyra* and *A*. cf. *andalusiensis*, suggests increasing river input associated with the cooling of climate and increase of humidity confirmed by pollen data from the studied cores. The period from 2800 to 2500 cal yrs BP coincides with a prominent cooling and increase of humidity in north Europe (Wanner et al. 2008). According to van Geel and Berglund (2000), the cooling in the Third millennium BP can be related to the abrupt decrease in solar activity around 2900 to 2800 cal. yrs BP. Freshening of the Black Sea surface water during the Late Holocene after 2500 cal yrs BP was previously assigned in the Core GGC-18 of the south-western Black Sea by Filipova-Marinova et al. (2013). Decrease in the abundance of fully marine dinoflagellate species and occurrence of the brackish-water indicator *P. ponticum* indicates seawater freshening after 2500 cal. yrs BP, also recorded in core 22-GC3 (Shumilovskikh et al. 2013). Substantial freshening of the Black Sea surface waters from 29 to approximately 19‰ after 3000 cal. yrs BP is evidenced by lower δD values of C^{37} alkenones produced by haplophyte algae *Emiliania huxleyi* (van der Meer et al. 2008). Additionally, combined lipid biomarkers and fossil DNA analyses of alkenones of *E. huxley* from the GGC-18 sediment core indicate a gradual cooling from 19–15 °C after 2570 cal. yrs BP (Coolen et al. 2009).

Conclusions

Vegetation successions and environmental changes along the north-western Black Sea coastal area during the last 26000 years were reconstructed by multi-proxy analysis including radiocarbon dating of sediments from three new marine cores. The following main conclusions from this study are: (1) The coastal landscape during the Late Pleniglacial (25903–17092 cal. yrs BP) was dominated by steppe vegetation composed of *Artemisia*, Chenopodiaceae, Poaceae and other cold-resistant and heliophilous herbs suggesting cold and dry environments. Sparse stands of *Pinus* and *Quercus*, partly enlarged during the melting pulses (19.2–14.5 cal. ka BP) and during the Late Glacial interstadials Bølling and Allerød reflecting warming and humidity increase. (2) During the Younger Dryas (13257–11788 cal. yrs BP), enlargement of steppe vegetation dominated by *Artemisia* and the shrubland of *Juniperus-Ephedra* indicates return to the coldest and driest climate. (3) In the Early Holocene (Preboreal-Boreal) (11788–8004 cal. yrs BP), pioneer forests of *Quercus* with groups of *Ulmus*, *Tilia*, *Alnus* and *Betula* spread and clearly confirm the presence of refugia of these taxa in the coastal mountains and their rapid migration due to the climate warming. (4) The short-term decline of arboreal pollen, particularly manifested by *Quercus*, between 8.5–8.3 ka BP can be explained as a vegetation response to the known in north Atlantic region '8.2 ka cold event'. This climatic oscillation is confirmed for the second time in Black Sea sediments. (5) During the Atlantic chronozone (8004–5483 cal. yrs BP), species-rich mixed oak temperate deciduous forests developed in the lowlands following climate optimal conditions (high humidity and increased mean annual temperatures). (6) During the Subboreal chronozone (5483–2837 cal. yrs BP), mixed oak forests dominate alongside a slight enlargement of *Carpinus betulus*. (7) During the Subatlantic chronozone (2837 cal. yrs BP – pre-industrial time), a specific vegetation succession manifested by the increased abundance of *Alnus*, *Fraxinus excelsior* and *Salix* along with lianas and the formation of flooded riparian forests (e.g. 'Longoz') lining the river valleys along the Black Sea suggests a climate shift (an increase of humidity and a cooling of the climate). (8) The first indications of farming and other human activities along the Black Sea coast were recorded during the Late Eneolithic (6790–6320 yrs BP). (9) Two main dinoflagellate cyst assemblages were distinguished: one dominated by stenohaline freshwater/brackish-water species and the successive one dominated by euryhaline marine species. (10) During the Early Holocene, *Pyxidinopsis psilata* revealed a wide ecological range and demonstrated its ecological optimum of growth concerning the increased sea surface temperature reaching a maximum relative abundance at 9475 cal yrs BP. (11) An abrupt short-term cooling centred between 8.5 and 8.3 ka BP associated with the '8.2 ka cold event' is evidenced by an abrupt decline in the abundance of *P. psilata* and *Spiniferites cruciformis* to values close to those characteristic for the Younger Dryas. This climate oscillation is described for the first time in dinocyst records from Black Sea sediments (Core Akad 11-17). This finding confirms that the high amplitude temperature anomaly, associated with the '8.2 ka cold event' may have also occurred in a southern direction possibly through atmospheric transmission of the signals. (12) The change in the composition of dinocyst assemblages occurred at 7668 cal yrs BP. The abrupt disappearance of freshwater/brackish-water species *Pyxidinopsis psilata* and

Spiniferites cruciformis was followed upwards by a gradual increase in euryhaline marine species *Lingulodinium machaerophorum*, *Spiniferites belerius*, *S. bentorii* and acritarch *Cymatiosphaera globulosa*. (13) A certain freshening of the Black Sea waters after 2837 cal. yrs BP has been established.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Unveiling prey preferences of endangered wild Malayan tiger, *Panthera tigris jacksoni*, in Peninsular Malaysia through scat analysis via *COI* DNA metabarcoding

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Abstract

Understanding the prey preference of Malayan tiger (*Panthera tigris jacksoni*) in Malaysia is important to guide conservation planning initiatives. The utilisation of DNA metabarcoding provides valuable insights, particularly in the field of carnivora diet research. This technique has been proven to be effective for identifying various species within complex mixtures such as scat materials, where visual identification is challenging. The Cytochrome c oxidase subunit I (*COI*) locus has been selected as it is a widely used as an effective non-invasive approach for diet studies. Hence, given this advance approach, Malayan tiger scats were collected on the basis of existing records of their presence in two types of habitats, namely, protected areas (PA) and human–tiger conflict (HTC) areas. This study aimed to identify prey species in Peninsular Malaysia, based on Malayan tiger scat samples using DNA metabarcoding. Based on the partial mitochondrial *COI* region, DNA metabarcoding led to the taxonomic resolution of prey DNA remnants in scats and the identification of prey species consumed by Malayan tiger, which were predominately small-to-medium-sized prey, including livestock. The dominant DNA prey detected belongs to the family Canidae, followed by Bovidae, Vespertilionidae, Homonidae, Felidae, Phasianidae and Muridae. A significant difference ($p < 0.05$) was observed in alpha and beta diversity using the Shannon index and PERMANOVA with regard to prey richness and evenness in two different habitat groups, namely, PA and HTC. Our finding provides insights into Malayan tiger dietary requirements, which can be used to develop conservation plans and strategies for Malayan tiger, particularly for habitat priorities.

Key words: Diet, faecal, mitochondrial DNA, next-generation sequencing, tiger

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Introduction

The Malayan tiger (*Panthera tigris jacksoni*) is a prominent apex predator, which has received considerable attention in Malaysia. Peninsular Malaysia constituted approximately 6% of the land areas in Malaysia and is designated as totally protected, which comprises national parks and wildlife reserves managed by the Department of Wildlife and National Parks (PERHILITAN), as well as state parks under the supervision of state governments (Kawanishi et al. 2010). According to Kawanishi et al. (2010), 51% of Malaysia's land cover was identified as a potential tiger habitat, which encompasses 29% confirmed tiger habitat, 9% expected tiger habitat and 13% possible tiger habitat. The potential and expected tiger habitats have decreased in recent times. Malayan tiger requires large prey and vast habitats to survive. However, continuous anthropogenic activities, such as illegal hunting, industrial and agricultural expansion and the establishment of human settlements, have resulted in habitat fragmentation and degradation, which have led to a significant decline in tiger population in Malaysia (Topani 1990; Elagupillay et al. 2001; Kawanishi et al. 2006; Shevade et al. 2017; Ten et al. 2021). At present, less than 200 Malayan tigers are left in the wild habitat (Halim et al. 2019). Other factors that led to the decline of Malayan tiger population include continuous depletion of prey species in the Malayan tiger habitat, which are considered as the current leading threats to tigers. The availability of prey species will determine the behaviour, health, social structure and survival of the Malayan tiger. Tigers can live in a diverse habitat and environment, in which they can consume prey that is of varying sizes and their hunting strategies will change depending on the habitat, prey type and prey size (Karanth 2003).

Predators play a crucial role in shaping the structure of food webs within ecosystems (Ritchie et al. 2012). The selection of prey can alter the composition and function of communities and the predator–prey relationship can affect the structure of habitats, behaviour of populations and their survival rates. Habitat loss and fragmentation also influence the relationship between predator and their prey (Haapakoski et al. 2013). In addition, habitat degradation greatly affects large predators that require a large habitat. The social structure of large predators, such as big cats, depends on the availability of prey biomass (Simcharoen et al. 2014) and the availability of the prey in the wild indicates the resources for their growth, survival and reproduction. The selection of their prey determines their eating habits, which, in turn, determines their life history strategies, including movement, habitat selection, social structure, geographical distribution and reproductive success (Sunquist and Sunquist 1989). However, information regarding real-time prey selection by the Malayan tiger in Peninsular Malaysia is lacking.

Determining the real-time prey selection by Malayan tiger is necessary to recognise the essential conservation needs of this endangered species in the future. Studies on prey selection of predators have been conducted in different ways, such as direct observation and scat analysis through undigested prey items remains in scats, such as hair, fur, bone and nails by using microscopic morphological analysis (Reddy et al. 2004; Ramesh et al. 2009; Kumar 2015; Upadhyaya et al. 2018; Matthews et al. 2020) and a molecular DNA approach (Xiong et al. 2017; Thuo et al. 2019; Hacker et al. 2021; Lu et al. 2021). Direct observation on the feeding behaviour of Malayan tiger is challenging because of their elusive habitat and limited accessibility in the wild habitat such as feeding areas, time-consuming and low population density. This species is also considered dangerous. Meanwhile, microscopic morphology scat analysis faces misidentification to sympatric analysis (Monterroso et al. 2018) and obtaining an accurate prey list particularly for closely-related prey taxa for species without the hard component that remains in their scat is difficult (Da Silva et al. 2019).

Both approaches have limitation in our study, such as safety during direct observation and the lack of a reference library database for hair, fur and other parts of wildlife. The database remains to be developed by forensic morphology teams at the National Wildlife Forensic Laboratory (PERHILITAN). Apart from scat analysis for undigested prey items to detect a tiger's diet, recent advances on molecular genetics using high-throughput next-generation sequencing (NGS) employing DNA metabarcoding have shown great application potential in determining the current Malayan tiger prey selection. At present, the molecular identification of prey items in scats is used to complement morphological analysis. In recent years, DNA metabarcoding has been widely used to determine various animal taxa diets. A molecular approach to detect prey items from scats is a time- and cost-effective tool (Mumma et al. 2015), which can address the difficulties of detecting prey species consumed by the predator through conventional methods, such as direct observation of feeding behaviour and through microscopic analysis.

The research trend of faecal analysis using DNA metabarcoding in Malaysia has been conducted to various species, such as primate, elephant, bats, birds and insect (Aziz et al. 2017; Chan et al. 2020; Osman et al. 2020, 2022; Fahimee et al. 2021; Abdullah-Fauzi et al. 2022; Mansor et al. 2022; Mohd-Radzi et al. 2022). However, lack of research has been conducted on the wild big cat family using DNA metabarcoding approach, with a specific focus on identifying the gut microbiome composition in the Malayan tiger (Khairulmunir et al. 2023). Considering that alternative diet approaches are difficult to adopt for this species, DNA metabarcoding provides an opportunity to establish the current prey preference of Malayan tigers in Peninsular Malaysia. This study used DNA metabarcoding by targeting the partial *COI* region to determine prey preference of Malayan tiger in their natural habitat. *COI* has been used to identify prey selection by various species, such as snow leopard, primate, giant otter, sea lion, bat, bird and wood mouse in previous studies (Srivathsan et al. 2016; Berry et al. 2017; Bohmann et al. 2018; Rytkönen et al. 2018; Hacker et al. 2021; Quéméré et al. 2021; Tercel et al. 2021; Sato et al. 2022) because of its high resolution of species identification and conserved gene. Therefore, our research aims to detect the prey in Malayan tiger scat using *COI* metabarcoding. Tigers are known to have a diverse habitat preference. In Malaysia, tigers roam areas across a range of forest vegetation types from mountainous to lowland forests. Tigers may be found near forest edges, which are often adjacent to human settlements. These areas can provide a mixture of natural habitat and human-modified landscapes, such as oil palm plantation. Occasionally, tigers may venture into human settlements, leading to conflicts as they may prey on livestock or come into contact with humans. This study categorised habitat preferences into two groups: protected areas (PA) and the forest edge near human settlement areas (human–tiger conflict, HTC), based on the collected samples. Therefore, this study could provide an insight into their current diet in two different habitats and the information can be used to emphasise the importance of an effective tiger conservation planning strategy.

Methods

Field sampling and sample collections

A total of 33 scat samples were collected from areas known to be inhabited by the Malayan tiger between the years 2021 and 2022. Fig. 1 shows the sampling location in three states of Peninsular Malaysia. Sampling was conducted by trekking and patrolling in the PA and HTC. Field identification of scats during scat sample collections was based on size and shape; however, this strategy is sometimes inconsistent and unreliable as the body size of a carnivore can vary greatly within a species and age group. The difficulty in finding the scat of Malayan tigers in forests may be attributed to the decrease in tiger population in the region or forest fragmentation, which also restricts their movement. During field sampling, all relevant data were collected, including the presence of other animal species and their traces, to determine the potential prey selection of the Malayan tiger in the specific study areas. All potential feline scats found during trekking and searching on the forest floor were collected, regardless of how old they were assumed to be (probably 2 weeks old). Along with the observation of scat size and shape, most scat size was more than 2.5 cm in diameter for tigers and below 2.5 cm for leopard species. In addition, scats were obtained from Malayan tigers that were captured or rescued because of HTC. Scat samples were kept in an icebox during field sampling and preserved at −80 °C prior to DNA extraction.

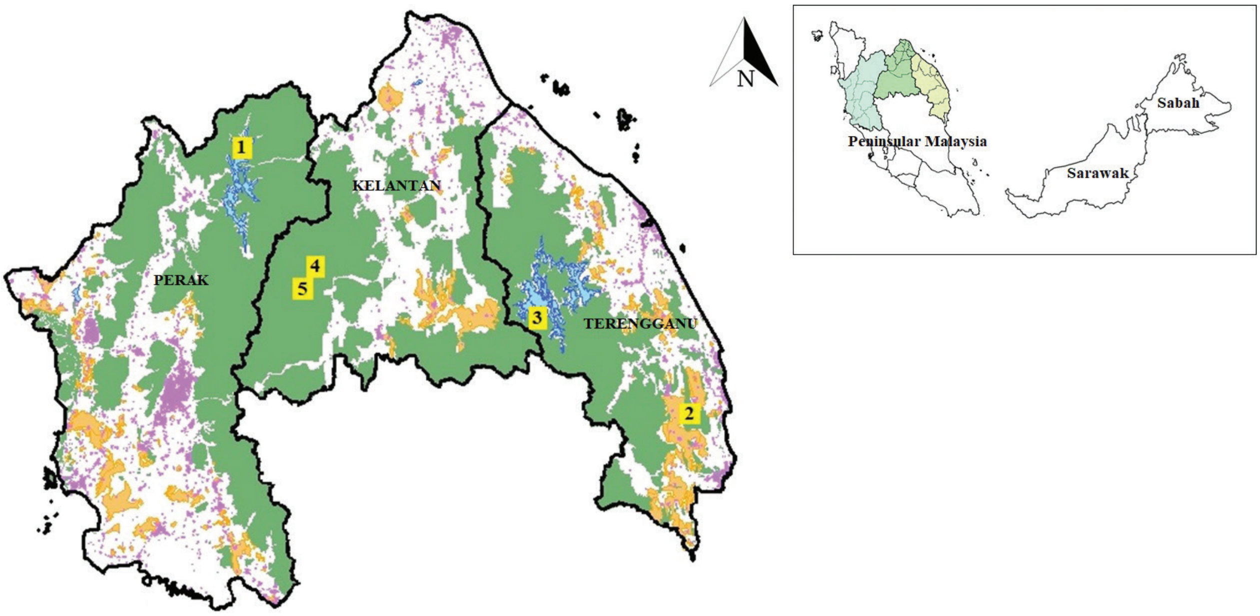

Figure 1. Sampling locations of Malayan tiger scats. (1: Royal Belum State Park, Perak; 2: Felda Kerteh, Kemaman, Terengganu; 3: Taman Negara Terengganu; 4: Pos Bihai Gua Musang, Kelantan; 5: Hutan Simpan Kekal Perias, Kelantan. Map on the left side: green, forest coverage; brown, oil palm plantation; purple, human settlement).

DNA extraction and species identification

All scat samples were extracted using the QIAmp FAST Stool Kit (Qiagen, Germany) and QIAmp DNA Blood and Tissue Kit (Qiagen, Germany). A slight modification was performed during lysis by incorporating DTT to effectively lyse harder components, such as hair and nails present in the scats. In ensuring the absence of cross-contamination, negative controls were included in all DNA extraction procedures and in polymerase chain reactions (PCR). The extracted genomic DNA was visualised using 1% agarose gel electrophoresis and the DNA concentration was determined using Nanodrop ND-1000 (Nanodrop, Wilmington, DE, USA).

Scat samples were amplified using the partial control region (Dloop): MGCR560F (5'-GTGTACCTCTTCGCTCCG-3') and MGCR873R (5'-TGTTGTACGTGGAACCCC-3') for species identification. Of the 33 samples, 13 were successfully amplified and identified as Malayan tiger scats. Given the low concentration of obtained DNA, only 10 Malayan tiger samples were subjected for DNA metabarcoding analysis. Fig. 1 and Table 1 show the scat sample information collected for prey detection data analysis.

PCR and library construction

A total of 10 samples were proceeded with amplicon sequencing as these samples passed the quality control (QC) with the minimum concentration of 10 ng/µl. A two-stage PCR was used to amplify and prepare sequencing libraries. The PCR amplification using the *COI* primer pairs m1COIintF and dgHCO2198 (Geller et al. 2013; Leray et al. 2013). The forward primer, mlCOIintF, was constructed using the Illumina 5' overhang adapter sequence (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG), followed by the forward primer sequence (GGWACWGGWTGAACWGTWTAYCCYCC). Meanwhile, the reverse primer, dgHCO2198R, was constructed using another 5' overhang adapter sequence (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG), followed by the reverse primer sequence (TAAACTTCAGGGTGACCAAARAAYCA). The first PCR step was performed in a 25 µl reaction volume containing 2.5 µl (5 ng/µl) of DNA template, 12.5 µl of KAPA Hifi HotStart ReadyMix (KAPA Biosystem), 0.5 µl (10 µM) of each millilitre

Table 1. Scat sample information of Malayan tigers.

No.	Individuals/Sample ID	Locations tag on map	Habitat type	Habitat type
1.	Syamilla Mek Bihai	4	Kampung Orang Asli	HTC
2.	Sau Bihai	4	Kampung Orang Asli	HTC
3.	PT-BH01	4	Kampung Orang Asli	HTC
4.	Awang Rasau	2	Oil palm plantation	HTC
5.	Atan Kerteh	2	Oil palm plantation	HTC
6.	PSC01	2	Oil palm plantation	HTC
7.	PSC12	3	National Park	PA
8.	PSC13	3	National Park	PA
9.	HSKP-21-13	5	Forest Reserve	PA
10.	SgKejar	1	Forest Reserve	PA

of m1COIintF and dgHCO2198R primers and 9 µl of nuclease-free water. The PCR conditions were as follows: 95 °C for 3 min, 25 cycles of 95 °C for 30 s, 54 °C for 30 s and 72 °C for 45 s and (final extension at?) 72 °C for 10 min. The amplification products of the first PCR were purified using 0.8X AMPure XP beads.

The second round of amplification was performed to incorporate Illumina i5 and i7 adapters and 8-bp barcodes. The PCR mixture was performed in a 10-µl reaction containing 5 µl of KAPA HiFi HotStart Ready Mix, 1 µl of each primer index (i7 and i5) and 3 µl of PCR products from the first PCR. The second PCR conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 8 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 1 min. The libraries were purified using 0.7X AMPure XP beads and quantified using a Qubit 2.0 fluorometer (Life Technologies). The size distribution of the libraries was assessed using TapeStation 2200 (Agilent). All libraries were pooled and loaded on to an Illumina Miseq 2 × 250 bp flow cell at 11 pM. All NGS laboratory works were conducted in Monash University Malaysia Genomics Platform (MUMGP).

Sequences data analysis

Fastq-formatted reads were imported into Geneious Prime® 2022.1.1. The assembly of raw reads of *COI* sequences was run using Geneious Prime® 2022.1.1. Raw sequencing data were trimmed using BBDuk in the Geneious Prime platform to remove any remaining Illumina adapters, bases below the average quality and reads that are less than 150 bp after end-trimming. Then, the trimmed sequences were merged by using the BBMerge tool in the Geneious Prime platform to create a single consensus sequence for each pair. Chimeric sequences were checked and eliminated on the basis of UCHIME (https://drive5.com/uchime/uchime_download.html) and "Gold" database (https://drive5.com/uchime/gold.fa).

Reads were clustered into operational taxonomic units (OTU) using a de novo assembler. Then, the sequences were assigned with a minimum overlap identity of no less than 97%. The taxonomy database was created by blasting the OTUs against a *COI* database from the National Center for Biotechnology Information (NCBI) nucleotide database with some entrez filters targeting on Eukaryote sequences and excluding environmental, uncultured and unclassified sequences. After creating a sequence classifier database from BLAST hits, the full amplicon dataset was classified using the Sequence Classifier in Geneious Prime 2022.1.1. Then, the filtered reads were analysed using R version 2022.07.2. All analyses, including the determination of relative read abundance (RRA), as well as alpha and beta diversity analyses, were performed to evaluate within and between sample habitat types by using the phyloseq (McMurdie and Holmes 2013) and ggplot2 (Wickham 2016) packages in Rstudio. Alpha diversity indices were estimated to indicate the species richness, abundance and evenness of each sample. The alpha diversity using Chao1 and Shannon–Weaver indices was calculated to describe the population diversity in scat samples (Kim et al. 2017). Beta diversity was calculated using weighted unifrac and pairwise Bray–Curtis index to construct Principal Coordinate Analysis (PCoA), which represents the differences between prey selection and habitat groups in HTC and PA. PERMANOVA with 999 permutations was also used to estimate and assess significant differences in diet amongst habitat groups.

Data accessibility

Raw sequence reads have been archived on the NCBI Sequence Read Archive (SRA) with project number PRJNA954969, Biosamples submission numbers SAMN34161777, SAMN34161791, SAMN34161871, SAMN34162214, SAMN34162338, SAMN34162396, SAMN34163286, SAMN34163462, SAMN34163485 and SAMN34163613.

Results

Scat species identification and data filtering

Of the 33 felid scats collected during field sampling, only 13 were confirmed to be Malayan tigers. However, only 10 gDNA products of Malayan tiger scats were proceeded to NGS because of low DNA concentration. DNA metabarcoding analysis was run in Illumina Miseq platform and a total of 2,132,729 raw reads were generated after filtering. Then, low-quality sequences, primer adapter and chimeric reads were removed and 440,272 reads remained. The remaining metabarcoding *COI* data were blasted against the NCBI nucleotide database. The rarefaction curve with 13,885 reads (Fig. 2) showed that the samples reached an asymptote and sampling depth was sufficient to estimate the diversity. Table 2 shows the read count summary generated during filtering and rarefaction.

Malayan tiger dietary profiling

A total of 10 Malayan tiger scat samples indicated the presence of 416 OTUs and yielded 17 phyla in the kingdom of eukaryotes (Fig. 3A). This study aims to identify the potential prey species predated by Malayan tiger are in phylum Chordata only. Therefore, 21 OTUs were generated from the 97% OTU cluster in phylum Chordata. Three classes of prey DNA were identified in 10 Malayan tiger scats, comprising seven orders and eight families and only 12 genera were identified.

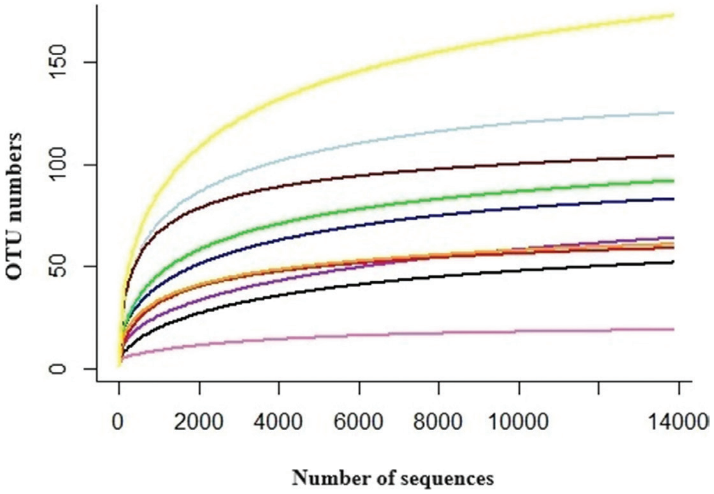

Figure 2. Rarefaction curve based on 10 samples of wild Malayan tiger scats.

Table 2. Read count summary of 10 Malayan tigers.

Sample ID	Habitat type	OTU numbers	Raw sequence reads	Non-chimeric reads
Syamilla Mek Bihai	HTC	81	187,410	80,100
Sau Bihai	HTC	73	146,716	43,777
PT-BH01	HTC	116	159,839	59,362
Awang Rasau	HTC	71	176,472	55,069
Atan Kerteh	HTC	110	203,531	26,232
PSC01	HTC	26	247,962	32,600
PSC12	PA	73	249,590	35,862
PSC13	PA	131	246,986	13,885
HSKP-21-13	PA	120	182,621	51,093
SgKejar	PA	219	331,602	42,382

Despite unknown and unidentified Chordata sequences, Malayan tiger scats were dominated by the class Mammalia (99.96%), Aves (0.01%) and Reptile (0.03%). These percentages were derived from the 3.56% representation of the Chordata phylum, which was generated from all COI sequences obtained. The three classes identified consist of seven orders which are Artiodactyla (22.52%), Carnivora (71.38%), Chiroptera (5.67%), Galliformes (0.04%), Primates (0.34%), Rodentia (0.05%) and Squamata (0.01%).

Considering the habitat type, Malayan tigers at PA preyed most on the family Canidae (66.21%), followed by Vespertilionidae (5.66%), Bovidae (1.75%) and Rhinolophidae (0.01%). Meanwhile, the dominant prey DNA was identified in Malayan tiger scats that are living in areas categorised as HTC mostly from the family Bovidae (20.74%), Homonidae (0.34%), Felidae (Felinae, 0.06%), Muridae (0.044%) and Phasianidae (0.038%, Fig. 4A). Fig. 5 shows the summary of the overall RRA of prey DNA in 10 tiger scats within the two habitat groups based on relative abundance at genus level. The HTC and PA groups showed different patterns of prey selection by Malayan tigers. In the HTC group, the primary prey

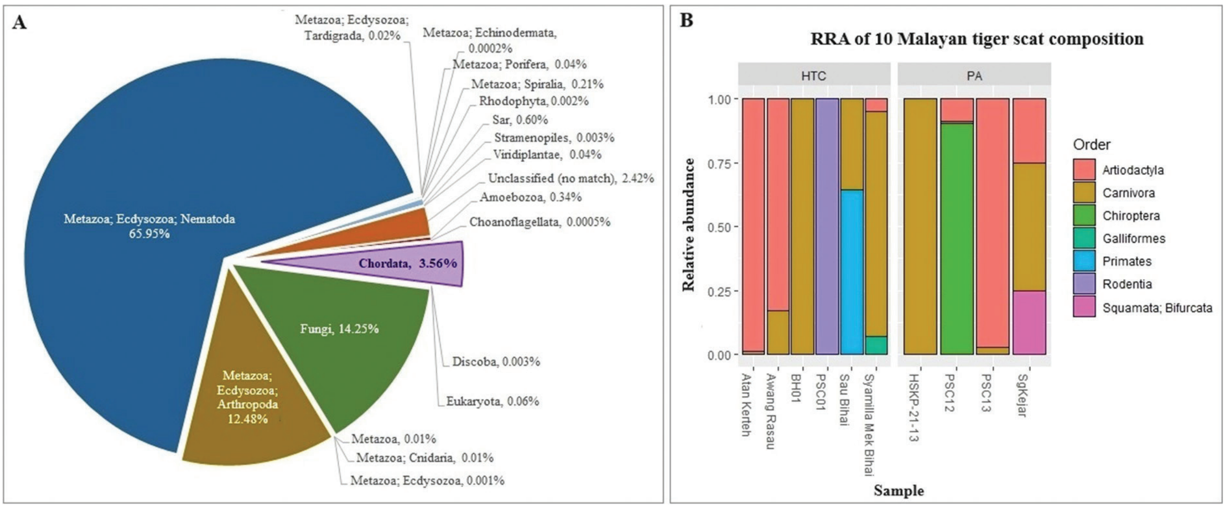

Figure 3. Scat composition of 10 Malayan tigers **A** relative read abundance (RRA) of scat composition at phylum level, based on 416 OTUs and **B** RRA of scat composition from two habitat groups of Malayan tigers at order level, based on 3.56% Chordata phyla identified.

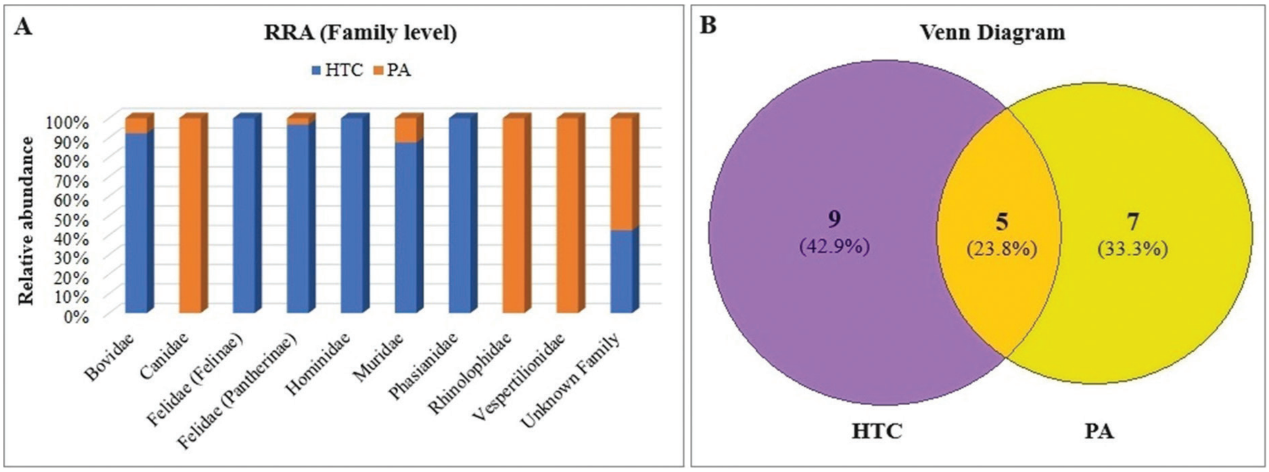

Figure 4. Prey taxa composition in scat samples of Malayan tigers, based on 21 OTUs **A** relative read abundance (RRA) of prey taxa at family level in Malayan tiger scats and **B** venn diagram from two habitat groups of Malayan tigers.

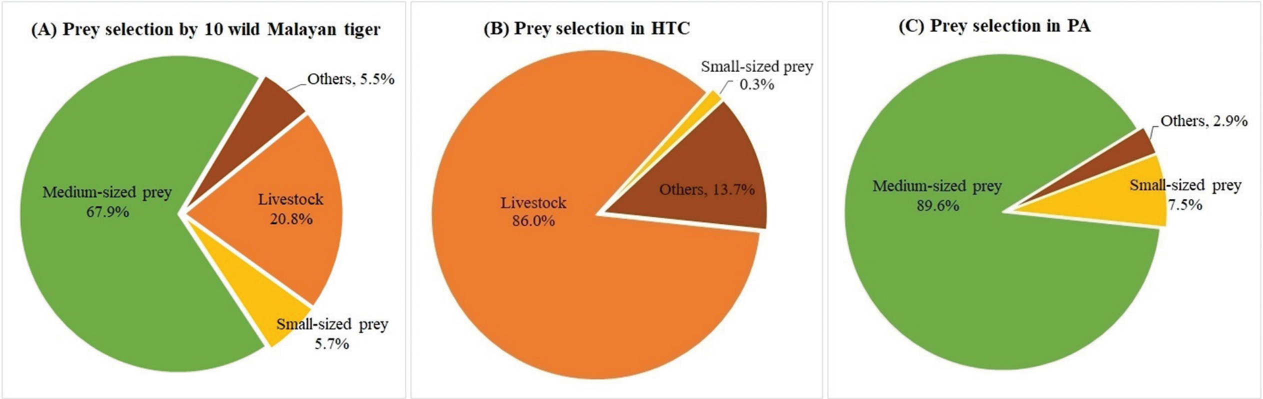

Figure 5. Summary of prey species identified in **A** all Malayan tiger scats **B** human–tiger conflict areas (HTC) and **C** protected forest areas (PA).

selection of tigers primarily consists of livestock (86.0%), small prey (0.3%), followed by other prey species (14%). The livestock consist of genus *Bos*, *Bubalus*, *Capra* and *Gallus*. Small-size prey identified include genus *Mus* and *Felis*. Meanwhile, for the other group, this consists of genus *Homo*, Malayan tiger DNA itself and unknown genus. The PA group showed the absence of livestock species and preyed on medium (89.6%) and small (7.5%) prey. The medium prey consists of genus *Canis* and *Capricornis*; meanwhile, small prey consists of genus *Kerivoula*, *Rhinolophus* and *Mus*. The Venn diagram shows (Fig. 4B) the 97% OTU cluster overlap within the HTC and PA groups. The size of the circle represents the relative OTU richness of each habitat type, PA (7 OTUs) and HTC (9 OTUs). A total of five shared OTUs between PA and HTC indicate that preys were commonly consumed by Malayan tigers in both habitat types.

Alpha and beta diversity

Species richness in each sample was assessed for alpha diversity analysis using the Chao1 and Shannon indices. The Shannon index showed a significant difference in prey selection by Malayan tigers at two different habitat groups

($p < 0.05$), whereas the Chao1 index showed no significant difference ($p > 0.05$). The high value of the Shannon index indicates the high diversity in richness and evenness. Meanwhile, the Chao1 index is a non-parametric method for estimating the number of species in a community. Table 3 shows the score values of Chao1 and Shannon indices and Fig. 6A shows the Chao1 and Shannon indices within the HTC and PA groups. The Chao1 and Shannon indices showed higher prey diversity in the PA group.

Beta diversity was displayed using a PCoA plot of *COI*-rarefied RRA data of prey DNA in Malayan tiger scats in different habitat types (Fig. 6B). A Bray–Curtis distance was calculated on the basis of the pairwise taxonomic profile of 10 scat samples and used to generate PCoA coordinates of each sample. The distance linking two samples is shorter, indicating higher similarity between these samples. The first (PCoA1) and second (PCoA2) axes explained the total variation of 13.8% and 12.2%, respectively. Samples collected from the two habitats

Table 3. Values of Chao1 and Shannon indices of 10 samples.

Samples ID	Chao1 index	Shannon index
Syamilla Mek Bihai	88.5	1.47674
Sau Bihai	106.0	0.67834
PT-BH01	129.6	2.38472
Atan Kerteh	141.6	2.37876
Awang Rasau	76.6	0.97147
PSC01	32.0	0.78397
PSC12	82.2	2.03072
PSC13	143.4	2.88448
HSKP-21-13	130.1	3.13198
SGKejar	235.1	3.38908

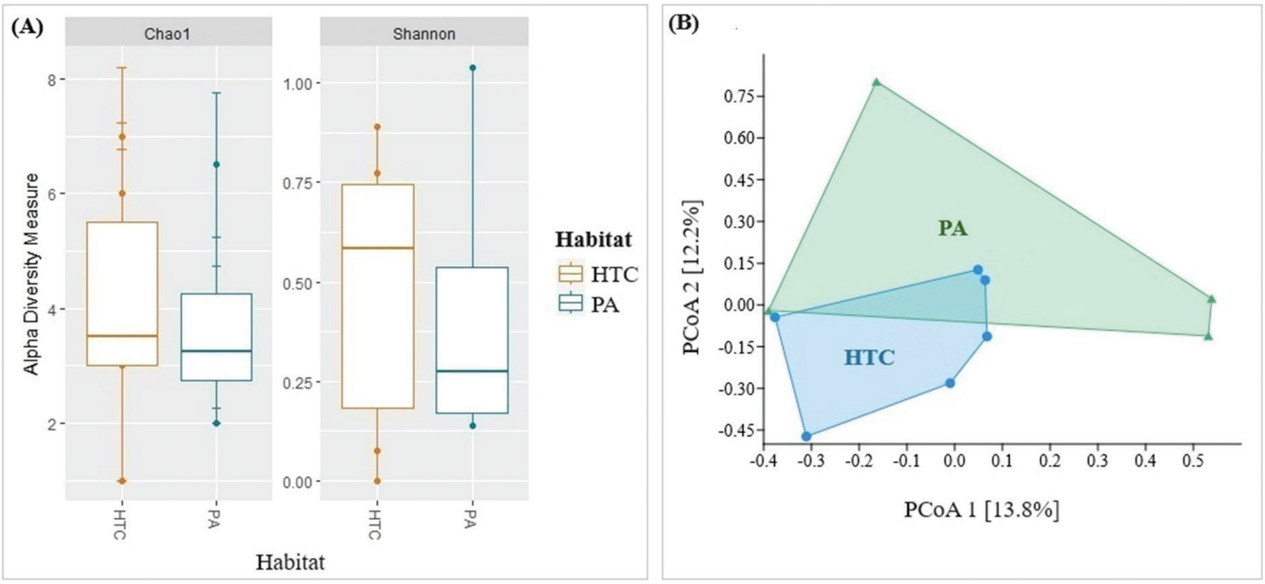

Figure 6. Diversity of prey taxa in Malayan tiger scat samples **A** alpha diversity, based on Chao1 and Shannon indices. **B** beta diversity displayed in the PCoA plot, based on the Bray–Curtis distance.

were illustrated by different colours. The PCoA plot data indicated there is a significant difference between HTC and PA. This result was supported by PERMANOVA analysis, which showed significant difference between the PA and HTC groups (Pr(> F) = 0.326; $R^2 = 0.117$; F = 1.0624, p-value = 0.026, < 0.05).

Discussion

Malayan tiger is an apex predator that plays a vital role in the ecosystem in Malaysia. The abundance and occurrence of prey species in tiger's habitat are associated with the predator–prey interaction, including survival, behaviour and their movement areas. In this study, scat sample collection was conducted in the wild habitat of Malayan tiger to understand the selection of prey by Malayan tiger in Peninsular Malaysia. Fig. 7 illustrates 10 scat samples confirmed to be from Malayan tiger, identified through molecular detection of the mitochondrial DNA (mtDNA) D-loop region. The taxonomic classification analysis in this study indicates that only 3.56% of phylum Chordata *COI* sequences were generated in 10 scat samples. The most sequences generated in this study are largely identified *COI* sequences of the fungi, Choanoflagellata, Amoebozoa, Discoba, Sar, Viridiplantae other metazoas and unknown eukaryotes (94.02%). Another 2.42% of *COI* sequences could not be assigned to any taxonomic group (unknown/unclassified sequences). This is probably due to sample quality and primer sensitivity to detect DNA prey items in scats of Malayan tiger.

This study found that the scat composition of the Malayan tiger in the selected areas of Peninsular Malaysia was dominated by medium prey (67.9%), livestock (20.8%) and small prey (5.7%, Fig. 5A). Based on our findings, Malayan tiger prey varies in HTC compared with that in PA areas. Malayan tiger prey selection in PA areas comprises dogs, bats, serows, rodents and squamates. Meanwhile, the dominant prey DNA identified in the HTC areas comprises prey taxa from livestock species (cow, buffalo, goat, chicken), rodent, human and cats. Alpha (Shannon index) and beta diversity (PERMANOVA) analysis showed significant difference ($p < 0.05$) between the PA and HTC groups. This finding might be due to the habitat characteristics, which influence the selection of prey availability in each habitat group. Therefore, the significant differences between the PA and HTC groups are also because of the differences in prey composition found in tiger scats in the HTC group, which are livestock, as well as the detection of human DNA in the HTC group. As shown in Fig. 5, livestock and human DNA were detected in the HTC group; meanwhile, these prey taxa were not found in the PA group. In addition, DNA from humans was discovered in Malayan tiger scat samples collected from the HTC group. This finding is attributed to two scat samples that originated from tigers involved in previous human attacks. The changes in behaviour and habitat structure to the food requirement bring Malayan tigers into conflict with humans and livestock. Department of Wildlife and National Parks (2008) reported that Malayan tigers also hunt livestock that are near to tiger habitats. Recently, Malayan tigers were found closer to human settlement areas and oil palm plantations because of the availability of livestock where they can easily hunt and readily prey on the livestock. The occurrence of tigers hunting livestock can be ascribed to the migration of Malayan tigers towards areas where livestock is present, as well as with their inability to fulfil their essential dietary requirements within their native

Figure 7. Collected Malayan tiger faecal samples **A** Syamilla Mek Bihai **B** Sau Bihai **C** PT-BH01 **D** Atan Kerteh **E** Awang Rasau **F** PSC01 **G** PSC12 **H** PSC13 **I** HSKP-21-13 **J** SgKejar.

habitats (Kumar 2015). Consequently, tigers shift from forested areas to buffer zones as they are compelled to do so when they find more livestock accessible and easy to prey upon (Kolipaka et al. 2017).

Scat composition analysis of Malayan tigers inhabiting PA areas detected the presence of dogs DNA in HSKP-21-13 sample, serow's DNA from PSC12 and PSC13; meanwhile bats, rodent and squamate DNA have been found in the scat sample named PSC12. Notably, the *COI* taxonomic classification analysis in this study did not detect any common prey by Malayan tiger as reported in previous studies (Lynam et al. 2007; Kawanishi et al. 2010) such as wild pig, barking deer and sambar deer. There is no large prey species found in Malayan tiger scat samples in the PA group. In the forest area, probably large prey was scarce; thus, hunting large prey is also scarce. Therefore, when the main prey was scarce, tigers will alter their diet. This factor could explain why tigers prefer to non-selectively prey on small-to-medium-sized species. We hypothesised that Malayan tigers in Peninsular Malaysia have a wider range of available prey sizes to meet their dietary requirements. Small sized prey consumed by tigers were rarely reported by previous studies. However, based on a study conducted by Schaller (1967), Royal Bengal Tigers also engage in predation on smaller species such as civet cats, water monitors, birds, fish and frogs, apart from their high consumption of spotted deer, wild boar, rhesus monkeys and water monitors. This finding is coherent with our study, demonstrating that tigers across different subspecies can prey on smaller animals, irrespective of the analysis method used. Bats have been identified in a scat sample labelled as PSC12. The probable explanation for detecting these bat species in the analysis of Malayan tiger scats is the significant bat population present reported in the PSC12 location (Pounsin et al. 2018; Azmir et al. 2022). The area comprises of hill dipterocarp forests and nearby karst caves. The vicinity of karst caves to the collected scat samples suggests the possibility of Malayan tigers preying on bats in their roosting areas. According to National Tiger Survey (NTS) programme which was coordinated by PERHILITAN, the Malayan tiger has been recorded roaming around the cave areas through the camera trap (Halim et al. 2019). Therefore, tiger prey preference may vary depending on locations. Another possibility is that the Malayan tiger indirectly ingested the small-sized animal while predating on another prey.

In addition to the above, during field sampling, wild boar is found to be present in PA and HTC areas; however, no wild boar DNA were detected in tiger scats in our analysis. Kawanishi et al. (2003) assumed that the abundance of wild boar and omnipresence in almost all vegetation, including oil palm plantation, can be the main option of prey by Malayan tigers, although some of the deer populations were depleted. However, tigers preferred to prey on livestock and small-to-medium-sized species to meet their food dietary requirement in PA and HTC areas. The reason why tigers prefer livestock, small and other medium species rather than wild boar in this study remains unknown. Thus, investigating the assertion and the degree to which the tiger's diet is augmented by livestock is important to understand the real productivity of the tiger's habitat beyond what is naturally available (Kawanishi et al. 2003). As mentioned before, tiger prey may vary depending on locations; however, in general, wild boar and sambar deer are the foremost kinds of herbivorous mammals that have been linked to tigers as prey (Reddy et al. 2004; Ramesh et al. 2009; Mukherjee and Sarkar 2013; Kumar 2015; Upadhyaya et al. 2018).

Based on the findings of this study, the prey DNA in Malayan tiger scats may be influenced by other factors such as the scat sample quality, predation time, and selection of gene used. The detection of DNA prey depends on several factors, particularly the time when Malayan tigers consumed their prey species. Some DNA were degraded because of long exposure to the environment and the freshness of the scat sample affected the detection and identification of prey species (Upadhyaya et al. 2018). Thuo et al. (2019) conducted a study on the influence of various factors on prey DNA detection, such as the time of prey consumption (feeding day), scat age, prey species and meal size. The study revealed that prey DNA detection was weaker on day 0, the day the prey was consumed by cheetahs. However, DNA was high on day 1 and then it declined on the following day. Moreover, chicken, deer and horse were easily detected on the day of consumption compared with quail and rabbit, based on the prey species. In addition, the assessment of prey species identification in scats was affected by prey size. According to Ackerman et al. (1984), small prey species have a high amount of hair per unit body weight, which results in undigested hair found in their scat. The presence of a large amount of hair in the scat could result in an exaggerated observation of the occurrence of smaller prey species in the diet of the carnivore. This study revealed a lower frequency of prey species occurrence in each scat. Several factors could influence this frequency, including: 1) samples size, 2) sample quality or 3) the timing of predation (whether the prey species had been recently hunted or had been hunted for an extended period). Therefore, this study recommends future studies to increase sample number by conducting a longer duration of field sampling and covering a wider area of study locations.

Another factor influencing the result is the selection of gene or primers for prey DNA detection in scat samples. In this study, taxonomic classification analysis indicates that 2.42% of *COI* sequences generated could not be assigned to any taxonomic group (unknown/unclassified sequences). The partial *COI* gene (mlCOIintF and dgHCO2198R) can identify only 3.56% of the Chordata taxa in all samples and another 94.02% of the sequences generated largely identified other eukaryotes, such as fungi, amoebozoa, viridiplantae and another metazoan other than Chordata. The partial region of *COI* used in this study is an insufficient taxonomic coverage of *COI* barcoding primers. Hacker et al. (2021) conducted a study utilising the *COI* region on *Panthera uncia* to analyse MT-*COI* and identify the prey items, particularly Caprinae, which is present in snow leopard scats. However, they found that only goat and sheep DNA were successfully amplified, whereas the DNA of other ungulate families or mammalian orders was not detected. DNA metabarcoding has its own shortfalls and it depends on the accuracy and coverage of the DNA reference database (Nielsen et al. 2018; Traugott et al. 2020). The *COI* primer might be used in identifying prey species from invertebrates, but not much for vertebrate prey species. Moreover, using a single set of primers focusing on specific prey groups may produce biased results (Da Silva et al. 2019). According to Quéméré et al. (2021), the use of multiple primers can produce more comprehensive results. Utilising multiple markers enhances the precision in identifying individual taxa and contributes to the overall precision of dietary profiles (Ando et al. 2020). Therefore, this study suggests that future studies should consider incorporating multiple loci or other alternative loci to investigate the elusive prey selection by the Malayan tiger in Peninsular Malaysia.

Conclusions

This study was the first to investigate and describe the prey selection of Malayan tigers by DNA metabarcoding of scats. Despite the relatively small number of scats analysed in this study, the result demonstrated the overview of the current situation of prey selection by Malayan tigers in Peninsular Malaysia. In HTC and PA areas, Malayan tigers mostly consumed livestock and medium-to-small-sized prey species. Human expansion might be the major cause of the alteration of prey selection to livestock and medium-to-small-sized prey species. Prey selection by Malayan tigers is affected by human-induced alterations to the environment. Forest fragmentation causes the roaming areas of Malayan tiger to move towards the human areas, particularly oil palm plantation areas and human settlements. Usually, livestock owned by local people are often placed within their oil palm plantations area and most of the oil palm plantations in Peninsular Malaysia were near to the forest making the area a HTC area. When livestock is available, tigers will readily prey on them. Based on our results, future studies on tiger diet should consider using multi-locus DNA metabarcoding and conducting field sampling for a longer duration and should cover a wide area to understand the spatiotemporal variation in tiger diet. This approach will provide other opportunities to study their preference in the wild. Although this study cannot be used to quantify true abundance or proportion of prey species, it provides an important first step towards identifying prey taxa and spatial–temporal patterns in Malayan tiger diets. Less attention has been paid to medium and small prey than to large ones, leading to a shortfall in knowledge regarding their ecological roles. This limitation should be considered to help in planning strategies of conservation effort to Malayan tigers in Peninsular Malaysia. The data obtained in this study will improve dietary insight, which can be used to develop conservation plans and strategies for Malayan tigers, particularly for habitat priorities, protection and restoration in specific areas.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

Wildlife Permit No.: P06/10/2020 -Ethical Permit: UKMAEC approval number: FST/2021/BADRUL MUNIR/22-SEPT./1198-OCT.-2021-OCT.-2023-NAR-CAT2).

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Author contributions

MG, FTS, ZK, SSS, NMZA, HNMS, MW, BS and NFKAH conducted field sampling and collected samples. MG conducted DNA extraction and laboratory works. LSY and LFY performed library construction and sequencing. MG analysed all the data and collected information of the samples. MG drafted and edited the manuscript. ARMR, SY and BMMZ critically revised the intellectual content. All authors read and approved the final version of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Spatial distribution models and biodiversity of phytoplankton cysts in the Black Sea

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Abstract

The current study employed diverse statistical and machine learning techniques to investigate the biodiversity and spatial distribution of phytoplankton cysts in the Black Sea. The MaxEnt distribution modeling technique was used to forecast the habitat suitability for the cysts of three potentially toxic microalgal taxa (*Lingulodinium polyedra*, *Polykrikos hartmannii*, and *Alexandrium* spp.). The key variables controlling the habitat suitability of *Alexandrium* spp. and *L. polyedra* were nitrates and temperature, while for the *P. hartmannii* cysts, nitrates and salinity. The region with the highest likelihood of *L. polyedra* cyst occurrence appears to be in the western coastal and shelf waters, which coincides with the areas where *L. polyedra* red tides have been documented. The projected habitat suitability of the examined species partially overlapped, perhaps as a result of their cohabitation within the phytoplankton community and shared preferences for specific environmental conditions, demonstrating similar survival strategies. The north-western region of the Black Sea was found to be the most suitable environment for the studied potentially toxic species, presumably posing a greater risk for the onset of blooming events. Two distinct aspects of cysts' ecology and settlement were observed: the dispersal of cysts concerns their movement within the water column from one place to another prior to settling, while habitat suitability pertains to the particular environment required for their survival, growth, and germination. Therefore, it is crucial to validate the model in order to accurately determine a suitable habitat as well as understand the transportation patterns linked to the particular hydrodynamic properties of the water column and the distinct features of the local environment.

Key words: Black Sea, cyst assemblages, habitat suitability, harmful algal blooms, MaxEnt, potentially toxic phytoplankton

Introduction

As essential primary producers, phytoplankton biodiversity has a profound ecological impact on the state and dynamics of marine ecosystems and can influence their functioning via global biogeochemical cycling of carbon, nitrogen, phosphorus, and silicate, in addition to primary productivity (Ptacnik et al.

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2008; Ellegaard and Ribeiro 2018). A considerable number of phytoplankton species undergo benthic dormant phases throughout their life cycles (Belmonte and Rubino 2019). These dormant stages may result from either sexual reproduction, such as the resting cysts of dinoflagellates and the resting spores of some diatoms, or vegetative division, such as the resting spores or resting cells of most diatoms (Ellegaard and Ribeiro 2018). Some of the species forming benthic resting stages may produce harmful algal blooms, which can have detrimental impacts on the environment and result in substantial economic losses (Hallegraeff 1993). Phytoplankton cysts that have accumulated in sediments may remain viable for several decades or potentially up to a century (Lundholm et al. 2011), serving as potential seed banks, maintaining biodiversity over time (Kremp et al. 2018), and contributing to bloom dynamics (Lundholm et al. 2011; Anderson et al. 2014; Castañeda-Quezada et al. 2021). Therefore, benthic seed banks hold significant ecological importance by playing a vital role in enhancing the resilience of phytoplankton populations, particularly in changing environments (Picoche and Barraquand 2022). Dinoflagellate cysts have been extensively utilized as a proxy for studying alterations in sea-surface conditions (Sgrosso et al. 2001; Godhe and McQuoid 2003; Pospelova et al. 2004; Richter et al. 2007; Kremp et al. 2009; Zonneveld et al. 2013; Mudie et al. 2017; Shin et al. 2018). Given the discontinuous presence of phytoplankton species in the water column (Lundholm et al. 2011), studying the structure and distribution of cysts' communities can provide important insights into the diversity of phytoplankton (specifically those that form cysts) over time, environmental shifts, and potential harmful algal blooms associated with cysts (Mudie et al. 2017). The latter could have major implications for vulnerable ecosystems such as the Black Sea, which is characterized by its high level of isolation from the global ocean, substantial inflow of freshwater, pronounced vertical stratification, low salinity, and elevated concentrations of hydrogen sulfide below depths of 150–200 meters.

The composition and survival of cyst assemblages in pelagic communities rely heavily on the species' distinct morphological, biochemical, and physiological characteristics, as well as the fluctuations in biological, physical, and chemical oceanographic conditions in both surface water and bottom sediments (Ellegaard and Ribeiro 2018 and the references therein). The Atlas of modern dinoflagellate cyst distributions in the Black Sea Corridor (Mudie et al. 2017) provides a thorough reference for enhancing our knowledge of the connections between surface water conditions and the distribution, diversity, and morphological variations of dinoflagellate cysts in the Black Sea. Additional research on cyst assemblages and their correlation with environmental variables may aid in the evaluation and modeling of the distinct distribution patterns of cysts in sediments, as well as in the expansion of our knowledge of these complex interactions. This is particularly essential for the mapping of cyst banks containing potentially harmful/toxic species.

Spatial distribution modeling (SDM) has gained significant recognition in recent years as a highly effective method for assessing the spatial status of biodiversity (Cayuela et al. 2009). It is widely acknowledged as an accurate approach for identifying the habitat preferences of species in response to certain environmental variables (Guisan and Thuiller 2005). SDMs are predictive models that utilize the spatial distribution of documented species occurrences and analyze them in relation to different environmental factors. This analysis is facilitated through the use of statistical algorithms that aim to elucidate the influence of the environment on the patterns of species presence or absence (Guisan and Thuiller 2005; Coops et al. 2009; Elith and Leathwick 2009). The present research employed the Maximum Entropy (MaxEnt) modeling technique. The latter refers to a machine learning algorithm that has been specifically designed to make predictions using incomplete data (Baldwin 2009). The method is to determine the sampling sites' distribution that is the most uniform in comparison to the background locations, taking into account the constraints related to the data. The maximum entropy algorithm is a deterministic approach that exhibits convergence towards the probability distribution with the highest entropy (Baldwin 2009). Hence, the resultant output indicates the degree to which the model presents a more precise alignment with the location data in comparison to a uniform distribution. The MaxEnt algorithm has several advantages, one of the most notable being its capability to model both continuous and categorical variables. In addition, MaxEnt performs variable transformation and feature selection, maximum entropy model fitting, and regularization techniques in order to avoid the likelihood of model overfitting. Furthermore, the output generated by the model is rather easy to interpret. The predictive accuracy of MaxEnt has consistently demonstrated comparability to the most efficient modeling techniques, and it has been successfully employed to model the biogeographic distributions of species in various regions of the world (Yost et al. 2008; Elith et al. 2011; Smith et al. 2012; White et al. 2012; Urbani et al. 2015; Mamun et al. 2018; Wang et al. 2018; Holder et al. 2020; Yan et al. 2020).

In this study, the biodiversity of modern cyst assemblages was examined in sediment samples collected from 30 sites (coastal and shelf) in the Black Sea. The species composition, abundance, diversity indices, and association of species with sites and sediment types were analyzed. The MaxEnt distribution modeling technique was employed to accurately fit habitat suitability models for the distribution of cysts of three potentially toxic microalgal taxa (*Lingulodinium polyedra*, *Polykrikos hartmannii*, and *Alexandrium* spp.) in the Black Sea basin. The objective was to evaluate the extent to which their distribution is influenced by specific environmental variables.

Methods

Sampling

The study region encompassed the Black Sea waters of Bulgaria (BG), Romania (RO), Ukraine (UA), Georgia (GE), and Turkey (TR) (Fig. 1, Suppl. material 1: table S1). A total of 47 surface sediment samples (top 2 cm) were collected at 30 sites, ranging in depth from 7.5 to 101 meters. These samples were obtained throughout various campaigns conducted between April 2008 and June 2016, primarily in the spring and/or summer seasons. The collection methods used either a multicorer or a Van Veen Grab sampler. The sediment samples were stored in a light-free environment at a temperature of 4 °C without any preservatives until they were processed.

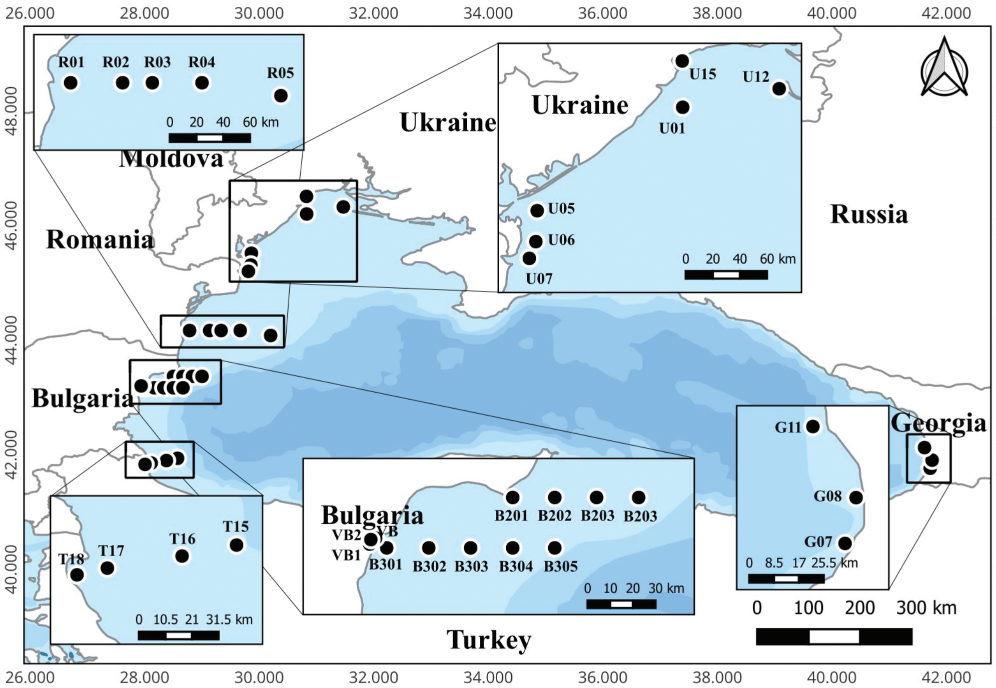

Figure 1. Map of sampling sites and locations of stations in the Black Sea.

Sediment treatment, qualitative, and quantitative analysis of cysts

An aliquot of homogenized sediment (from 2.0 to 2.2 cm^3) was taken from each sample for cyst analysis. Additionally, a separate aliquot (≈ 10 cm^3) was obtained to determine the water content. The wet aliquots were weighed and screened through a 10 μm mesh (Endecotts Limited steel sieves, ISO3310-1, London, England) using natural filtered (0.45 μm) seawater (Montresor et al. 2010). The material retained on the sieve was ultrasonicated for 1 min at low frequency and screened again through a sieve battery (200, 75, and 20 μm mesh sizes). A fine-grained fraction (20–75 μm), mainly containing protistan cysts, was obtained. The material retained on the 75 and 200 μm mesh was not considered in this study. No chemicals were used to dissolve sediment particles in order to preserve calcareous and siliceous cyst walls.

Qualitative and quantitative analyses were carried out under an inverted microscope (Zeiss Axiovert 200M) equipped with a Leica MC170 HD digital camera at ×320–400 magnification. Both full cysts with cytoplasmic content (i.e., presumably viable) and empty, already germinated cysts were enumerated, but the empty cysts were not considered in this study; a minimum of 200 viable cysts were counted for each sample to obtain abundance values as homogeneous as possible and evaluate rare species too.

To estimate the water content of sediment, an aliquot from each sample (≈ 10 cm^3) was weighed and dried out at 70 °C. Quantitative data are reported as cysts per gram of dry sediment (cysts g^{-1}). The results from the repetitive stations (which were sampled more than once for the study period in separate expeditions) were compiled, and the highest abundance values were utilized for the analyses.

All resting-stage morphotypes were identified using previously published descriptions. The organic dinocysts were analyzed using the images and keys supplied in Mudie et al. (2017). The currently accepted biological taxonomic (motile-cell) names were used preferentially according to WoRMS (2023).

Statistical analyses

Biodiversity indices were utilized to conduct a robust evaluation of the distinct patterns and preferences of species with regard to habitat or site groups. This included assessing the species richness per habitat/sediment type, examining the association between species and habitat/sediment type, and determining the effectiveness of species as indicators of site groups. The habitat types were defined according to Vasquez et al. (2021). The analyses were performed using the R environment version 4.1.2 (R Core Team 2022), utilizing the 'BiodiversityR' package (Kindt and Kindt 2019) and the 'indicspecies' package (De Cáceres and Jansen 2016).

In addition, the present study examined the spatial distribution of cyst assemblages of three potentially toxic taxa, specifically *L. polyedra*, *P. hartmanii*, and *Alexandrium* spp., utilizing the Maximum Entropy machine learning algorithm. The models were initially fitted and cross-validated using MaxEnt software Version 3.4.4 (Phillips et al. 2017) and thereafter developed in the Jupiter Notebook environment (Kluyver et al. 2016) using the Python programming language (Van Rossum and Drake 1995) version 3.9.0 through the implementation of elapid-species distribution modeling tools for Python (Anderson 2023) that offer a customized implementation of the Maximum Entropy machine learning modeling technique and a collection of methods for analyzing biogeography data. It enables the incorporation of spatial components into the model through features such as sample weighting and geographic k-fold cross-validation. Elapid enhances user control and comprehension of the complex MaxEnt approach, thereby improving flexibility. Consequently, this assists users with developing and evaluating their models more effectively. The latter facilitates the process of modeling data that varies over time across multiple scales, fitting models that consider geographic weighting, creating ensembles of models, accurately defining the distribution of background points, and summarizing the predictions made by the model.

The input data for the MaxEnt model includes a collection of species' presence-only (PO) locations and a set of environmental predictors within a spatial extent selected by the user. The MaxEnt algorithm selects a set of background locations, which are then compared to the known presence locations. Based on this comparison, MaxEnt produces an estimation of the probability of species presence or relative environmental suitability. This estimation ranges from 0 (indicating the least likelihood) to 1 (indicating the most likelihood) (Phillips et al. 2006, 2017).

Species data were compiled by using the present sampling data and additional published data (Mudie et al. 2017) for potentially toxic taxa cyst assemblages of interest (*P. hartmanii*, *L. polyedra*, and *Alexandrium* spp.), considering only sampling stations at depths up to 200 m, encompassing the coastal and shelf waters (0–200 m) of the Black Sea basin. As a result, the species datasets were constructed by taking into consideration the PO locations: 72 records for *Alexandrium* spp. cyst occurrence in sediment samples, 80 PO records for *L. polyedra*, and 38 records for *P. hartmanii*.

Furthermore, another suite of Python libraries commonly employed for data processing and visualization was utilized: the GDAL/OGR library (GDAL/OGR contributors 2022), numpy (Harris et al. 2020), rasterio (Gillies 2019), sklearn (Pedregosa et al. 2011), pandas (McKinney 2010), geopandas (Jordahl et al.

2020), matplotlib (Ari and Ustazhanov 2014), shapely (Gillies 2013), xarray (Hoyer and Hamman 2017), and cmocean (Thyng et al. 2016). The properties of the water column were studied in a Python programming environment through the use of the GSW Oceanographic Toolbox, the version developed for Phyton (McDougall and Barker 2011).

Environmental variable selection

The selection of abiotic factors as predictors was based on previous research into the extent of their influence and ecological principles concerning species preferences for habitat, as well as their impact on the variability of phytoplankton biomass. The variables of interest include surface sea temperature, salinity, current velocities, concentrations of chlorophyll *a* and dissolved oxygen, as well as pH levels, phosphates, and nitrate concentrations in seawater, proven as primary factors affecting benthic cyst assemblages (Godhe and McQuoid 2003; Ribeiro and Amorim 2008; Mudie et al. 2017; Li et al. 2019; García-Moreiras et al. 2021). The suite of selected predictor variables was processed as digital layers to be utilized when fitting the models. While it was expected that some of the chosen predictors might be correlated, the maximum entropy algorithms used in training the model are robust to collinearity among predictors. MaxEnt also accounts for redundant variables through regularization implementation (Feng et al. 2019). Therefore, the initial exclusion of highly correlated variables through feature dimension reduction methods had minimal impact on improving the model. Considering the latter, the initial set of predictor variables was retained.

The Copernicus Marine Environmental Service (CMEMS) data portal, accessed on 20 December 2023, was used to acquire monthly mean environmental data layers for the selected variables spanning from 1993 to 2016. These data layers correspond to the sampling expeditions and additional published data (Mudie et al. 2017) that were employed to compile the final datasets of species-presence-only (PO) localities.

CMEMS data were produced by numerical simulation models that combine in situ and satellite data for the Black Sea profile. The models used are the hydrodynamic NEMO (Nucleus for European Modeling of the Ocean) and the BAMHBI (Biogeochemical Model for Hypoxic and Benthic Influenced Areas) (Grégoire et al. 2008; Grégoire and Soetaert 2010; Capet et al. 2016). The data were constructed by utilizing two products: The Black Sea Physics Reanalysis (Lima et al. 2020), covering the time span from 1993 to 2016, with a spatial resolution of 0.037° × 0.028°, and the Black Sea Biogeochemistry Reanalysis (Grégoire et al. 2020). The latter offers monthly climatology fields for the specified time period, with a spatial resolution of 0.025° × 0.025°.

The datasets were averaged over the study period using MATLAB (The Math Works, Inc. MATLAB, version 2020a) for the Black Sea region. The original spatial resolution was maintained for the data obtained from the Black Sea Biogeochemistry Reanalysis, while the data obtained from the Black Sea Physics Reanalysis were resampled from 0.037° × 0.028° to a denser resolution of 0.025° × 0.025°. The data for each layer was extracted in ESRI ASCII grid format (subsequent conversion to GeoTIFF data format took place in the maximum entropy models' implementation in Python).

Model evaluation and validation

The resultant SDMs were cross-validated with ten replicate model runs in MaxEnt and checkerboard geographic structuring in the Python implementation for an adequate evaluation of their performance. Additionally, 25% of PO data was set aside to be utilized as a randomly selected test sample for every model run in MaxEnt and 50% in the Python implementation. Checkerboard partitions provide an effective solution by implementing geographical structuring and masking at the finer level, henceforth minimizing spatial correlation between training and testing data (Pearson et al. 2013; Muscarella et al. 2014; Anderson 2023).

The performance of the resulting SDMs was assessed using the area under the curve (AUC) of receiver operating characteristic (ROC) metrics (Fielding and Bell 1997), and additionally, the model accuracy score and the misclassification rate were evaluated for the Python implementation of the models. Both metrics (AUC and accuracy) are being used to evaluate SDM performance; nevertheless, they capture different aspects of model performance. AUC metrics measure the model's ability to discriminate between positive and negative instances, while accuracy measures the overall correctness of predictions (Hossin and Sulaiman 2015). Conversely, the misclassification rate, which is equal to 1 minus the accuracy (*missclassification rate* = 1 – *accuracy score*), is a measure of the likelihood of misclassification based on the model's predictions (Bekkar et al. 2013).

Additionally, the stability of the water column was studied through the Python implementation (McDougall and Barker 2011) of the Gibbs SeaWater (GSW) Oceanographic Toolbox of TEOS-10 (IOC, SCOR, and IAPSO 2010) to address the specifics of the hydrodynamic conditions and cyst transportation patterns. Considering the latter, the in situ density of the water column $\rho = \hat{\rho}(S_A, \Theta, p)$ was calculated for the whole spatial extent along the depth gradient (31 depth levels) as a function of absolute salinity (S_A) conservative temperature (Θ), and pressure (p) using the 75-term expression implemented in GSW toolbox functions (Roquet et al. 2015). The absolute salinity $\mathrm{S_A}$ was derived by the practical salinity S through the conversion functions available in the toolbox, and the conservative temperature (Θ) (ITS-90) by the potential temperatures θ and S_A, using the computationally efficient expression for specific volume in terms of S_A, Θ, and p (Roquet et al. 2015). The vertical stability of the water column over the latitudinal gradient was examined by the Brunt-Vaisala (buoyancy) frequency: $N^2 = g^2 \rho \frac{\beta^{\Theta} \Delta S_A - \alpha^{\Theta} \Delta \Theta}{\Delta P}$, where ΔS_A and $\Delta\Theta$ are the differences between the absolute salinities and conservative temperatures of vertically adjacent seawater parcels separated by pressure ΔP measured in Pa. The density and the saline concentrations, including the thermal expansion coefficients β^{Θ} and α^{Θ}, were evaluated at the average values of S_A, Θ, and p by using the functions implemented in the GSW toolbox (IOC, SCOR, and IAPSO 2010; Roquet et al. 2015). The Turner angle Tu and the stability ratio R_p of the water column were evaluated using the 75-term expression $\hat{v}(S_A, \Theta, p)$, implemented in GSW toolbox functions (IOC, SCOR, and IAPSO 2010; Roquet et al. 2015).

All maps and graphs were created using QGIS version 3.34 (QGIS Development Team 2009) and the Python library Matplotlib (Ari and Ustazhanov 2014).

Results

Species Composition and Abundance of Resting Stages in Sediments

The assemblages of phytoplankton resting stages discovered in the sediments of the Black Sea were highly diverse, consisting of a total of 71 distinct taxa, with 41 identified at the species level. These taxa were classified into 23 genera, which belonged to six orders and two classes (Table 1). Furthermore, 15 uncertain dinoflagellate taxa and six species described as fossils whose active stage is not known were determined in the samples but excluded from the analyses. The majority of the detected taxa belonged to Dinophyceae (91.5%), and only 8.5% were representatives of Bacillariophyceae. The highest taxonomic diversity (including several unidentified species-level taxa differentiating in form and size) was observed within the genera *Scrippsiella* (13 taxa), *Alexandrium* (13 taxa), and *Protoperidinium* (11 taxa).

Overall, the distribution of most species in the studied area was not uniform. The most prevalent taxa, detected at 70% or more of the stations, were *Scrippsiella acuminata* (found at all stations), followed by *Scrippsiella* sp. 1, *Pentapharsodinium tyrrhenicum*, *Pentapharsodinium dalei*, *Lingulodinium polyedra*, *Gonyaulax* sp., *Protoperidinium* sp. 1, *Scrippsiella* sp. 5, *Calciodinellum albatrosianum*, *Scrippsiella* sp. 4, and *Chaetoceros* sp. 1. A total of 17 additional taxa, on the contrary, were recorded as a single entry.

Considerable spatial variability in total cyst concentration has been observed, ranging between 5 cysts g^{-1} (st. VB and B202/June 2008) and 11,929 cysts g^{-1} (st. B305/July 2013) (Suppl. material 1: table S2). The most abundant taxa, in terms of relative abundance, were the diatom resting stages of *Chaetoceros* sp. 1, which made up over 40% of the total cyst count in 13 samples, and *Chaetoceros* sp. 2, which accounted for up to 28% of the total cyst count in one sample. Additionally, *Scrippsiella acuminata* dinoflagellate cysts were present in relative abundance, ranging from 10% to 100%; *Scrippsiella* sp. 1 cysts presented between 12% and 33% in 13 samples; and *Lingulodinium polyedra* cysts were between 12% and 25% in 4 samples. The diatom species *Chaetoceros* sp. 1 and *Chaetoceros* sp. 2 had the highest concentrations of 6793 cysts g^{-1} and 1598 cysts g^{-1} at station B305 in July 2013. The dominant dinoflagellates *Scrippsiella acuminata*, *Lingulodinium polyedra*, and *Scrippsiella* sp. 1 had the highest concentrations at station U01 in May 2016, with 2741 cysts g^{-1}, 1722 cysts $g^{-1`}$, and 1463 cysts g^{-1}, respectively. The diatom resting stages of *Chaetoceros* sp. 2 were quite abundant (exhibiting peaks in certain samples); however, their occurrence was sporadic, being found at only 7 sites. In contrast, several common taxa (*Calciodinellum albatrosianum*, *Gonyaulax* sp., *Pentapharsodinium dalei*, *Protoperidinium* sp. 1, *Pentapharsodinium tyrrhenicum*, *Scrippsiella* sp. 4, and *Scrippsiella* sp. 5) found in over 57% of the samples, despite being present in a wide range of cyst reservoirs, do not reach high abundances, with the highest concentrations being less than 190 cysts g^{-1}.

Out of the resting stages that were detected, eight types were assigned to potentially toxic microalgae species: *Alexandrium minutum*, *A. pseudogonyaulax*, *A. tamarense*, *A. taylorii*, *Gonyaulax spinifera*, *Lingulodinium polyedra*, *Polykrikos hartmannii*, and *Protoceratium reticulatum*. The cysts of these potentially toxic dinoflagellates, except for *L. polyedra*, were in low abundance, with the highest concentrations not exceeding 81 cysts g^{-1}. The majority of the species exhibited sporadic distribution, being present in just 2–23% of the samples (3–33% of the sampling stations). However, *Polykrikos hartmannii* and *Alexandrium minutum* were more widespread, being detected in 40% and 49% of the samples and 50% and 60% of the stations, respectively.

Diversity indices and species association to sites and sediment type

The species richness detected at each station exhibited significant variability (Suppl. material 1: table S3), ranging from 2 (st. VB) to 45 (st. B305), with just one species, *Scrippsiella acuminata*, consistently present in the cyst assemblages. The Shannon diversity index (H) varied between 0.41 (st. G11) and 2.05 (st. U06) (Suppl. material 1: table S3). The highest values (> 1.89) were calculated for the Ukrainian coastal stations (U06, U05, U15, and U12). The stations exhibiting the lowest diversity indices were G11 (H = 0.41) and VB (H = 0.45), mostly due to the dominant presence of *Scrippsiella acuminata* and *Scrippsiella* sp. 1, which accounted for 91% of the overall abundance at both stations. The lower values of Pielou's evenness index (J), ranging between 0.21 (st. G11) and 0.71 (st. B204), in 80% below 0.6 (Suppl. material 1: table S3), indicated the dominance of specific taxa in the cyst assemblages.

The species richness per sediment type showed a high degree of similarity, with an average of 9.7 ±0.4 species per sediment type. Additionally, the Fishers' alpha diversity index values were comparable across all five sediment types, ranging from 1.256 (sand) to 1.596 (muddy sand) (Suppl. material 1: table S4). The beta diversity displayed a range of values from 0 ("Muddy sand") to 0.418 ("Fine mud"), with an average of 0.234.

The indicator species analysis displayed a statistically significant association between specific sediment types and cyst species (Suppl. material 1: table S5). Eight species were associated with the group (sediment type) "muddy sand" (*Chaetoceros* sp. 3, *Chaetoceros* sp. 6, *Protoperidinium* sp. 9, *Diplopsalis* sp., *Protoperidinium claudicans*, *Protoperidinium* sp. 6, *Alexandrium margalefii*, and *Pyrophacus horologium*), whereas one species (*Alexandrium tamarense*) was associated with the group "mixed sediment + muddy sand." The remaining types of sediments showed low indicator values (*ind val*) and no statistically significant results; hence, no associated species were detected.

A statistically significant association was found between certain site groupings (areas) and certain cyst species (Suppl. material 1: table S6). Three species (*Protoperidinium* sp. 9, *Scrippsiella kirschiae*, and *Scrippsiella* sp. 2) were indicative for the Bulgarian sites, six species (*Alexandrium margalefii*, *Alexandrium pseudogonyaulax*, *Calciodinellum albatrosianum*, *Oblea rotunda*, *Scrippsiella lachrymosa*, and *Scrippsiella* sp. 8) were associated to Romanian sites, three species (*Alexandrium taylorii*, *Archaeperidinium minutum*, and *Scrippsiella* sp. 9) were indicator species for Turkish sites, and 13 species (*Alexandrium* sp. 10, *Alexandrium* sp. 4, *Alexandrium* sp. 7, *Chaetoceros* sp. 4, *Diplopsalis lenticula*, *Dissodinium pseudocalani*, *Ensiculifera carinata*, *Gymnodinium impudicum*, *Levanderina fissa*, *Lingulodinium polyedra*, *Pentapharsodinium dalei*, *Scrippsiella* sp. 1, and *Scrippsiella* sp. 5) were related to Ukrainian sites. No indicator species were found for the Georgian site group.

Table 1. Cyst species identified in the samples and number of stations (%) within different geographic locations. In the table, the different cyst types unidentified at the species level were pooled as spp. and their types noted in brackets. (* potentially toxic species; $^{+}$ species not reported in Mudie et al. 2017). The taxonomic nomenclature is according to WoRMs (https://www.marinespecies.org/; accessed on 23 April 2024).

Class	Order	Species	% Stations Where Detected				
			BG	RO	UA	GE	TR
Dinophyceae	Gonyaulacales	*Alexandrium* cf. *margalefii* Balech, 1994 $^{+}$	33	100	17	0	100
		Alexandrium cf. *taylorii* Balech, 1994 * $^{+}$	0	0	0	0	25
		Alexandrium minutum Halim, 1960 * $^{+}$	42	100	67	0	100
		Alexandrium pseudogonyaulax (Biecheler) Horiguchi ex K.Yuki & Y.Fukuyo, 1992 *	25	40	0	0	100
		Alexandrium tamarense (Lebour) Balech, 1995 * $^{+}$	33	80	0	0	50
		Alexandrium spp.(8 different cyst types/ species)	8, 8, 8, 25, 0, 17, 0, 8	0	17, 0, 0, 83, 17, 67, 17, 33	67, 0, 0, 33, 0, 33, 0, 0	0, 0, 0, 0, 0, 0, 0, 25
		Gonyaulax sp.	67	100	67	100	100
		Gonyaulax spinifera (Claparède & Lachmann) Diesing, 1866 *	0	40	17	0	50
		Lingulodinium polyedra (F.Stein) J.D.Dodge, 1989 *	67	100	83	67	100
		Protoceratium reticulatum (Claparède & Lachmann) Bütschli, 1885 *	8	20	0	67	25
		Pyrodinium bahamense L.Plate, 1906	17	60	0	0	50
		cf. *Pyrophacus horologium* F.Stein, 1883 $^{+}$	17	60	0	0	0
	Gymnodiniales	*Gymnodinium* cf. *litoralis* A.ReÃ±é, 2011 $^{+}$	25	60	33	33	25
		Gymnodinium impudicum (S.Fraga & I.Bravo) Gert Hansen & Moestrup, 2000 $^{+}$	50	20	67	0	100
		Gymnodinium nolleri M.Ellegaard & Ø.Moestrup, 1999	42	100	83	100	50
		Gymnodinium spp. (4 different cyst types/species)	0, 0, 17, 8	20, 20, 40, 0	0, 0, 17, 0	0	0, 0, 25, 0
		Nematodinium armatum (Dogiel) Kofoid & Swezy, 1921 $^{+}$	0	0	0	0	25
		Polykrikos hartmannii W.M.Zimmermann, 1930 *	42	80	17	67	75
		Warnowia rosea (Pouchet) Kofoid & Swezy, 1921 $^{+}$	25	40	0	0	50
	Dinophyceae incertae sedis	*Levanderina fissa* (Levander) Moestrup, Hakanen, Gert Hansen, Daugbjerg & M.Ellegaard, 2014 $^{+}$	25	0	17	0	0
	Peridiniales	*Archaeperidinium minutum* (Kofoid) Jørgensen, 1912 $^{+}$	8	0	0	0	25
		Calciodinellum albatrosianum (Kamptner) Janofske & Karwath, 2000 $^{+}$	58	100	67	67	100
		Diplopelta parva (T.H.Abé) K.Matsuoka, 1988 $^{+}$	0	0	17	0	0
		Diplopsalis lenticula Bergh, 1882 $^{+}$	42	80	83	33	75
		Diplopsalis sp.	17	0	0	0	0
		cf. *Ensiculifera carinata* Matsuoka, Kobayashi & Gains, 1990 $^{+}$	42	100	83	33	100
		Kryptoperidinium foliaceum (F.Stein) Lindemann, *1924* $^{+}$	0	20	50	0	50
		Oblea rotunda (Lebour) Balech ex Sournia, 1973 $^{+}$	33	100	50	0	25
		Pentapharsodinium dalei Indelicato & Loeblich III, 1986	75	100	67	67	100
		Pentapharsodinium tyrrhenicum (Balech) Montresor, Zingone & Marino, 1993 $^{+}$	100	100	67	67	100
		Protoperidinium claudicans (Paulsen, 1907) Balech, 1974	17	40	0	0	0
		Protoperidinium compressum (Abé) Balech, 1974 $^{+}$	8	20	0	0	0
		Protoperidinium conicum (Gran) Balech, 1974	42	100	33	0	25
		Protoperidinium oblongum (Aurivillius) Parke & Dodge, 1976	25	100	17	0	50

Class	Order	Species	% Stations Where Detected				
			BG	RO	UA	GE	TR
Dinophyceae	Peridiniales	*Protoperidinium parthenopes* A.Zingone & M.Montresor, 1988 $^{+}$	33	60	50	33	50
		Protoperidinium steidingerae Balech, 1979 $^{+}$	0	0	17	0	0
		Protoperidinium thorianum (Paulsen, 1905) Balech, 1973 $^{+}$	25	40	0	33	50
		Protoperidinium spp. (4 different cyst types/species)	58, 8, 25, 8	100, 40, 20, 0	100, 33, 0, 0	33, 0, 33, 0	100, 25, 25, 0
		Scrippsiella acuminata (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S.Soehner, Kirsch, Kusber & Gottschling, 2015	100	100	100	100	100
		Scrippsiella kirschiae Zinssmeister, S.Soehner, S.Meier & Gottschling, 2012 $^{+}$	8	0	0	0	0
		Scrippsiella lachrymosa J.Lewis, 1991 $^{+}$	33	100	0	0	50
		Scrippsiella ramonii M.Montresor, 1995 $^{+}$	8	0	17	0	0
		Scrippsiella spinifera G.Honsell & M.Cabrini, 1991 $^{+}$	17	20	0	0	25
		Scrippsiella trifida J.Lewis, 1991	42	40	0	0	50
		Scrippsiella spp.(7 different cyst types/species)	83, 8, 58, 58, 25, 25, 0	100, 0, 100, 80, 40, 40, 0	100, 0, 83, 100, 50, 0, 0	100, 0, 33, 33, 33, 0, 0	100, 25, 75, 100, 100, 25, 25
	Pyrocystales	*Dissodinium pseudocalani* (Gonnert) Drebes ex Elbrachter & Drebes, 1978 $^{+}$	25	60	50	0	50
Bacillariophyceae	Chaetocerotanae incertae sedis	*Chaetoceros* spp. (6 different cyst types/species)	33, 25, 8, 8, 8, 0	100, 60, 0, 0, 0, 0	100, 0, 0, 17, 0, 0	67, 0, 0, 0, 0, 33	100, 25, 0, 0, 0, 0

SDM and model validation

The grid output of the maximum entropy species distribution models (SDMs) uses a gradient color scale to represent the mean predicted probability (ranging from 0 to 1) of the most suitable habitat for the species being studied. The models produced clear visual representations (Fig. 2A–C) (MaxEnt Version 3.4.4 gridded outputs are shown in Suppl. material 1: fig. S1A–C) that showed a concentrated area with a high likelihood of the species being present, based on its preferred habitat. This area mainly occurred within the depth range of 2.5–100 meters and extended across the entire western coast of the basin. The most distinct distribution patterns were observed on the North-Western shelf. Moreover, the region that exhibits the highest suitability is evidently coincident with each of the studied species.

Model performance evaluation

In general, AUC values ranging from 0.8 to 0.9 are regarded as very good, while values over 0.9 are considered excellent (Peterson et al. 2012) (Table 2). The aforementioned conditions are equally applicable to the accuracy of the model. Caution should be applied when interpreting the AUC and accuracy results for imbalanced sets. However, the objective of the current study was to model habitat suitability rather than anticipate projections under various environmental conditions or introduce novel grounds.

According to MaxEnt outcome on predictor variables contribution to SDMs relative predicted probabilities (Table 3), the mean values of nitrates, temperature, salinity, and phosphates have the highest contribution in modeling the training data. Additionally, the permutational importance of variables was assessed in the SDMs Python implementation, and the variables that had the highest fitting test data were phosphates, temperature, and salinity.

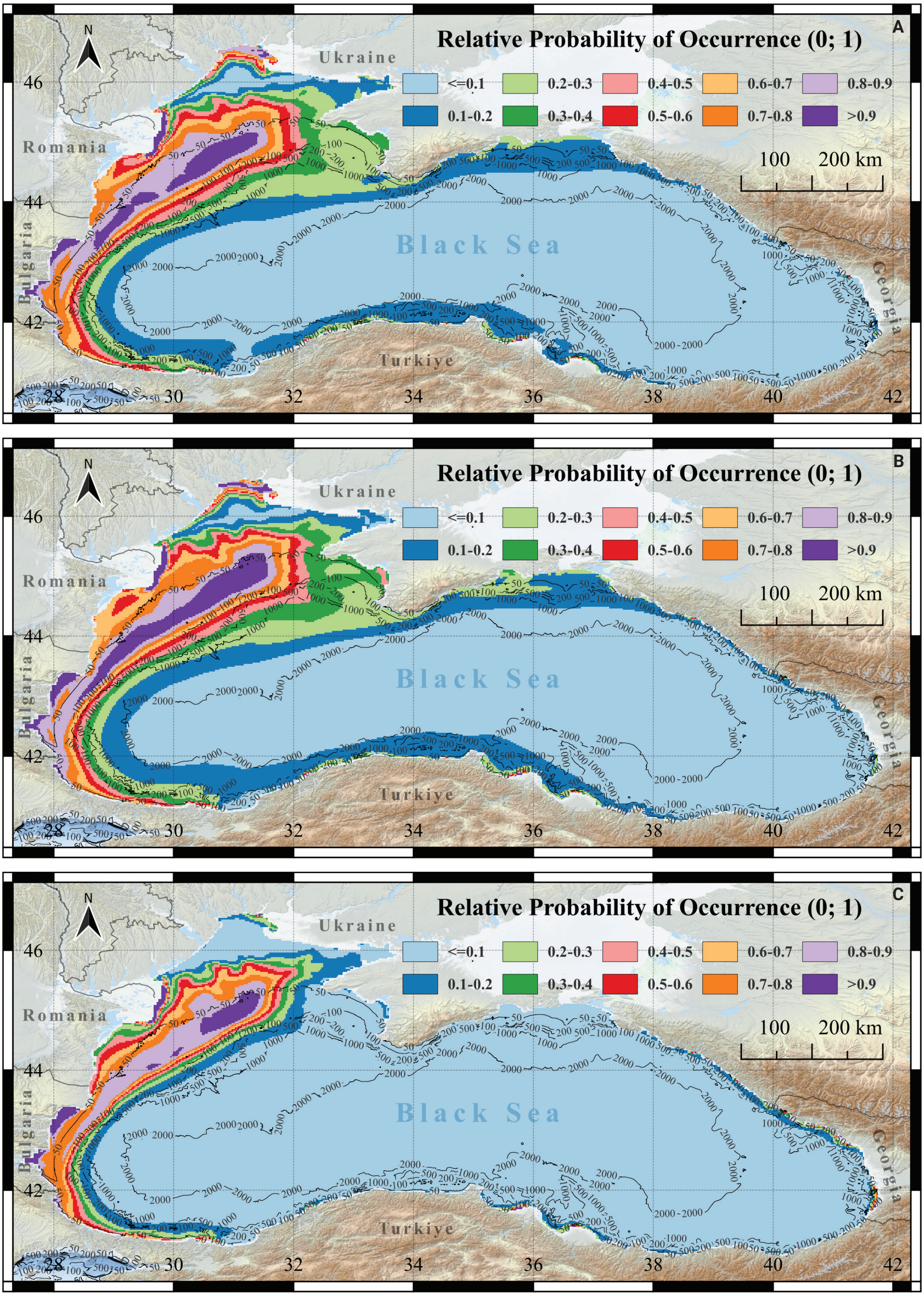

Figure 2. Maximum Entropy Habitat suitability maps (representing the elapid (python implementation tools for SDM) models' outcome) of **A** *Alexandrium* spp. **B** *L. polyedra* **C** *P. hartmanii* in the Black Sea coastal and shelf waters (represented with a color scheme, with light blue indicating the least likelihood of suitable conditions, light orange indicating conditions matching those where species were found, and purple corresponding to the highest predicted probability of a suitable environment).

Table 2. SDM performance evaluation metrics.

		***Alexandrium* spp.**	***L. polyedra***	***P. hartmanii***
MaxEnt Version 3.4.4	Avg AUC – replicated SDMs overall performance	0.904±0.056	0.901±0.030	0.920±0.033
Maxent Python Elapid	Unweighted naïve* AUC score – training data	0.935	0.901	0.913
Maxent Python Elapid	Weighted naive AUC score (training data with samples' geographic weights)	0.925	0.887	0.989
Maxent Python Elapid	Checkerboard Cross-validation AUC score – test data	0.926	0.896	0.904
Maxent Python Elapid	Checkerboard Cross-validation AUC score (test data with samples' geographic weights)	0.882	0.862	0.868
Maxent Python Elapid	Model accuracy	0.935	0.911	0.920
Maxent Python Elapid	Misclassification rate	0.065	0.089	0.080

* The term "naive" refers to a basic measure of a model's ability to distinguish between positive and negative instances, with higher AUC values indicating better performance.

Table 3. Variables contribution to species spatial dispersal MaxEnt Version 3.4.4.

***Alexandrium* spp.**		***L. polyedra***		***P. hartmanii***	
Variable	**Percent contribution (%)**	**Variable**	**Percent contribution (%)**	**Variable**	**Percent contribution (%)**
mean_NO_3	78.3	mean_NO_3	78.5	mean_NO_3	64.3
mean_temp	10.2	mean_temp	7.8	mean_sal	27.3
mean_PO_4	5.3	mean_sal	6.5	mean_PO_4	5.4
mean_sal	3.0	mean_PO_4	4.6	mean_temp	2.7
mean_pH	1.6	mean_DO	1.2	mean_pH	0.1
currents_speed	0.8	mean_Chl	1.0	currents_speed	0.1
mean_Chl	0.6	mean_pH	0.3	mean_Chl	0
mean_DO	0.3	currents_speed	0.2	mean_DO	0

Buoyancy frequency (N^2), Turner angle (Tu) and stability ratio (R_p) were obtained to address the specifics of the hydrodynamic conditions over the latitudinal gradient in the studied region. The calculations were performed at pressure midpoints ranging from a depth of -5.005 m to -2001.135 m, covering the latitudinal gradient of the Black Sea region based on mean annual datasets (potential temperature and practical salinity) obtained by CMEMS for three years: 2011, 2013, and 2015 (only the results for 2013 are presented).

High positive buoyancy frequency values indicate stable stratification and minimal vertical mixing, while lower positive N^2 values indicate a gradual change in density with depth (Monin 1990; McWilliams 2006), which can lead to weaker stratification and increased vertical mixing in the upper layer (0–25m), coinciding with the average mixed layer thickness (Fig. 3) over the latitudinal gradient in the spatial range confined within 41–45N. Low to moderate values of N^2 (evident in the latitudinal range from 45–46N) imply a moderate level of stratification, with a balance between stability and vertical mixing, suggesting specific water column dynamics in the north-western region. Furthermore, lower to moderate levels correspond to conditions promoting vertical exchanges in the water column, resulting in more effective nutrient transfer and increased biological production in the top layer.

The Turner angle reveals shifts in the orientation of water velocity at depths near 50 meters (Fig. 4), potentially impacting the patterns of horizontal transport. The values of the stability ratio (R_p) (Fig. 5) showed unstable stratification, implying a potential for vertical mixing and overturning of water masses at the surface to depths of 50 meters. A stability ratio of 0 is a precise point denoting a neutral state (Monin 1990; McWilliams 2006; Traxler et al. 2011), and the

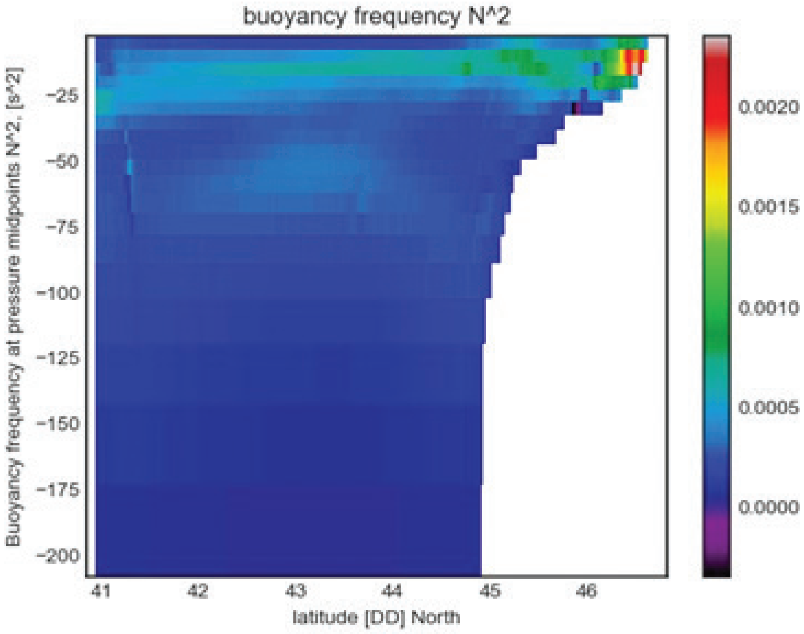

Figure 3. The buoyancy frequency (*N*), estimated using the Gibbs SeaWater (GSW) Oceanographic Toolbox of TEOS-10 (IOC, SCOR, and IAPSO 2010) implementation for Python.

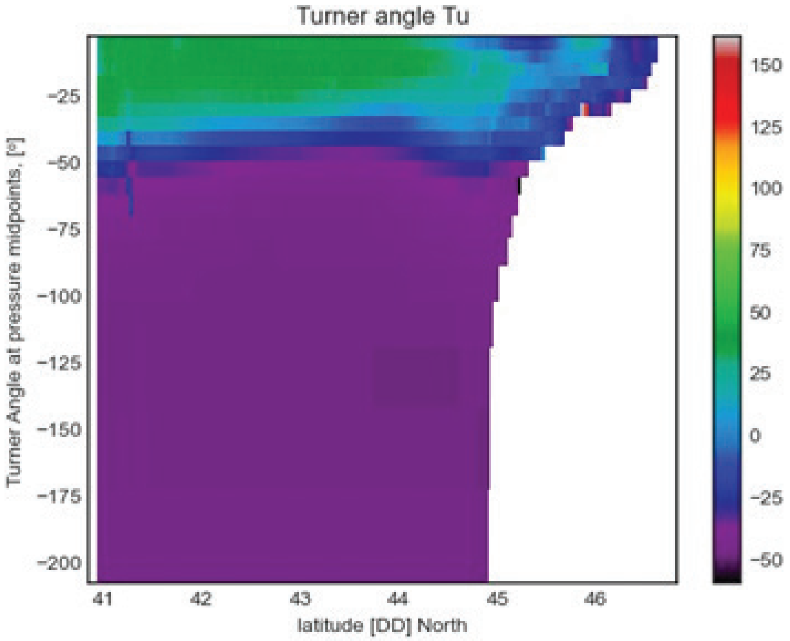

Figure 4. The Turner angle (*Tu*), estimated using the Gibbs SeaWater (GSW) Oceanographic Toolbox of TEOS-10 (IOC, SCOR, and IAPSO 2010) implementation for Python.

behavior of the water column will be influenced by the entirety of the oceanographic conditions in the area.

The combined effect of stability and stratification patterns in the water column is expected to affect the settling, vertical distribution, and horizontal transportation of cysts. The presence of a strong stratification can result in the formation of stable layers that facilitate the accumulation of cysts. Moreover, changes in the water mass flow direction, as indicated by the Turner angle, can affect the horizontal dispersal of cysts.

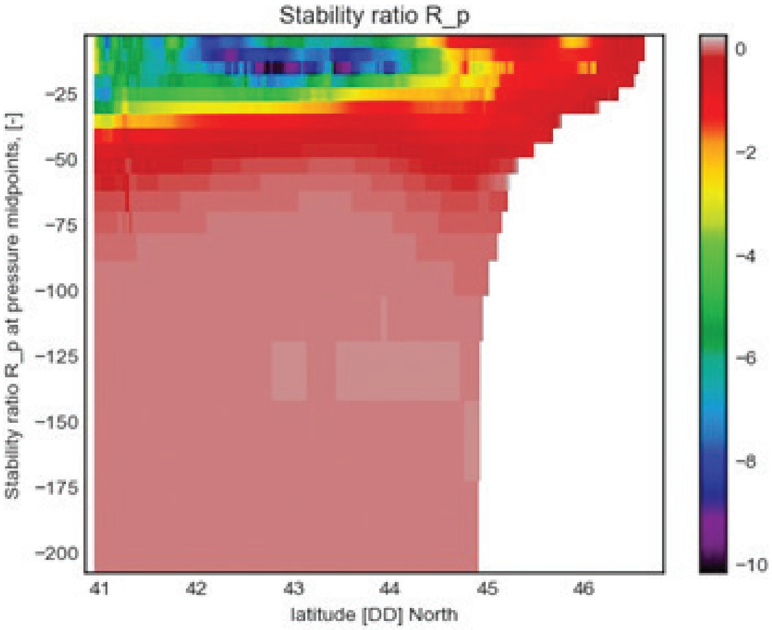

Figure 5. The stability ratio (R_p), estimated using the Gibbs SeaWater (GSW) Oceanographic Toolbox of TEOS-10 (IOC, SCOR, and IAPSO 2010) implementation for Python.

Discussion

Knowledge regarding the spatial dispersal, abundance, and diversity of cyst assemblages holds significant value in accurately evaluating phytoplankton biodiversity. It aids in comprehending how it is associated with biological, physical, and chemical oceanographic conditions of the surface water, identifying hot spot areas where resting cysts accumulate (cyst banks), and predicting potential harmful algal blooms (Genovesi-Giunti et al. 2006; Anderson et al. 2014; Mudie et al. 2017). This study provides a valuable supplement to the extensive mapping of dinoflagellate cysts in the surface sediment of the Black Sea (Mudie et al. 2017). It contributes new information regarding the occurrence, distribution, and abundance of 65 different taxa of dinoflagellate cysts, covering 27 species that were not previously documented in the Atlas, including 6 dormant forms of *Chaetoceros* diatoms (Table 1).

The presence of a large number of recorded cyst species confirms that Black Sea sediments have the ability to sustain significant biodiversity (Mudie et al. 2017). The Ukrainian coastal stations had the highest diversity indices (Suppl. material 1: table S3), which aligns with the abundance of phytoplankton species documented in this region (Moncheva et al. 2019). Peaks in total cyst concentration were observed in some samples, primarily due to the dominance of particular taxa (mainly *Scrippsiella* and *Chaetoceros*). The resting spores of *Chaetoceros* were both abundant and broadly distributed in the samples collected in the current research. The findings align with molecular data, which indicated that the genus has been among the most abundant and diverse taxa (Dzhembekova et al. 2018). Similar tendencies have been observed in other areas where seed banks were similarly characterized by predominant resting stages of *Chaetoceros* (Montresor et al. 2013; Casabianca et al. 2020). The seed banks of *Chaetoceros* spp. ensure their survival in unfavorable conditions (Pelusi et al. 2020) and enhance their ability to sustain the planktonic population in the water (Itakura et al. 1997; Ishikawa and Furuya 2004). Confirming these findings, *Chaetoceros* spp. are among the most dominant species in the plankton community in the Black Sea, often forming blooms (Nesterova et al. 2008; Moncheva et al. 2019). Resting stage formation appeared to be an advantageous strategy for other dominant cyst species, such as *Scrippsiella acuminata*, to dominate both sediment assemblages and plankton communities in the Black Sea (Dzhembekova et al. 2022a, and references therein). In contrast, certain species (*Pentapharsodinium tyrrhenicum*, *Pentapharsodinium dalei*, and *Calciodinellum albatrosianum*) that were present in the sediment samples are rarely observed or documented in the plankton of the Black Sea (Krakhmalnyi et al. 2018). The latter demonstrates the benefits of conducting cyst surveys for detecting rare species, hence uncovering the 'hidden flora' in the basin (Persson et al. 2000; Godhe et al. 2001). Previously, there has been a documented disparity between the species that were dominant in the sediment's cyst assemblages and the typical members of the plankton community in the water column (Persson et al. 2000; Godhe et al. 2001; Rubino and Belmonte 2021). The latter can be attributed to several factors: a limited number of species that produce resting stages (McQuoid and Hobson 1996; Bravo and Figueroa 2014); many species spend longer periods resting in the sediment compared to the water column (Godhe and McQuoid 2003); there is a temporal and spatial mismatch between the occurrence of vegetative cell blooms and resting stage peaks, which can be caused by horizontal transfer in the water column or bioturbational mixing (Kirn et al. 2005; Ribeiro and Amorim 2008; Balkis et al. 2016); and incomplete inventories. Nevertheless, the benthic seed banks provide substantial genetic diversity and likelihood for survival, making a considerable contribution to the long-term viability of species that produce dormant stages (Lundholm et al. 2011, 2017).

Eight cyst taxa of potentially toxic dinoflagellates were identified in this study (Table 1). Despite the fact that the majority of them had a low concentration and a limited spread, *Lingulodinium polyedra*, *Alexandrium* spp., and *Polykrikos hartmannii* exhibited a higher likelihood of influencing bloom dynamics. The interaction between benthic cyst assemblages and pelagic active stages (one aspect of the benthic-pelagic coupling) is critical in encystment and excystment cycles, connecting blooms and subsequent cyst abundance, and vice versa (Anderson et al. 2014; Ishikawa et al. 2014). Sediment seed banks not only serve as historical records of past bloom occurrences, but they also signal a specific basin's future productivity potential and could be used to forecast forthcoming bloom events (Rubino et al. 2000; Anderson et al. 2014).

On a global scale, the dinoflagellate genus *Alexandrium* is one of the foremost harmful algal bloom-causing genera in terms of diversity, scale, and impact of blooms (Anderson et al. 2012). In this study, the genus *Alexandrium* was represented by a total of five determined and eight unidentified species (Table 1, Suppl. material 1: table S2), which exhibited variations in their morphology and size. In order to gain a more comprehensive understanding of the spread of the genus, *Alexandrium* cysts were combined and referred to as *Alexandrium* spp., due to the challenges associated with species identification. Several taxa were found to coexist in sediment samples in both the current and previous studies (Aydin et al. 2015; Mudie et al. 2017) and also in plankton samples (Dzhembekova et al. 2022b). Although *Alexandrium* spp. often do not occur in large abundances, blooms of *A. monilatum* and *A. ostenfeldii* have been documented (Moncheva et al. 2001; Mavrodieva et al. 2007). The occurrence of toxins linked to *Alexandrium* species has been recorded in the Black Sea, albeit at low concentrations (Vershinin et al. 2006; Kalinova 2015; Peteva et al. 2019).

Polykrikos hartmannii cysts, another potentially harmful dinoflagellate widely distributed in the Black Sea (Mudie et al. 2017), as well as identified in the plankton community with the application of a molecular approach (Dzhembekova et al. 2022b), were detected in 40% of the samples, encompassing all specified locations (Table 1, Suppl. material 1: table S2). The highest abundances were registered in samples collected from the western region of the basin. Although there have been no reports of blooms at the regional level, bloom densities and ichthyotoxicity have been observed in a brackish estuary (Tang et al. 2013).

The potentially toxic dinoflagellate *Lingulodinium polyedra* was found to be widely distributed and abundant in the sediments of the Black Sea, as shown in both the current study (Table 1, Suppl. material 1: table S2) and prior research (Nikonova 2010; Aydin et al. 2015; Mudie et al. 2017). It is also prevalent in phytoplankton communities (Moncheva et al. 2019). A significant correlation has been discovered between the abundance of *Lingulodinium polyedra* cells and the presence of yessotoxins (YTXs) in the Black Sea, thereby substantiating the ability of local strains to produce toxins (Dzhembekova et al. 2022b). The latter emphasizes further the significance of delineating regions that represent the highest environmental suitability to serve as cyst beds.

The results of our study indicate that the distribution of *Lingulodinium polyedra* cysts relative probability of occurrence in terms of suitable habitat is significantly influenced by nitrates and temperature. The key variables controlling the habitat suitability of *Alexandrium* spp. were nitrates and temperature, while for the *Polykrikos hartmannii* cysts, the main factors were nitrates and salinity (Table 3). The availability of nutrients, temperature, and salinity are typically regarded as the primary triggers for the encystment processes (Genovesi-Giunti et al. 2006 and references therein). Comparable findings were documented for other basins (Godhe and McQuoid 2003). The formation of resting cysts is a proven survival strategy employed by *L. polyedra* in response to adverse environmental conditions, such as nitrogen depletion and fluctuations in temperature (Ganini et al. 2013). The region with the highest likelihood of *L. polyedra* cyst occurrence appears to be in the western coastal and shelf waters (Fig. 2A), which includes areas where *L. polyedra* red tides have been documented (Terenko and Krakhmalnyi 2021 and references therein). The projected habitat suitability of the other studied taxa, *Alexandrium* spp. and Polykrikos hartmannii (Fig. 2B–C), partly coincided with the modeled distribution of *L. polyedra* cysts, likely due to the coexistence of the three genera and their shared preferences for environmental conditions, productivity, and survival strategies. Additionally, local hydrodynamic conditions and the transportation of cysts before their settlement in sediments could also contribute to this overlap.

Understanding the interplay among the water column dynamics, the sedimentation processes, and cyst settling is essential for investigating the ecology and life cycles of organisms that form cysts. The latter necessitates the evaluation of various elements, including water column stability, currents, mixing patterns, and the properties of the sediments. In general, the density of the water column plays a role in the larger environmental context that affects how cyst assemblages are distributed in the aquatic sediments (Nehring 1993; Harland et al. 2004; Genovesi-Giunti et al. 2006; Ribeiro and Amorim 2008).

Moreover, the overall hydrodynamic processes and sediments' characteristics have been indicated as having a major impact on cyst concentrations in the sediments (Ribeiro and Amorim 2008; Li et al. 2019; García-Moreiras et al. 2021). The vertical stability of the water column, which is advantageous for many dinoflagellate species, has been recognized as a pivotal factor affecting the seasonal variations in cyst production (Harland et al. 2004; Ribeiro and Amorim 2008). Additionally, the density of the water column might impact the sedimentation rates, therefore influencing the distribution of cysts (Cho and Matsuoka 2001). A water column with a lower density may undergo more intense vertical mixing. The process of mixing can impact the dispersion of particles, such as cysts, throughout the water column prior to their final settlement into sediments. Therefore, areas with minimal water current velocities exhibited higher cyst densities (Azanza et al. 2004). Considering the distinct vertical structure of the Black Sea (Oguz et al. 2000; Yakushev et al. 2008; Capet et al. 2016; Kaiser et al. 2017; Zatsepin and Podymov 2021), our findings can be concisely stated as follows: The water column exhibits unstable stratification in the upper layer (0 to 25 m, coinciding partly with the mixed layer depth thickness) along the latitudinal gradient, potentially influencing the vertical distribution of water properties and the settling of organisms, including cysts. The fluctuations in buoyancy frequency (N^2) indicate alterations in the intensity of stratification, exhibiting a prominent maximum at a depth close to 25–35 meters (the average thermocline layer depth) (Fig. 3). The Turner angle reveals shifts in the density gradients and the orientation of water velocity at depths near 50 meters in the latitudinal range from 45–46N (Fig. 4), potentially impacting the patterns of horizontal transport. Furthermore, riverine freshwater input plays a significant role in influencing the Western Black Sea by creating stratification and nutrient gradients, having further impact on the primary production, abundance, and composition of phytoplankton communities, including cyst-forming species (Moncheva et al. 2019; Dzhembekova et al. 2022a). The latter is related to the significant nutrient input (including nitrogen and phosphorus), the transportation of sediments (cysts, being relatively heavy, can settle in these sediments), and the sedimentation process, which can influence the spatial distribution of cysts along the river's plumes and in the adjacent Black Sea region.

The distribution of cysts is also significantly affected by sediment composition (Cho and Matsuoka 2001). Dinoflagellate cysts, due to their similar mass and hydrodynamic properties, are often distributed and deposited in sediment alongside fine-grained particles (Godhe and McQuoid 2003; Rachman et al. 2022), and generally higher concentrations of dinoflagellate cysts are observed in muddy sediments than in sandy sediments (Cho and Matsuoka 2001; Anderson 2023). Consistent with these findings, the area in the Western region of the Black Sea exhibiting the highest habitat suitability > 80% (at depths 50–100 m) is primarily represented by mixed sediments (shelly mud) for all modeled species (Suppl. material 1: table S7).

The north-western region of the Black Sea was identified as the most favorable habitat for the examined potentially toxic species (Fig. 2), posing a greater risk for the onset of algal blooms. Furthermore, this region aligns with predicted zones of very high risk of mass outbreaks of potentially toxic dinoflagellate *Prorocentrum cordatum* in the Black Sea (Goncharenko et al. 2021). Considering the aforementioned results, the identified hotspots provide potential inoculum for bloom initiation (seed banks) and a suitable environment for vegetative growth and mass development of toxic dinoflagellates in the water column.

In conclusion, the accumulation of cysts pertains to how they are dispersed/transported within the water column from one location to another before settlement in sediment, whereas habitat suitability refers to the specific environmental conditions required for their survival, growth, and germination. Cysts can be dispersed in aquatic ecosystems by water currents, facilitating their colonization of new habitats. Both aspects, particularly the autoecology of the species and their life cycle, are crucial factors to comprehend when studying organisms that undergo cyst formation. However, the problem of modeling habitat suitability becomes challenging due to the spatial dispersal caused by horizontal transportation. This is because the specimens that have been sampled and documented as occurrences may have reached areas that are unsuitable or novel areas that could be suitable through horizontal transportation in a highly stratified environment. Therefore, model validation (Fig. 2, Table 2) is essential for accurately identifying suitable habitat and understanding the prospective transportation patterns associated with the local water column hydrodynamic properties and unique characteristics of the environment.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: SM, ND, IZ. Organization of samplings: SM. Sediment treatment and analysis of cysts: FR, MB. Environmental data: IZ, IP, VD. Statistical analysis: IZ. Visualization: IP, IZ, VD, ND. Model evaluation and validation: IZ. Writing: original draft: ND, IZ, SM. Writing - review and editing: ND, IZ, FR, MB, VD, IP, SM. All authors contributed to the final version of the manuscript and approved the submitted version.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary information

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Data type: docx

Explanation note: **table S1.** Sampling stations (location, sampling date, geographic coordinates, depth). **table S2.** Range of the cysts species concentration (cysts g-1) per different geographic locations. In the table the different cyst types unidentified at the species level were first combined as spp. (marked in grey) and bellow the different taxa were listed (* potentially toxic species; + species not reported in Mudie et al. 2017). **table S3.** Diversity indices. **table S4.** Species diversity per sediment type (only the sediment types where species have been sampled was included in the analysis). **table S5.** Indicator species analysis – species association to sediment type. **table S6.** Indicator species analysis – species association indices (indices for assessing the strength of association between species and groups of sites) to geographic area (BG – Bulgaria; GE – Georgia; RO – Romania; TR – Turkey; UA – Ukraine), with bold are marked the species with association to sites *Indval* > 0.5. **table S7.** Sediment types for habitat suitability > 80% (relative probability of occurrence > 0.8) for *Polykrikos hartmanii*, *Lingulodinium polyedra*, and *Alexandrium* spp. **fig. S1.** MaxEnt Version 3.4.4 gridded outputs in terms of Habitat suitability maps of A) *Alexandrium* spp. B) *L. polyedra* C) *P. hartmanii* in the Black Sea coastal and shelf waters (represented with a colour scheme, with light blue indicating the least likelihood of suitable conditions, light orange – indicating conditions equal to those where species were found, and purple corresponding to the highest predicted probability of suitable environment).

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Research Article

Applying landscape ecological principles in comprehensive landscape protection: Šumava National Park as a case study

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Abstract

In the face of ongoing anthropogenic pressure and biodiversity loss, there is a need to protect nature more effectively. Therefore, we propose a comprehensive and consecutive approach utilising landscape ecological principles and methods for effective landscape protection and spatial nature conservation. Methods applicable in various conditions are exemplified through case studies from the Šumava National Park, the largest NP in Czechia. Using a set of spatial environmental, landscape ecological and geographical data we can:

1) Characterise the area of interest from the physical-geographical, socioeconomic, and management point of view to create the concept´s framework and review important background information for analysis of the area. Therefore, the key factors for landscape protection and biodiversity conservation are defined.
2) Analyse trends and processes of landscape dynamics in terms of land cover, landscape structure and habitat fragmentation and connectivity, which helps us to set main objectives of landscape protection and nature conservation.
3) Use data about environment conditions and key species and habitat occurrence to model habitat suitability, identify their suitable areas, and thus improve their protection. As a result, areas of high conservation value are distinguished.
4) Synthetize outputs of the above-mentioned steps and prioritise the target goals of landscape protection and biodiversity conservation in the area of interest. This leads to the effective zonation, which is a necessary condition for the application of appropriate management measures.

Key words: Conservation planning, landscape ecology, nature conservation

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Introduction

Territorial nature protection is a well-known and widely used approach to preserve landscape and biodiversity (Antrop 2005). The most common tool of landscape protection is establishing a network of protected areas (PAs). In recent decades, PAs total extent has expanded greatly around the world (Watson et al. 2014). Despite the enlargement of PAs territories, it has not met the target of 17% of land and inland waters protected by 2020 (Aichi Target 11). Moreover, Kunming-Montreal Global Biodiversity Framework has targets for 2030 as to plan and manage areas to reduce biodiversity loss and to conserve 30% of the Earth. Furthermore, the current PAs set is not representative; not all ecoregions and endangered species are protected sufficiently (Maxwell et al. 2020). This is because the establishment of PAs and their management decisions are rather driven by political, economic, and other interests than scientific knowledge (Müller and Opgenoorth 2014). Therefore, it is complicated to design reserves with regard to all natural values and processes and allow coexistence of conservation targets with other activities within the area to delimit appropriate land-use management (Bengstsson et al. 2003; Bekessy et al. 2012).

PAs are recognized as the best tool to preserve endangered species populations and their habitats; more generally, biodiversity, and ecological functions of the area (Watson et al. 2014). Furthermore, PAs also mitigate the negative effects of the development of human society, such as built-up areas or unsuitable land use and land cover changes (Martin-Lopéz et al. 2011; Sharafi et al. 2012; Montesino Pouzols et al. 2014; Ceauşu et al. 2015; Fuente et al. 2020).

The oldest PAs have protected mainly iconic species and landscapes (Martín-López et al. 2011); later, biodiversity and ecosystem services became the subjects and, more recently, also human activities such as tourism and sustainability of local communities with social and economic aims are also included as parts of the mission of PAs (Moilanen et al. 2011; Watson et al. 2014). These wider objectives and diversification of PA aims can create conflicts of different interests and priorities within some PAs (Soliku and Schraml 2018). There is a general assumption that human activities in PAs should be restricted or regulated by law to maintain nature quality (Bengstsson et al. 2003). However, various visions of the PAs' future including large human exploitation are often proposed and even applied, while appropriate data and relevant negotiation are lacking (e.g., Yakusheva 2019).

Yellowstone National Park, the first national park in the world, was founded in 1872. Roughly, one hundred years later the number and area of PAs started to grow quickly as a response to degradation of the environment (Watson et al. 2014). In Central Europe, a region with a long-term anthropogenic presence, the first PAs were already established in the 1820s (Ceauşu et al. 2015; Hausner et al. 2015). The first nature reserves in Czechia were established in 1838 (Čihař 1997) and, nowadays, the number and area of PAs are increasing in Czechia as well (Pelc 2018). However, ensuring effective nature protection and long-term stability in the management of protected areas is a big challenge for Czechia as well as other countries, where distrust of local actors and lack of communication between local communities and conservation authorities are common (Yakusheva 2019). There is an evident problem especially in post-communist Eastern Europe; priorities of nature conservation are not commonly agreed on and in the hunt for economic development, the preservation of biodiversity is put to one side (Kindlmann and Křenová 2016).

Therefore, facing biodiversity loss and ongoing anthropogenic pressure, the effective prioritisation of conservation goals in naturally valuable areas, as well as the delimitation of PAs and their zonation and management, are crucial tasks for contemporary nature conservation. In the context of the above-mentioned problems, we are persuaded that the general approach and methods presented within this paper can help to bridge the gaps and help to manage PAs more properly. In this paper, we propose adopting methods of geography and landscape ecology for effective nature conservation planning and appropriate management of PAs. We stressed issues of delimiting the PAs, integrating biodiversity and natural processes and dealing with spatial features affecting the management of PAs.

Our aim is to bridge the gap of various approaches dealing with conservation planning and prioritisation with easy, innovative and objective data-driven four-steps methods especially based on landscape ecology approaches using appropriate data and tools. We considered dilemmas of social-ecological systems (Soliku and Schraml 2018; Yakusheva 2019), conservation based on species or ecosystems methodology (Mace et al. 2007; Ceauşu et al. 2015) and tried to plan systematically and evidence-based (Margules and Pressey 2000; Sutherland et al. 2004).

Landscape ecology deals with space and its development, dynamics, patterns, and how all these aspects together influence relationships between natural elements (energy, material, species). Furthermore, landscape ecology is a science on the edge of several scientific disciplines, from which ecology and geography are the nearest ones (Turner 1989; Turner 2005; Cushman et al. 2008). Landscape ecology connects the landscape scale with ecological processes (Tscharntke et al. 2012). It implies that landscape ecology brings interesting and very useful information for land-use planning, spatial landscape protection, and nature conservation (Turner 1989). Effective protection of natural processes, ecosystem quality, and diversity, which is now in high demand, can also benefit from landscape ecology knowledge because anthropogenic pressure brings habitat loss, fragmentation, and land-use intensification, and also causes subsequent biodiversity loss (Martin-Lopéz et al. 2011; Sharafi et al. 2012). Therefore, in this article, we review landscape ecological principles and indicate the most effective of them for improving the state of PAs.

In this paper, we use our large experience and results of long-term research conducted in Šumava National Park (**ŠNP**), Czechia, to introduce this new **LEP** (landscape ecological principles) approach and discuss opportunities, challenges, and potential of its use in a common overview.

Methods

Landscape ecological principles

Landscape ecological principle) are defined here in accordance with three main aims on landscape ecological studies (*in sensu* Forman and Godron 1986): (i) landscape structure, (ii) changes of land cover, and (iii) functions of landscape. We used LEP in a four-step approach. Landscape function was especially assessed in the first step investigating abiotic and socio-economic conditions of the area. Land cover changes were analysed in the second step and landscape structure is crucial for the second and third step as well. Finally, all these steps were integrated into the fourth step, creating a proposal for territorial nature conservation priorities. The individual steps are described below as (also in Fig. 1, Table 1):

1. Summarising conditions of a study area from natural, socio-economic and management points of view;
   a. Characterization of the study area based on landscape or physical-geographical typology (Chuman and Romportl 2010; Romportl et al. 2013; Janík and Romportl 2016). This also serves as a framework for further analysis.
   b. Mapping of human activities and management in the area (Brandon and Wells 1992; Mayer and Job 2008; Brown et al. 2015; Janík and Romportl 2017; Janík 2020).
2. Based on summarised conditions, landscape dynamics driven by rather human (Kupková et al. 2013; Janík et al. 2019; Janík et al. 2022) or rather natural processes (Turner 1989; Janík and Romportl 2018) on various scales were analysed. Broader context to highlight main trends, values, problems and differences, which are important for protection and management of the area, were included. By a synthesis of knowledge derived from step 1 and step 2, it is possible to evaluate landscape dynamics according to different existing management (e.g., ownership; Křenová et al. 2022).
3. Capacity for harbouring and enhancing species was assessed - habitats and species occurrence, habitat requirements of target species, and suitable habitat capacities of a study area were analysed (Janík et al. 2021b).
4. The last step is the integration of the previous ones. It shows landscape ecology in practice as a synthesis of knowledge about landscape and its values. Objective data about habitats and key species occurrence are essential tools for proper and effective protection resulting in prioritisation of protection (Moilanen et al. 2005; Moilanen 2007; Janík and Romportl 2023).

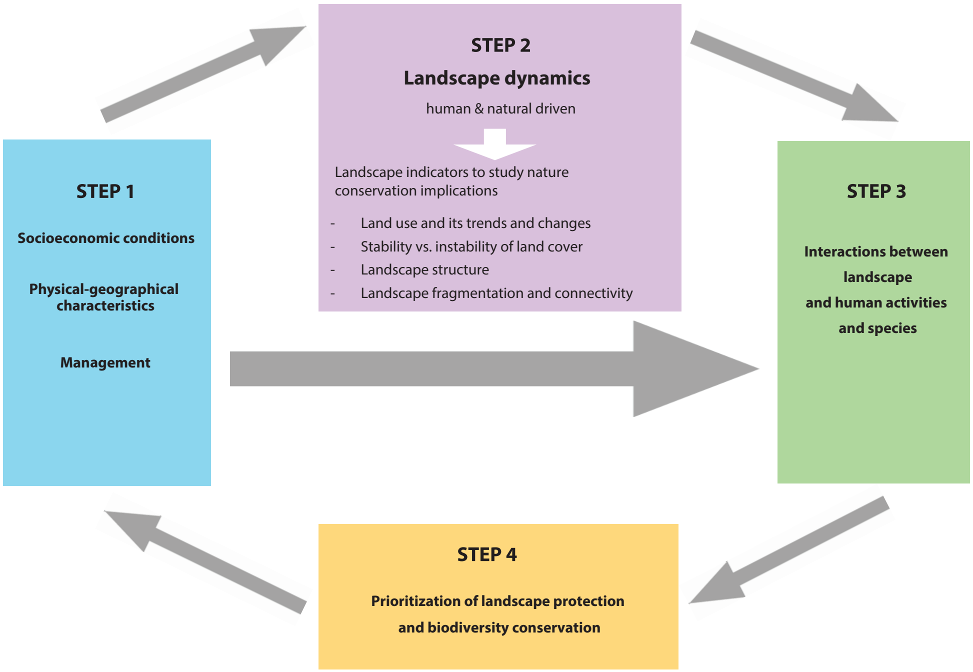


Figure 1. Diagram of relationships between steps within proposed LEP approach: The first step in our LEP approach is a blue part; conditions of the area influencing each other. Conditions as management, physical-geographical framework and human activities influence the second step (purple part, landscape dynamics). Blue and purple parts are inputs for interaction between landscape, human and species. This third step (green) consists of modelling biodiversity (habitat suitability models). All three steps together are inputs for synthesis – a yellow fourth step for prioritisation regarding the protected areas. It, again, influences management of the area and starts another circle of processes.

Table 1. Steps of the LEP approach and how they were used regarding ŠNP.

Steps	Used LEP	Used data	Used methods	Solved concerns	Integration with other steps	References (regarding ŠNP)
Step 1: study area conditions	Landscape function	Physical-geographical characteristics (topography and climate) Socioeconomic data (inhabitants, visitors, GDP) Management of the PA	Typology Literature and data review and comparison	SWOT analysis of the PA Differentiation of the PA (within the area and also with neighbourhood) Management	Inputs: • information on typology of the areas • management of the area • wildlife management • use of the area by human (recreation) Outputs: • typology of PA • human use of the PA	Janík and Romportl 2016; Janík and Romportl 2017; Janík 2020
Step 2: landscape dynamics	Landscape structure Land cover changes	Land cover data	Land cover change analysis	Land cover change and stability facing natural disturbances and anthropogenic activities	Inputs: • typology of PA • human use of the PA Outputs: • stability of the land cover • naturalness of land cover	Janík and Romportl 2018; Janík et al. 2019; Janík et al. 2022; Křenová et al. 2022
Step 3: protecting species and enhancing biodiversity	Landscape function Landscape structure	Environmental variables Anthropogenic structures Occurrence data	Habitat suitability modelling	Detection of valuable habitats for selected species	Inputs: • land cover data (stability, naturalness) Outputs: • habitat suitability models • anthropogenic risks for biodiversity (e g. landscape fragmentation)	Janík et al. 2021b; Bluhm et al. 2023
Step 4: landscape ecology in practise	Integration of all these steps	Land cover data Habitat suitability models Anthropogenic structures Management and zonation of the PA	Synthesis of data from previous steps (land cover, habitat suitability models) Prioritisation software (e.g. ZONATION)	Synthesis of previous steps and prioritisation of nature protection, comparison with current state	Inputs: • land cover data • habitat suitability models • human use of the PA Outputs: • prioritisation • comparison to management of the area from step 1	Janík and Romportl 2023

All four steps, further in this chapter, are described generally in detail and in the Results chapter as a case study from our model area. These steps are imprinted to nature protection and management of PAs. We propose integrating all these perspectives, bridge the gap between science and practical conservation (Müller and Opgenoorth 2014), and support evidence-based conservation (Sutherland et al. 2004).

Case study area

Šumava National Park was established in 1991, after the fall of communism, in the south-western part of Czechia. ŠNP was demarcated within Šumava Protected Landscape Area (**ŠPLA**), which now creates the surroundings of ŠNP (Janík and Romportl 2018). ŠNP covers 683.4 km^2 and it is almost three times larger than its German neighbour – Bavarian Forest National Park (**BFNP**) (Fig. 2). Formerly, ŠNP was divided into three conservation zones.

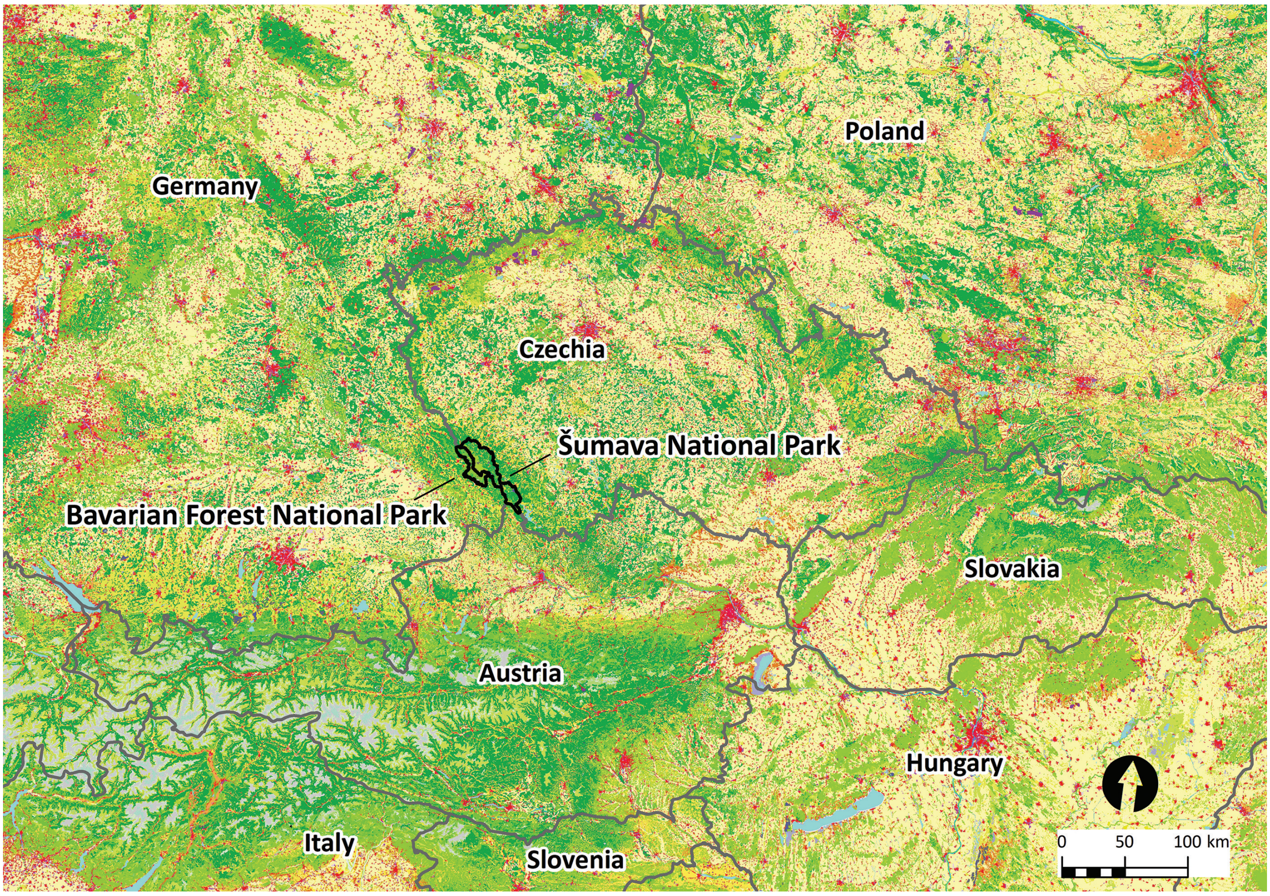


Figure 2. Case study area; Šumava and Bavarian Forest NPs in Central European landscape. Data sources: Corine Land Cover (European Environment Agency), Open Street map.

The first zone was the most protected; it covered 22% in 1991 and it even shrank to 13% in 1995 (Křenová and Hruška 2012). Large fragmentation of this strictly protected area, together with different forest management applied in different zones (including large-scale logging during the bark beetle outbreak), negatively affected ecosystems in ŠNP (Šantrůčková et al. 2010; Křenová and Hruška 2012). Therefore, these management steps led to the concerning interest of scientists and conservationists in the further development of ŠNP.

Scientists criticised these changes of zonation and forest management (Křenová and Kindlmann 2015; Zýval et al. 2016) and delivered a proposal of zonation based on mapping of habitats and species for the NATURA 2000 sites network: an easy-to-make proposal was made using an overlay of key layers (valuable habitats, core areas of capercaillie (*Tetrao urogallus* - the umbrella species)); the proposal delimited 51.9% of the area of ŠNP for the most valuable first zone (Bláha et al. 2013); however, this proposal has not been accepted. Based on an amendment of the Czech Nature Conservation Act, a new zonation with four zones came into force in 2020. A natural zone, the most strictly protected one with non-intervention management, currently covers 27.7% of ŠNP. Nevertheless, there are still some shortcomings regarding management of valuable habitats, especially in forests (Křenová et al. 2022). In the context of these turbulent changes, we systematically and continually focused on the research of landscape protection and nature conservation in ŠNP, as it is an interesting study site and a suitable laboratory for our approach.

Step 1: Study area conditions

To understand processes within a certain area, it is necessary to know how its geographical and environmental conditions vary in space. Therefore, GIS software enables using objective data and deploys analysis of abiotic environment or/and landscape parameters resulting in a typology of a study area from one (and a small) PA to national or even continental or worldwide scale, and helps to understand differences within the landscape on various scales (Chuman and Romportl 2010; Romportl et al. 2013). Based on the results of such a typology, we can see the study area through separate landscape types.

Moreover, other drivers of PA management are economic and social conditions. After abiotic and landscape conditions, which can be investigated through typology like our frameworks, anthropogenic presence and management is the next layer. PAs can be beneficial for the local community and can bring economic activities, such as tourism (Brown et al. 2015; Yakusheva 2019).

Step 2: Landscape dynamics

The above-mentioned abiotic conditions and anthropogenic activities imply characteristics of landscape changes that are crucial for ecosystem services and biodiversity (Feranec et al. 2010; Tappeiner et al. 2021). Value of the landscape and its change for protection was studied, e.g. by Němec et al. (2022). Basically, these indicators are recognised as important:

- Land use and its trends and changes
- Stability versus instability of land cover
- Landscape structure
- Landscape fragmentation and connectivity

Information on history, regarding nature protection, stability and instability, persistence and change of the landscape, from a temporal-historical view are a key for biodiversity conservation and nature protection (Guerra et al. 2019; Němec et al. 2022). Moreover, history can help to manage sustainability of the landscape for the future (Tappeiner et al. 2021). Long-term legacies of changes affect fauna and flora (Turner 2010).

The stability of natural development is crucial for biodiversity, however, in some parts of the area, the main value of landscape to protect can be heterogeneity of landscape structure (e.g., caused by natural disturbances) supporting biodiversity (Turner 1989; Bengstsson et al. 2003; Turner 2010; Janík et al. 2021a), even after windstorms and bark beetle outbreaks in the study area (Thorn et al. 2017).

Based on our results and literature review, this step should specify main drivers and types of landscape change regarding the conservation concern.

Step 3: Protecting species and enhancing biodiversity

Generally, mapping biodiversity is a proper and effective conservation tool and it is an inherent part of management of spatial nature protection (Margules and Pressey 2000). Linking knowledge about occurrence of species and habitats with information about quality of the landscape and environmental conditions significantly improves conservation knowledge. More detailed environmental variables, based on environmental and landscape evaluation from previous steps, can be analysed as predictors for suitability of habitats for certain species and occurrence of certain habitats. Data about species and habitat occurrence can be used to model potential distributions of target species (Phillips et al. 2006; Elith et al. 2011). Spatial distribution of habitats can be modelled as well (Mücher et al. 2009). This is especially suitable for larger areas for which we have less knowledge about habitats.

In addition to models, there is a necessity to evaluate anthropogenic activities and pressure negatively affecting dispersal of animals, which were gathered in the first step. Fragmentation of landscape and connectivity is, therefore, another topic of landscape ecology with practical impacts on spatial nature protection (Turner 1989). While fragmentation can lead to isolation of populations with many negative consequences or, on the other hand, to a more heterogeneous landscape, connectivity is needed and it can prevent the population from extinction (Tscharntke et al. 2012).

Step 4: Landscape ecology in practise

The last step of this proposed concept of LEP used for landscape protection is to integrate all the previous steps and give recommendations to PAs administrations on how to manage their area in the most appropriate way. By using both species and ecosystem data, with local knowledge on values and qualities for protection and threats and costs to consider, we can maximise the benefit for effective spatial protection prioritisation (Ceauşu et al. 2015).

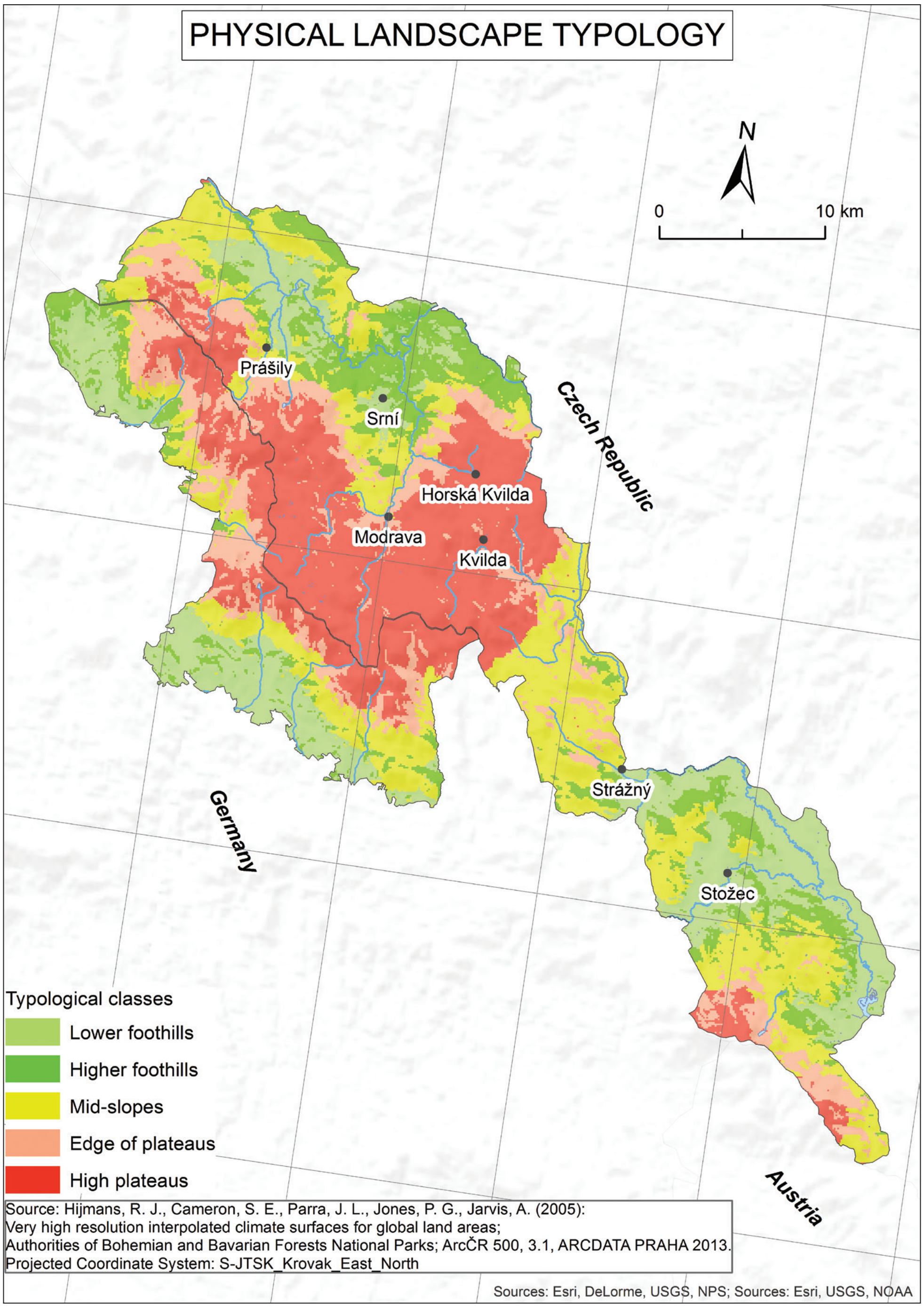
Fortunately, a prioritisation bringing complexity can consider nature protection as well as other activities within a target area to delimit appropriate land-use management (Bekessy et al. 2012). In this study, by using LEP concept and software ZONATION (Moilanen et al. 2005; Moilanen and Kujala 2006) in this last step 4, all relevant objectives including social, local and recreational, are also met (Moilanen et al. 2011; Watson et al. 2014). ZONATION works as a spatial conservation tool using hierarchical prioritisation as it iteratively removes cells of the data inputs (raster layers) (Moilanen 2007). Inputs could be data such as biodiversity features, qualities of the landscape, anthropogenic pressures (habitat suitability maps, biotope quality maps, anthropogenic transformation of the landscape). This concept can be used with various data and in various tasks; as well as in different geographical locations or scales (e.g., Rayfield et al. 2009; Carroll et al. 2010; Leathwick et al. 2010; Sirkiä et al. 2012; Srivathsa et al. 2023).

Results

For an unquestionable clarification of the presented concept and a better illustration of its use in PA management, in this chapter we present experiences with the implementation of this concept in the Šumava National Park.

Step 1: Case study area conditions

Typology of ŠNP and neighbouring BFNP was created using topography and climate data. It shows several different physical landscapes in the study area (Janík and Romportl 2016, see Fig. 3). There is a difference between the flatted core area of the upper mountain plateau along the borderline and subsequent steep parts and those with lower altitudes, in particular. This internal differentiation of the study area enables further investigation, for example for evaluation of forestry changes after disturbances (Křenová et al. 2022).



Source: Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., Jarvis, A. (2005):
Very high resolution interpolated climate surfaces for global land areas;
Authorities of Bohemian and Bavarian Forests National Parks; ArcČR 500, 3.1, ARCDATA PRAHA 2013.
Projected Coordinate System: S-JTSK_Krovak_East_North

Sources: Esri, DeLorme, USGS, NPS; Sources: Esri, USGS, NOAA

Figure 3. Landscape typology of Šumava and Bavarian Forest NPs (Janík and Romportl 2016).

ŠNP and BFNP, a transboundary area with two national parks, is a very suitable site for investigation of this layer. These two NPs share the same ecosystems, key species, and very similar conservation targets (Křenová and Kiener 2012). However, the historical development and path dependency of management, economy, and society differ; mainly in the period after World War II, political decisions resulted in a discontinuity of settlement, access restriction, and delay in upgrading the protection status of ŠNP on the Czech side (Janík and Romportl 2017; Janík 2020). Therefore, history led to different institutional environment, management, and nature protection between countries (Brown et al. 2015; Křenová and Kindlmann 2018). This is particularly evident in the Czech and German parts of the area, especially concerning wildlife management, management and zonation of the national parks with a lower level of protection in ŠNP (Janík 2020). Since the 1990s, following the transition to democracy and capitalism in Czechia, varying perceptions of ŠNP management among key stakeholders have persisted, leading to disagreements regarding the management and development of the area on the Czech side (Brandon and Wells 1992; Janík and Romportl 2017). It is also given by mixed ownership in ŠNP, which makes the management even more challenging. However, in case of transboundary harmonisation, coordination has improved, e.g. enlarging of a zone with strict non-hunting area regarding wildlife management (Janík 2020). From an economic perspective, tourism has been recognized as a significant part of the economy for BFNP and its profit is higher than from the potential timber industry (Mayer and Job 2008). The results of the similar study for ŠNP have not been published yet (Harmáčková et al. 2016). However, the Czech NP also yields significant economic benefits for the region, which could be further enhanced if tourists extended their stays (Dickie et al. 2014; Bílá et al. 2019). ŠNP is three-times larger, includes the settlements and also the number of visitors is higher than in BFNP; estimations are 760,000 for BFNP and 2,000,000 for ŠNP annually (Mayer and Job 2008; Perlín and Bičík 2010). Therefore, we identify main the anthropogenic structures such as built-up areas and recreational zones to characterise human activities.

Using this detailed knowledge on the specific PA (here ŠNP), its attributes, qualities, and values, as well as the threats and costs, can be determined for further steps (Kujala et al. 2018).

Step 2: Landscape dynamics

General trends show extensification and abandonment of agricultural activities in peripheral mountainous regions, coupled with forest growth across Europe and in Central Europe and Czechia as well (Latocha 2009; Kupková et al. 2013; Kupková and Bičík 2016; Ameztegui et al. 2021). The abandonment contributes to the ecological stability (Guerra et al. 2019) of the landscape but could threaten biodiversity by reducing habitat diversity in agricultural landscapes (Queiroz et al. 2014; Zakkak et al. 2015). On the other hand, land use of part of the mountainous regions, including ŠNP, is being intensified, for example by recreational activities (Verburg et al. 2009; Janík et al. 2019). These long-term (decades) changes of the landscape were caused predominantly by human-induced driving forces (political, economic and social processes) (Kupková et al. 2013).

In the case of ŠNP, a clear trend of land use extensification is evident, with agricultural practices shifting from intensive, such as arable land, to more extensive forms like permanent grassland, pastures, and meadows, resulting in an increase in forest cover (Janík et al. 2019, 2022). In ŠNP, the forest has remained stable over time roughly since protection was established (Janík et al. 2019). Regarding BFNP and ŠNP, this stable area can host species requiring large suitable habitats, such as large carnivores like wolves (*Canis lupus*) and lynx (*Lynx lynx*) and herbivores like moose (*Alces alces*), and it also leads to higher quality of habitats (Müller et al. 2014; Janík et al. 2021b; Bluhm et al. 2023).

However, in the geographical context of ŠNP, as one the largest Central European relatively natural and forested area, we can analyse also the dynamics after disturbance and subsequent natural and anthropogenic reactions (Dale et al. 2001; Bengstsson et al. 2003; Turner 2005; Turner 2010). In the forests of ŠNP, and similar natural mountainous forests, disturbances are an inherent part of their dynamics (Fischer et al. 2002; Grodzki et al. 2006; Heurich 2009; Brůna et al. 2013; Čada et al. 2013; Nováková and Edwards-Jonášová 2015). Forests in the case of ŠNP are able to regenerate (Brůna et al. 2013; Červenka et al. 2014; Janda et al. 2014). However, also human-induced interventions, such as logging or new clear-cuts were made in the vicinity of the area, which was affected by bark beetle infestation after the wind disturbance. It is a consequence of disagreement of main actors on future development of the area leading to its suboptimal management (Janík and Romportl 2018, see Fig. 4).

The second step provided us with data on landscape and its change as a key information for understanding ecosystems and their dynamics. In NPŠ, forest dynamics is primarily driven by natural disturbance. But human intervention, such as management practices, still plays a significant role, varying according to ownership (Křenová et al. 2022). It is also different in various geographical conditions according to framework originated in typology from the first step (Janík and Romportl 2016; Křenová et al. 2022).

Step 3: Protecting species and enhancing biodiversity

In the case of ŠNP, there are suitable habitats for some species. Therefore, we selected fifty key species and developed habitat suitability models as inputs for the fourth step to capture priority areas of their occurrence (Janík and Romportl 2023).

The region of our study and its core area of BFNP and ŠNP is a significant area for harbouring large mammals, because migration from the source population is impossible due to surroundings of the ŠNP with open cultural and inhabited landscape (Bluhm et al. 2023).

The suitability of this area for these species is high due to the presence of large forested areas and lower anthropogenic pressure (Anděl et al. 2010). Recently, a more detailed suitability analysis for moose (*Alces alces*) was assessed and published. It demonstrates that there is a suitable habitat for moose in ŠNP, yet the population has decreased (Janík et al. 2021b, see Fig. 5). Similar above-mentioned suitability maps were used as an indicator for suitability and consequence for protection of biodiversity.

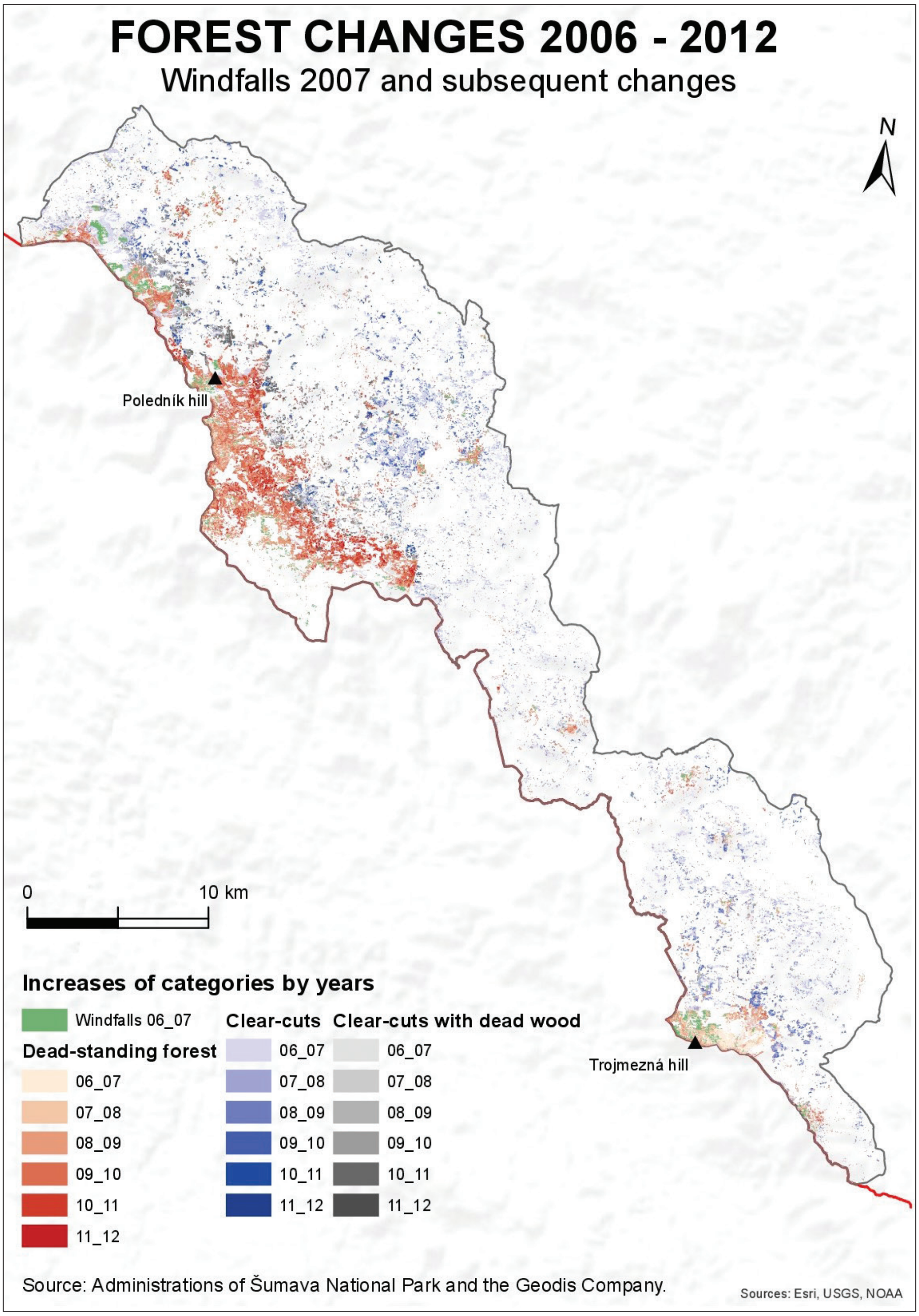
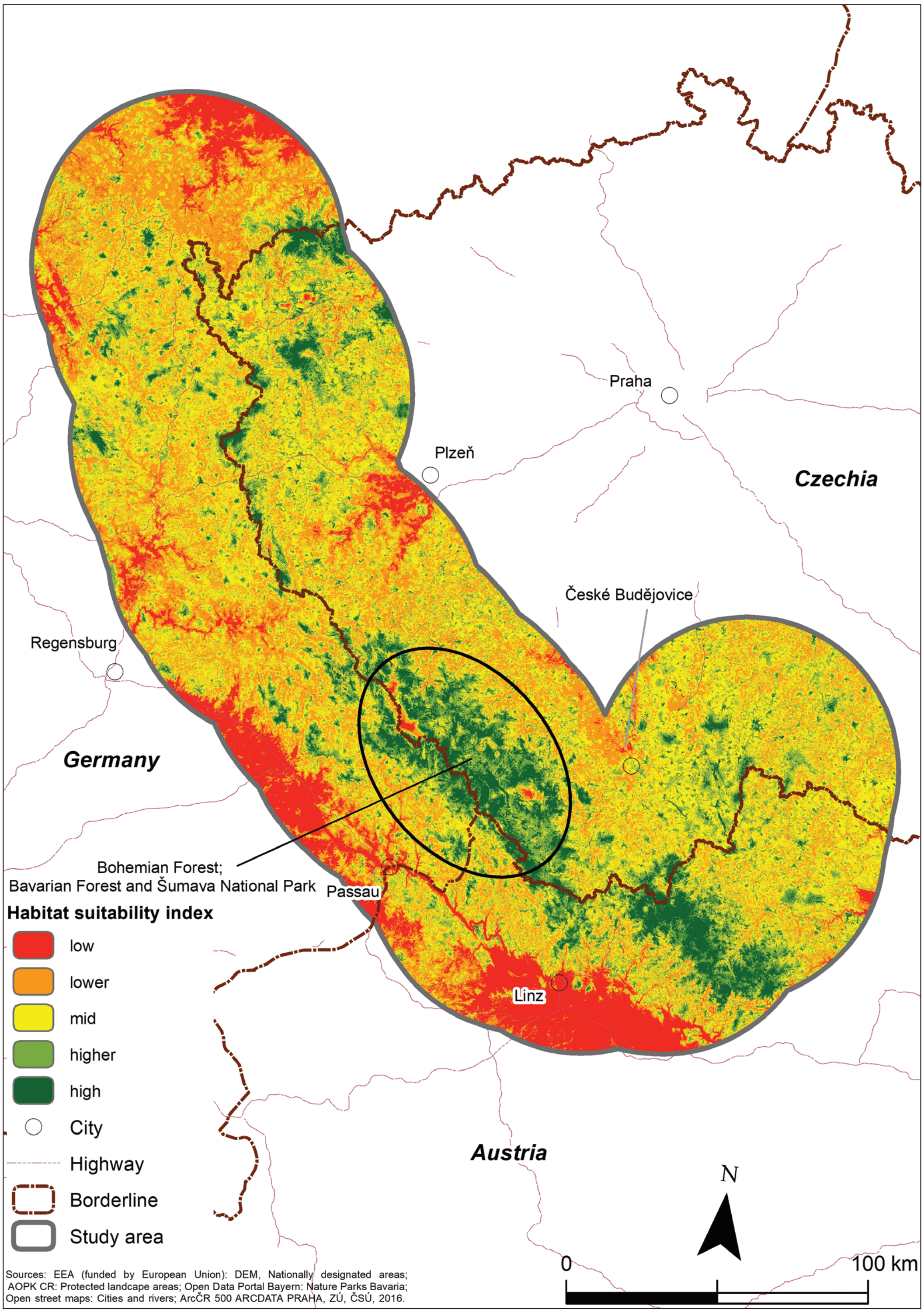


Figure 4. Forest changes 2006–2012 in Šumava NP (Janík and Romportl 2018).

Mapping anthropogenic activities and fragmentation of landscape by anthropogenic structures is another task crucial for preserving viable populations of target species regarding fragmentation of population or additional mortality. From this point of view, illegal hunting and wildlife-vehicle collisions pose serious threats to the lynx and moose populations in BFNP and ŠNP (Heurich et al. 2018; Janík et al. 2021b).



Sources: EEA (funded by European Union): DEM, Nationally designated areas; AOPK CR: Protected landcape areas; Open Data Portal Bayern: Nature Parks Bavaria; Open street maps: Cities and rivers; ArcČR 500 ARCDATA PRAHA, ZÚ, ČSÚ, 2016.

Figure 5. Habitat suitability index for moose (*Alces alces*) within the Bohemian Forest Ecosystem (Janík et al. 2021b).

Step 4: Landscape ecology in practise

In a case of ŠNP, after comprehensive analyses and based on knowledge and results from previous steps: 1a) typology (Janík and Romportl 2016), 1b) anthropogenic activities (Janík and Romportl 2017; Janík 2020), 2) analysis on landscape dynamics (Janík and Romportl 2018; Janík et al. 2019, 2022), 3) evaluation of habitat suitability for key species (Janík et al. 2021b), a synthesis of the three above-mentioned levels was prepared. Specifically, the NATURA 2000 habitat mapping layers (*in sensu* Chytrý et al. 2010) with detailed resolution and information on habitat quality and stability during the last circa 15 years and habitat suitability models for selected key fifty species were the main inputs used to create prioritisation of landscape protection (Janík and Romportl 2023).

However, in the geographical reality of Central Europe, with high human population density, human activities and social and economic interests must also be included into consideration (Brown et al. 2015). By balancing these parts, sustainability is a desired aim (Kušová et al. 2008). Therefore, in our case, built-up and recreational areas were placed in the prioritisation process and treated as areas used by humans.

The result shows a prioritisation map suitable for finding core areas for protection and also highlights the differences between the model and current management zonation of ŠNP. It can be used by the ŠNP administration as material for management of the PA to fill the gaps (Janík and Romportl 2023, see Fig. 6).

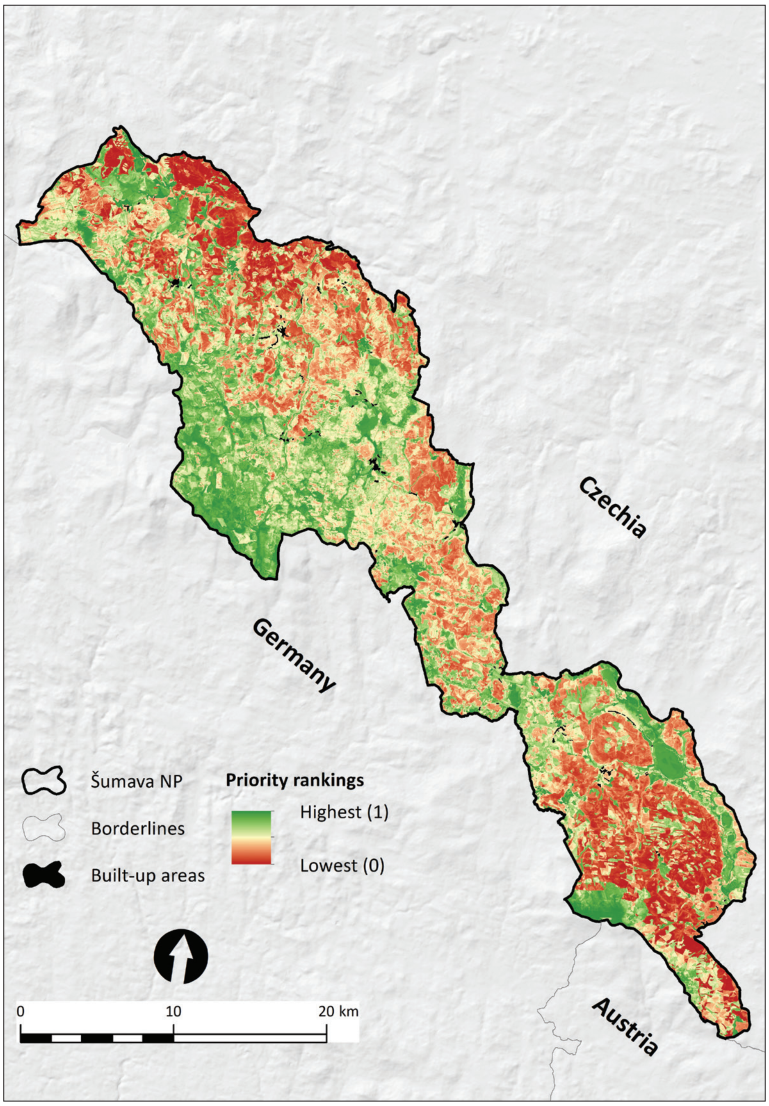


Figure 6. Prioritisation analysis of Šumava NP showing scale of priorities for protection from lowest (red) to highest (green) as a material for evaluation of NP´s zonation (based on Janík and Romportl 2023).

Discussion

There is an urgent need to protect key areas for biodiversity, supporting ecosystem services and landscape functions, as well as human wellbeing (O'Connor et al. 2021). A proposed set of sequential steps, based on essential landscape ecological principles (LEP), can help to successfully address current conservation challenges. The concept presented in this paper offers new opportunities to expand methodological approaches so far used in geography and landscape ecology for nature conservation and to improve conservation management (Fig. 1).

We propose applying a comprehensive approach using LEP and producing a final output – a model for prioritisation of spatial nature protection goals in the area of interest.

Our LEP approach incorporates all relevant features for protected areas' planning and designation. In the first step, we dealt with society and its relationship to ecosystems and protection of the area (Müller and Opgenoorth 2014; Soliku and Schraml 2018; Janík and Romportl 2017; Janík 2020), step two analysed landscape and ecosystem data (Ceauşu et al. 2015, Janík and Romportl 2018; Janík et al. 2019), step three stressed biodiversity (selected species) as some other studies e.g. Sirkiä et al. 2012; Jenkins et al. 2015; McGowan et al. 2020.

Therefore, species and ecosystem data are used to cover relevant factors as much as possible (Ceauşu et al. 2015), and costs and constraints are balanced in a clear scheme. Finally, step four integrated previous steps and prioritised conservation of the PA (Lehtomäki and Moilanen 2013; Janík and Romportl 2023).

In the face of today´s anthropogenic transformation, protected areas (PAs) hardly reach the spatial and temporal requirements for delimiting good quality PAs (Bengstsson et al. 2003), e.g. for some species with high spatial requirements, such as wolf (*Canis lupus*), lynx (*Lynx lynx*) or moose (*Alces alces*) in our case study, which are threatened by fragmentation and they need large-scale and connected landscape, thus detailed prioritisation is not crucial for them (Turner 2005; Heurich et al. 2018; Bluhm et al. 2023). Moreover, with other interests of stakeholders, the establishment of PAs is often a political, socio-economical, esthetical, or cultural-driven decision (Müller and Opgenoorth 2014; Soliku and Schraml 2018). Therefore, our approach can help to fulfil our worldwide targets from Aichi or Kunming-Montreal Global Biodiversity Framework using objective data and methods.

In addition, some activities, e.g. tourism and its intensity influencing negatively PAs, are hardly detectable (Verburg et al. 2009; Křenová and Kindlmann 2015). On the other hand, large-scale natural impacts (such as disturbance) can change regions of PAs rapidly but without being necessarily harmful, then to set an appropriate management is needed, however the decision to not intervene is an option as well (Turner 2005). In the Anthropocene setting and the context of Central Europe, we are facing negative influences of strong anthropogenic pressure on nature. However, using available high-quality data and the above-mentioned landscape ecological methods we may protect PAs more effectively and also find a sufficient solution for other valuable parts of the landscape and nature (Lehtomäki and Moilanen 2013).

Our proposed LEP approach can be widely used as a methodological framework in a diverse range of PAs, including NATURA 2000 sites, as well as in the fulfilment of other European objectives, such as the Biodiversity Strategy (European Commission 2021).

Moreover, the process of prioritisation can be used in the process of new PAs designation, but also in the expansion or reorganisation of existing ones. In our case, it is a supportive material for the zonation of ŠNP. Zones differ according to their management and values (Janík and Romportl 2023). Elsewhere, harmonisation with other land use or future risks was considered (Schuster et al. 2023; Tamburini et al. 2023) or wider landscape planning and priorities (Srivathsa et al. 2023). However, our aim was to clearly wrap the methodical procedure and illustrate its usability in a well-mapped area (Janík and Romportl 2023). Of course, the use of this concept is not limited geographically or regionally. Depending on the availability of relevant input data, the whole process can be applied both in countries with a high level of nature conservation as well as in countries where nature and landscape protection is still developing.

Due to their complementarity and complexity, these steps can be used for a wide range of tasks and generally wherever around the World with various types of available data: from prioritisation and delimitation of new PAs (Bekessy et al. 2012) and setting priorities as supportive material for appropriate zonation (Janík and Romportl 2023), to management recommendations (Křenová et al. 2022; Bluhm et al. 2023). We therefore recommend that the conservation community use the whole set of proposed steps to strengthen the science-based approach to nature and landscape conservation.

Conclusion

This study shows a newly and clearly organised approach using landscape ecological principles to prioritise nature protection. We tested it on the largest national park in Czechia (ŠNP) as a pilot site. Despite the good database, we would like to improve our approach in the next steps, e.g. to use modern technology and more detailed data, which can capture anthropogenic pressures such as intensity of tourism or biotope structure. Our aim was to propose a scientific, evidence-based approach, based on objective data and – above all – not influenced by the subjective view of the author, one that was needed as a methodological framework for spatial designing and prioritising of PAs. We hope that such an approach, consisting of several steps described above, can help to manage PAs and protect nature with regard to all relevant factors.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

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Data availability

All of the data that support the findings of this study are available in the text and original articles cited in this paper.

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Research Article

Rubus plicatus Weihe & Nees: resilience to pollution caused by stone quarries

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Abstract

This study aimed to analyze the effect of pollution caused by stone quarries on the morpho-anatomy, biochemistry, and physiology of a medicinal wild bramble *Rubus plicatus* Weihe & Nees. Samples were collected from two natural protected areas: Iron Gates Natural Park and Jiu Gorge National Park, both located in the southwestern part of Romania, and two unpolluted areas from these parks as background sites. We carried out the following analyses on the collected leaves of this taxon: morphology, micromorphology, anatomy, assimilating pigments, heavy metals (Pb, Ni, Cr, Fe), dry mass, bioactive compounds (total phenols and flavonoids), and antioxidant capacity. The results showed more stomata, higher amounts of assimilating pigments, higher amounts of heavy metals (especially lead), less dry mass, less phenols, and more flavonoids in *Rubus plicatus* leaves from polluted areas compared to areas without sources of pollution. The increased number of stomata and the amounts of assimilator pigments revealed the mechanisms developed by this species in order to survive in polluted conditions.

Key words: Chlorophyll, dust, flavonoids, metals, micromorphology, phenols, stomata, structure

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Introduction

Plants have been widely used in the food industry as well as in the pharmaceutical industry, with consumers and researchers showing an increasing interest in these products because they are an important source of flavonoids (Brodowska 2017; Bjørklund et al. 2018; Konieczynski et al. 2021). However, the quality and quantity of these plant chemical metabolites are influenced by a multitude of factors, most of them environmental (Ncube et al. 2012; Perrino et al. 2023). Plants constantly and rapidly interact with potentially harmful external environmental factors (Ncube et al. 2012). Being immobile organisms, throughout their evolution, plants had to develop alternative defense strategies, among which we now mention the biochemical ones, respectively secondary metabolites, which are important in helping plants adapt to the environment (Holopainen and Gershenzon 2010). On the other hand, these secondary metabolites offer a diverse range of benefits to humans, which include, among others, medicinal properties (Casella et al. 2023). Plant responses to environmental cues are specific (Ncube et al. 2012). A multidisciplinary analysis of this topic, combining ecology, biochemistry, and molecular physiology, would have great potential to reveal the extent to which plant-environment interactions contribute to phytomedicine. The medicinal quality of plants is determined in the field (Ncube et al. 2012). Heavy metals, in addition to direct their effects on the plant communities (Perrino et al. 2014), have the potential to induce the production of secondary metabolites in medicinal plants, however, high levels of this can suppress it (Nasim and Dhir 2010). So, heavy metals induce the production of secondary metabolites in medicinal plants. Metal contamination can change the chemical composition of plants, and modify the quantity, quality, safety, and efficacy of natural plant extract (Murch et al. 2003; Lajayer et al. 2017; Nobahar et al. 2021; Pandey et al. 2023), including the genus *Rubus* (Micu et al. 2016).

On the other hand, the biodiversity of medicinal plants is suffering a great loss globally, along with other animal and plant species (Halder and Jha 2023). Another alternative source to the collection from wild flora, which could potentially ensure the uniform supply of phytotherapy quality, would be their cultivation in vitro, in cell cultures, and in bioreactors, where the manipulation and maintenance of environmental factors are controlled (Bruce and West 1989; Ncube et al. 2011).

Chromium (Cr), iron (Fe), nickel (Ni), and other 14 trace elements are essential for the plants (Kabata-Pendias and Pendias 2001), while lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are non-essential, with unknown biological functions and are toxic to plants and human even at low concentrations (Shahid et al. 2017). Their presence can be caused by natural sources, but a dramatic increase in these elements is linked to anthropogenic activity (Sarma et al. 2012), such as transportation (De Lurdes Dinis and Fiúza 2011), and illegal waste dumping (Perrino et al. 2014). In the food chain, heavy metals from air, water, and soil can be absorbed by plants and cause their metabolism to malfunction, as primary producers, but also are a risk, already present in unmonitored territories, to human health as consumers (Shahid et al. 2017). Plants, including medicinal plants, use different strategies to deal with heavy metals that enter their cells, and the tolerance to a particular heavy metal is controlled by a complex interdependent of morphological, physiological, biochemical, and genetic mechanisms (Maleki et al. 2017), with some plants that have specialized in absorbing the heavy metals as the species belonging to the Brassicaceae family (choose reference). The most frequently highlighted and earliest result of heavy metal stress in plant cells is the excessive generation of ROS (Reactive Oxygen Species) (Schutzendübel and Polle 2002; Das and Roychoudhury 2014). Unlike physiologically non-redox-active heavy metals such as As, Cd, Co (Cobalt), Hg, Mn (Magnesium), Ni, Pb, and Zn (Zinc), free redox-active metals such as Cr, Cu (Cooper), and Fe directly increase ROS production (Kovacik and Backor 2008). To eliminate ROS overproduced by heavy metal stress, plants use specific mechanisms, including the activation of antioxidant enzymes (Blokhina et al. 2003), and non-enzymatic antioxidants such as carotenoids (Mittler 2002). Specifically, Cr represents a heavy metal that is very dangerous to humans when is linked to oxygen. Through the oxidation states of peroxo Cr(V) intermediates or through the action of ROS oxidative DNA damage occurs (Bokare and Choi 2011). Many phenolic compounds, including flavonoids and anthocyanins, are well known for their role in increasing tolerance to heavy metals (Keilig and Ludwig-Müller 2009).

Rubus plicatus Weihe & Nees (Rosaceae family) leaves are rich in tannins and also contain a notable amount of flavonoids, phenolic acids, but also triterpenes, mineral salts, and vitamin C (Gudej and Tomczyk 2004; Zia- Ul-Haq et al. 2014; Abu-Shandi et al. 2015; Oszmiański et al. 2015; Bhatt et al. 2023). This taxon has often been studied and recommended for biomonitoring of contaminated areas (Nujkić et al. 2016), but it accumulates Pb poorly (Steingräber et al. 2022). Air pollution is widespread in a consumerist society. The main air pollutants are gases such as carbon dioxide (CO_2), sulfur dioxide (SO_2), ammonia (NH_3), ozone (O_3), volatile organic compounds, and carbon monoxide, or heavy metals such as Cd, Cr, Fe, Mn, Ni, Pb, etc. (Sawarkar et al. 2023). The increase in demand for quality construction stones and sand has led to the intensive mining of hard rock in many parts of the world (Ukpong 2012; Vandana et. al. 2020). Size and crushed stones are the final output of such an industry, and they are used for various purposes, such as the construction of new roads and highways (Nartey et al. 2012). Many species of *Rubus*, including the one studied here, are present in different types of human impact: railways, roads, and quarries (Sargent 1984; Dehaan et al. 2007; Gentili et al. 2011). Unfortunately, these exploitations harm the environment because they involve the presence of heavy machines and explosives, exhaust gases, and dust; these activities are associated with air pollution, noise pollution, biodiversity damage, and habitat destruction (Lameed and Ayodele 2010). Sayara et al. (2016) concluded that it is recommended to develop a green belt in the surroundings of the exploitation site, using pollution-tolerant trees (usually with broad leaves) to limit the spread of quarry dust by intercepting, filtering, and absorbing pollutants. The high cost of instrumental monitoring methods and difficulties in sampling limited the studies on atmospheric contamination. These are the reasons why there is more interest in the use of indirect monitoring methods such as the use of bioaccumulators organisms, as higher plants (Bargagli 1998). The use of plant leaves, primarily as accumulators and biomonitors of trace metal pollution, has gained great ecological importance (Mulgrew and Williams 2000).

When stone quarries are near or within protected areas, protecting these ecosystems is as important as protecting humans from pollution (Ukpong 2012; Vandana et. al. 2020). This is the reason why in the present study we proposed and analyzed various parameters of the plants, to identify the impact of the pollution caused by the presence of stone quarries on them. The hypothesis we started from was that stone quarries affect the structure and growth of plants from the genus *Rubus*, with the possibility of developing morpho-functional resilience to survive.

Materials and methods

Plant material

The plant material consisted of *Rubus plicatus* leaves, taken in September 2023, from two protected areas in Romania, Jiu Gorge National Park (**JGNP**) and Iron Gates Natural Park (**IGNP**), both from unpolluted areas: Bratcu

Valley (DDM: 45°13.6933'N, 23°21.5983'E), respectively Sirinia Valley (DDM: 44°39.105'N, 22°4.3233'E), as well as around stone quarries in these parks: Meri Quarry (DDM: 45°13.08'N, 23°22.5967'E), and Eșelnița Quarry (DDM: 44°43.9883'N, 22°20.8767'E). We used Google Maps (2023) to take the GPS coordinates in the WGS84 geographic system. The leaves were taken from the middle of the stem of five shrubs at each collection point. They were kept cold in polyethylene bags during transport and storage in the laboratory. The analyses of morphology and assimilating pigments were carried out within a maximum of 48 hours from the sampling. For microstructure and anatomy analyses, leaf fragments were preserved in 70% ethyl alcohol. For quantification of bioactive compounds and antioxidant capacity, fresh leaves were dried at 40 °C for 7 days.

Samples for morphological, micromorphological, and anatomical study

The technique of imprinting the epidermis with a collodion film was used to determine stomatal density, and the counting was carried out under 200× magnification. The morphology of the leaf was analyzed with an Olympus SDF PLAPO 1XPF stereomicroscope with an Olympus UC 50 camera, and the morphology of the epidermis and the structure of the leaf limb were studied with an Olympus IX73 Inverted LED microscope with an Olympus UC50 camera. In addition, scanning electron microscope (SEM) observations for micromorphology were performed at the median level of the leaves, as follows: after dehydration, up to 96% alcohol concentration, leaf samples mounted on hubs, covered with 2 mm gold, in a sputter coater and examined at 8.00 KV, at SEM LEO 436VP. Transverse sectioning was done manually, at the median level of the leaf limb, in the direction of the main rib; 30 sections were analyzed for each sample. The abbreviations for morphological measurements are: **AdECL** adaxial epidermis cell length; **AdECW** adaxial epidermis cell width; **AdESN** adaxial epidermis stomata number; **AbECL** abaxial epidermis cell length; **AbECW** abaxial epidermis cell width; **AbESN** abaxial epidermis stomata number; **AbESL** abaxial epidermis stomata length; **AbESW** abaxial epidermis stomata width.

Assimilating pigments analysis

Fresh leaves were cut into small pieces, 2.5 mg weighted, and homogenized with 5 ml DMF (N, N-dimethylformamide). The mixture was kept at 4 °C for 72 h. At the time of the reading, a 10× dilution was also made with DMF. Measurements were performed in three replications with a T60 UV-VIS spectrophotometer.

For quantifying chlorophylls (Chl), namely Chl *a*, Chl *b*, or total Chl, the equations necessary were: Chl $a = 12.70A_{664.5} - 2.79A_{647}$; Chl $b = 20.70\ A_{647} - 4.62A_{664.5}$; total chlorophyll pigments Chl $a{+}b = 17.90A_{647} + 8.08A_{664.5}$, where in 1.00-centimeter cuvettes and Chl = chlorophyll in milligrams per liter (Inskeep and Bloom 1985). Total carotenoids (Car) were calculated with the formula: Car = $1000A_{480}$ – 0.89 Chl *a* – 52.02 Chl *b*/245 (Wellburn 1994). The extinction coefficients necessary for the quantification of Chl *a*, *b*, and total Chl were determined by the Moran (1982) method.

Heavy metals

The dried leaf samples were mineralized with a Speedway Xpert microwave digestion system in 10% nitric acid solution. The metals were analyzed by atomic absorption spectrometry (AAS), Cr (χ - 357 nm), Ni (χ - 232 nm), and Pb (χ - 217 nm), by GF-AAS (graphite furnace), and Fe (χ - 248 nm) by FL-AAS (air-acetylene flame), using ZEEnit 700P spectrometer. To plot the calibration curve, standard solutions of concentrations were prepared: for Pb - 3, 12, 30, 45, and 60 µg/L; for Ni: 2, 5, 10, 15, and 20 µg/L; for Cr: 2.5; 5; 7.5; 10 µg/L; for Fe 0.1; 0.5; 1; 2; 3 mg/L).

Total biomass

The dry biomass of the *Rubus* leaves was weighed after 2 g of fresh leaves from each category of leaves was desiccated.

Determination of bioactive compounds. The total phenols content (TPC), total flavonoids content (TFC), and antioxidant capacity (ferric reducing antioxidant power - FRAP and free radical scavenging capacity - DPPH)

The powder of each leaf sample (10 mg) was suspended for 48 h in ethanol 70%. 10 ml of this mixture was centrifuged for 20 minutes at 5000 rpm, and the resulting supernatant was collected for analysis. The **TPC** was determined using the Folin-Ciocalteu method (Singleton et al. 1999), with some modifications. Briefly, 100 µL diluted samples (1:20, v/v) were combined with 1700 µL of distilled water. Subsequently, 200 µL Folin-Ciocalteu reagent (freshly diluted 1:10, v/v) was added and mixed using a homogenizer. Then, 1000 µL of 7.5% Na_2CO_3 solution was mixed again. The mixture was incubated at room temperature, in the dark, for 2 h. The absorbance measured at 765 nm was noted in mg gallic acid equivalents (GAE)/g of dry weight (DW), based on the calibration curve. For **TFC** the aluminum chloride colorimetric method was used. Summarily, 1000 µL diluted extract sample, 4000 µL distilled water, and 300 µL $NaNO_2$ 5% were combined. After 5 minutes, 2000 µL of 1 M NaOH, and after another 6 minutes, 300 of µL 10% $AlCl_3$ were added. Lastly, distilled water was used to complete the volumetric flask to bring the volume up to 10 ml (Pekal and Pyrzynska 2014). The absorbance values measured at 510 nm were reported as mg quercetin equivalents (QE)/g DW. **FRAP** was performed according to Benzie and Strain (1996) with some modifications. Therefore, 100 µL diluted samples (1:20, v/v) were mixed with 2000 µL distilled water and 500 µL FRAP reagent. The mixture was held for 1 h in the dark, and after that the absorbance was measured at 595 nm. The **DPPH** (2.2-diphenyl-1-picrylhydrazyl) was performed according to Brand-Williams et al. (1995) with some modifications. A portion of the 100 µL diluted sample was mixed with 2.8 ml DPPH solution (80 µM) and kept for 30 minutes in the dark. The percentage of the DPPH scavenging effect of samples was calculated using formula 1.

$$\text{The percentage of DPPH scavenging effect (\%)} = [(A_0\text{-}A_s) \times 100]/A_0 \quad (1)$$

where A_0 is the absorbance of the blank, and A_S is the sample absorbance, both monitored at 517 nm. All absorbances were measured using a spectrophotometer Shimadzu UV mini-1240 UV-VIS.

Statistical analysis

The raw data were processed mathematically and statistically, calculating the mean and the standard deviation for three replications. The significance between polluted and unpolluted areas was determined by a Two-Sample *t*-test by MICROSOFT OFFICE16\EXCEL software.

Results

Morphologically, the stereomicroscope observations revealed major changes between the leaves taken from the unpolluted area and those from the quarry-polluted area (Fig. 1). Thus, compared to the control leaves (Fig. 1A, B, E, F), the leaves from the polluted areas, respectively the Meri quarry (Fig. 1C, D) or the Eșelnița quarry (Fig. 1G, H) showed lesions and necrosis. In Meri and Eșelnița quarries but also in Sirinia Valley, deposits of insect droppings were identified on the abaxial side of the leaves (Fig. 1D).

The upper epidermis is devoid of stomata, and these and numerous tector hairs are arranged on the abaxial surface (hypostomatic) (Fig. 2).

Following biometry of epidermal cells in the leaves of the blackberry bushes from both operating stone quarries from the two parks, smaller epidermal cells were observed, compared to those of the leaves from the unpolluted areas, both located on the adaxial and abaxial faces; some differences were significant from a statistical point of view (Table 1). On the other hand, in the polluted sites, the number of stomata per unit area was significantly higher compared to the control sites (Table 1).

In Sirinia Valley, the blackberry leaves had about 5 times larger epidermal cells than those grown in Bratcu Valley, and the number of stomata per unit area was lower (Table 1). All stomata were closed at all sampling points. The lesions were observed macroscopically and morphologically (Fig. 3). If in the unpolluted sites of the two valleys, the leaf tissues have a normal structural appearance, in the lamina sections of the leaves from plants grown in the quarries area, the parenchyma lesions were either subepidermal (Fig. 3B) or periphloemic (Fig. 3D). Also, in the quarry area, dust particles were caught (Fig. 3E) on the abaxial side of the leaf. On the upper part of the leaf, the dust particles were larger and fewer than on the lower part of the leaf, where they were smaller but numerous. Tector hairs were rare on the adaxial side and more numerous on the abaxial side of the leaves. Comparatively, between the sample of control leaves and those from the quarries, the tector hairs were more numerous in the latter, especially on the lower epidermis.

The amount of assimilating pigments was increased in the polluted areas compared to the unpolluted areas (Table 2), but in the Iron Gates Natural Park, at both points, it was higher than in similar areas in the Jiu Gorge National Park (Table 2). The amount of chlorophyll *a* was higher in all cases than the amount of chlorophyll *b* (Table 2), and the ratio between the two is relatively lower in both quarriers, versus the control (Table 2). We notice a more intense increase in the Chl/car ratio, of 2.75, in Sirinia Valley, caused by the low values of carotenoids, while in the Eșelnița quarry, we measured the highest value of carotenoids (Table 2).

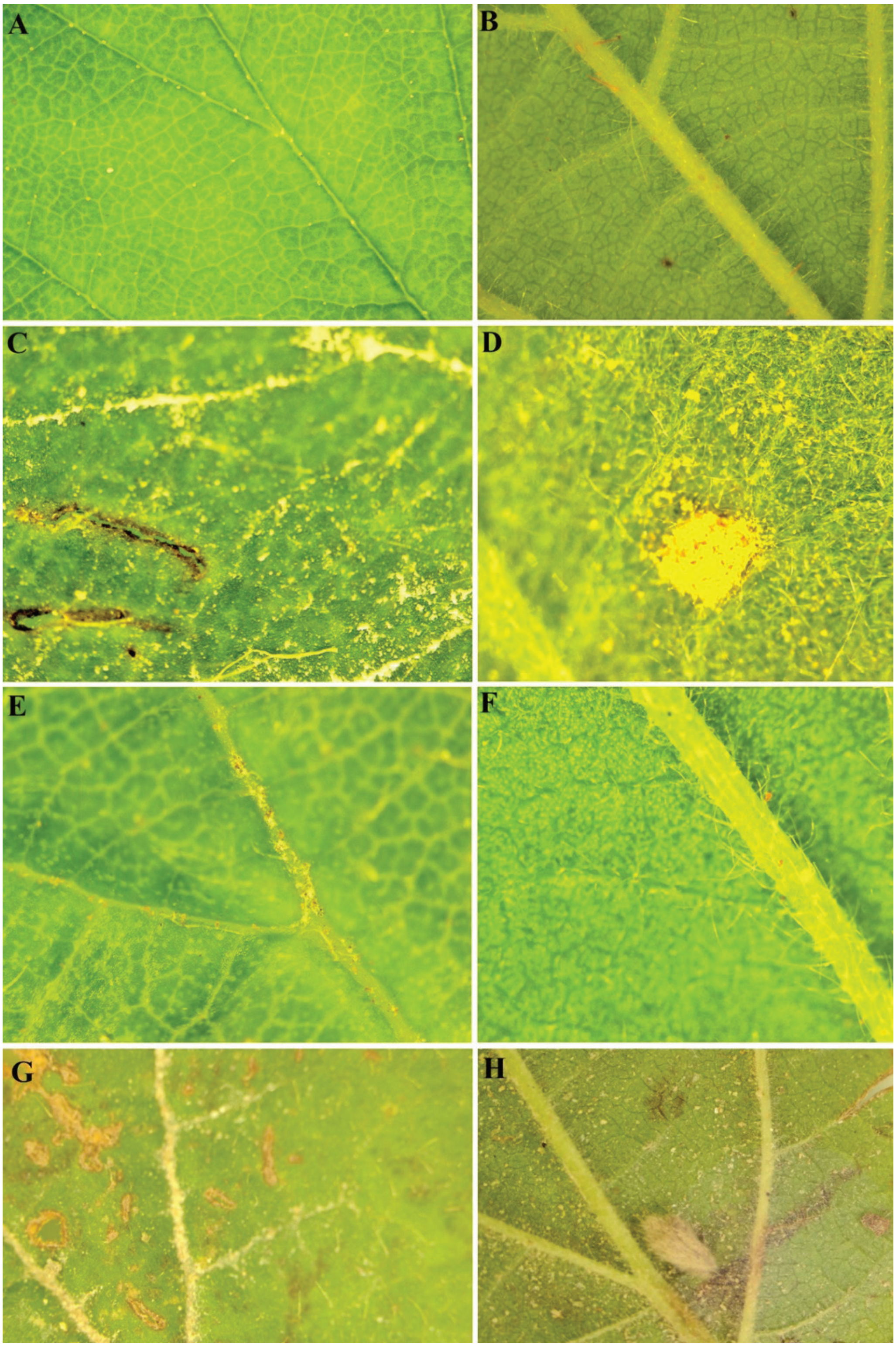

Figure 1. The morphology of the blackberry leaf (*Rubus plicatus Weihe & Nees*) captured with the stereomicroscope, depending on the sampling point. Jiu Gorge Natural Park: Bratcu Valley (control) **A** adaxial surface (4×) **B** abaxial surface (2×); Meri quarry **C** adaxial surface, with lesions (4×) **D** abaxial surface, with insect egg deposits (4×); Iron Gates National Park: Sirinia Valley (control) **E** adaxial surface (2×) **F** the abaxial surface (4×); Eșelnița quarry **G** the adaxial surface, with necrosis (2×), **H** abaxial surfaceface, with necrosis, insects and dust (1×).

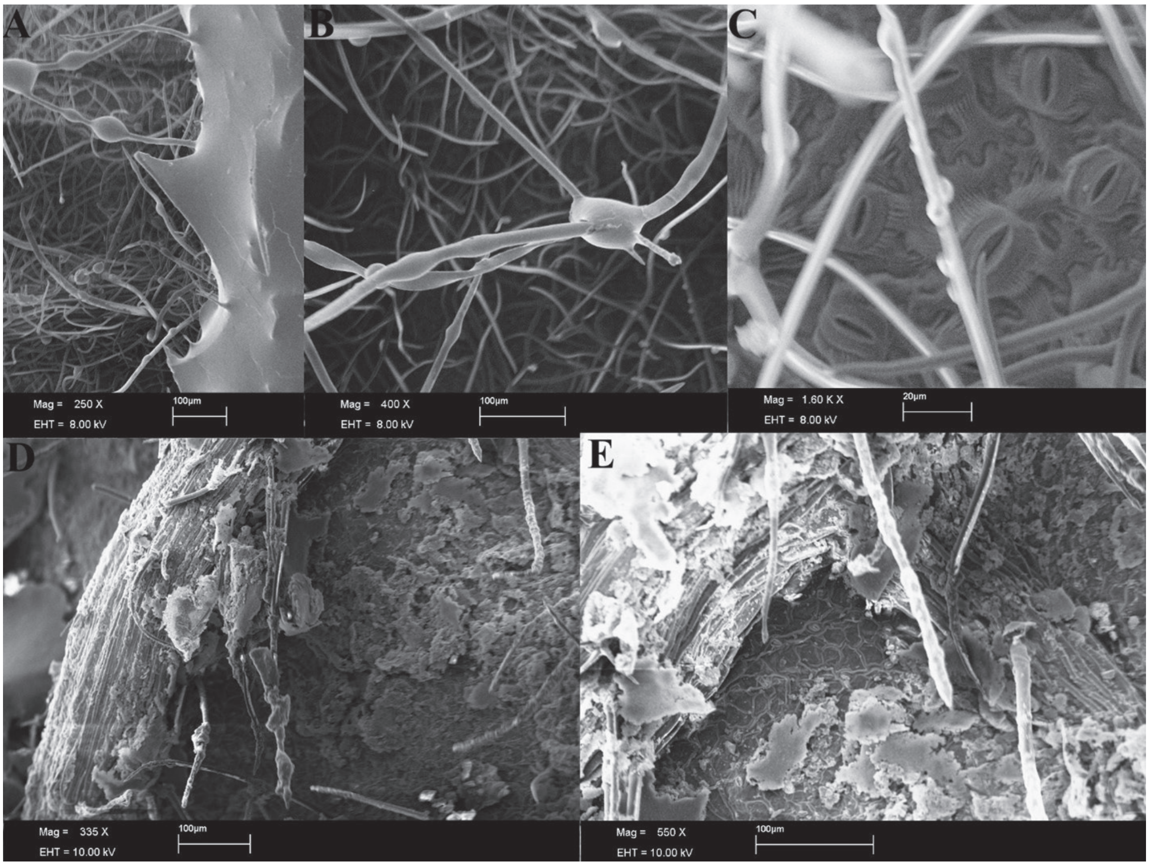

Figure 2. Photomicrographs with epidermal formations in the blackberry (*Rubus plicatus* Weihe & Nees). Unpolluted area **A** thorns (250×) **B** tector brushes (400×) **C** stomata on the abaxial epidermis (1.60 K×), and polluted area **D** epidermis with particle deposits in the leaves from the Eşelnița quarry (335×) and **E** Meri quarry (E – 550×). Scale bars: 100 µm (**A, B, D, E**); 20 µm (**C**).

Table 1. Parameters of the foliar lamina (AdECL adaxial epidermis cell length; AdECW adaxial epidermis cell width; AdESN adaxial epidermis stomata number; AbECL abaxial epidermis cell length; AbECW abaxial epidermis cell width; AbESN abaxial epidermis stomata number; AbESL abaxial epidermis stomata length; AbESW abaxial epidermis stomata width) (average ± standard deviation).

Protected area	Sample	AdECL (µm)	AdECW (µm)	AdESN	AbECL (µm)	AbECW (µm)	AbESN	AbESL (µm)	AbESW (µm)
Jiu Gorge	Bratcu Valley	33.37±1.15	21.99±2.21	0.00±0.00	20.302±2.45	14.05±2.75	19.1±0.55	21.62±2.23	17.11±2.91
	Meristone quarry	30.0±0.71	18.2±1.48	0.00±0.00	19.1±0.89	12.80±1.30	22.8±0.84	22.2±1.10	18.20±1.53
	t -test	0.001	0.01	-	0.34	0.39	0.0002	0.62	0.48
Iron Gates	Sirinia Valley	164.00±6.57	85.29±9.20	0.00±0.00	17.66±1.82	13.18±0.26	17.2±1.30	22.8±1.64	18.96±0.57
	Eşelnița stone quarry	155.0±0.71	72.8±1.48	0.00±0.00	16.4±1.34	12.2±1.10	23.6±0.89	23.6±1.14	20.26±1.24
	t-test	0.295	0.036	-	0.251	0.116	0.000	0.400	0.080

Note: p<0.01 – statistically very significant; p<0.05 – distinctly significant; p<0.1 – significant (s); p>0.1 ns– non-significant. The values measured in unpolluted areas were the reference (control).

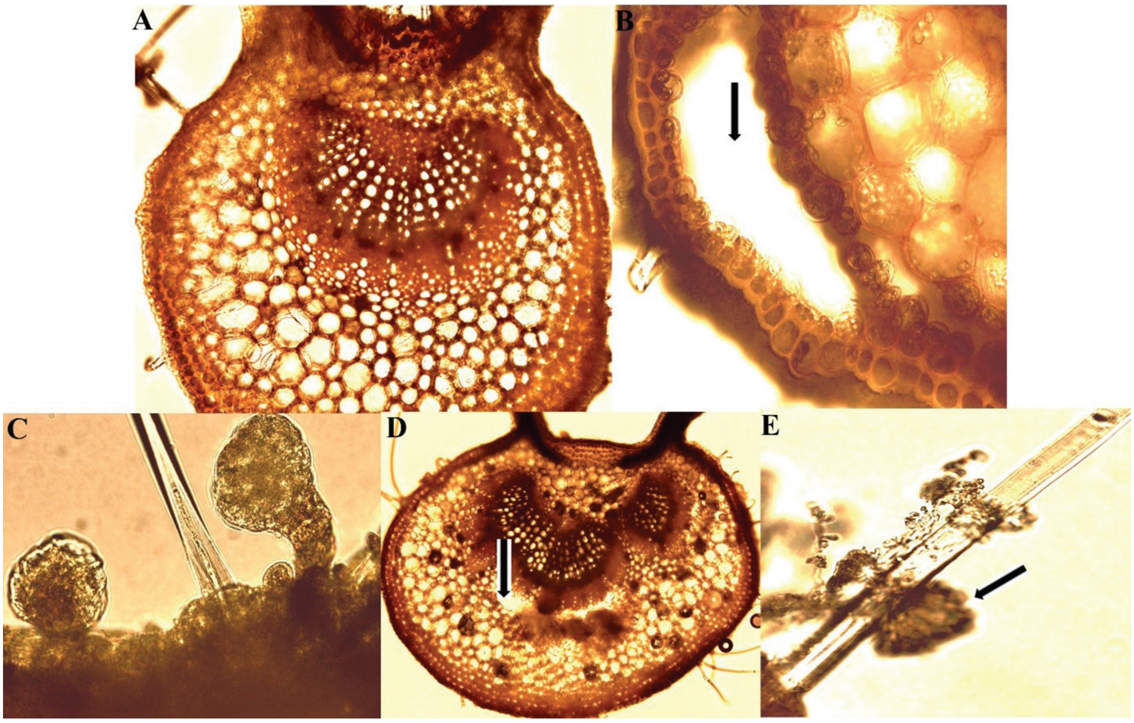

Figure 3. Blackberry leaves structure (*Rubus plicatus* Weihe & Nees). Provided from Jiu Gorge National Park **A** normal aspects in Bratcu Valley (200×) **B** with parenchyma lesions (row) from Jiu Gorge, Meri stone quarry (600×); from Iron Gates Natural Park **C** without dust in Sirinia Valley (1000×) **D** with lesions in the periphloem parenchyma (row) (100×), and **E** dust (row) on tector hair (1000×) in Eșelnița stone quarry.

Table 2. The assimilating pigment content of *Rubus plicatus* Weihe & Nees leaves from unpolluted and polluted areas (stone quarry) (average ± standard deviation).

Protected area	Sample	Chl *a* (mg L^{-1})	Chl *b* (mg L^{-1})	Car (mg L^{-1})	Chla/*b*	Chl *a*+Chl *b* (mg L^{-1})	Chl/Car
Jiu Gorge	Bratcu Valley	1.92±0.5	1.45±0.7	2.55±0.3	1.33	3.37±0.4	1.32
	Meri stone quarry	3.21***±0.6	2.51**±0.9	3.66**±0.6	1.28	5.72***±0.5	1.56
Iron Gates	Sirinia Valley	7.32±1.2	6.02±1.1	4.84±1.2	1.22	13.33±0.9	2.75
	Eșelnița stone quarry	9.38***±0.9	9.09***±1.4	9.26***±2.1	1.03	18.46***±1.2	1.99

Note: p<0.01 – ***statistically very significant; p<0.05 – **distinctly significant; p<0.1 – *significant (s); p>0.1 ns– non-significant. The values measured at unpolluted areas were the reference (control).

The anthropogenic activity in stone quarries increased the concentration of all heavy metals measured by us (Pb, Ni, Cr, Fe) in the vegetation and decreased the dry mass (Table 3). Among non-essential heavy metals, lead was in higher quantities in Iron Gates Natural Park, especially in the area of the stone quarry (Table 3).

The blackberry leaves from the Meri quarry contained higher amounts of flavonoids and DPPH than those of the control group (Table 4). The lowest amount of flavonoids was recorded in the Eșelnița quarry (Table 4).

Table 3. Median concentration (mg/100g dry leaf) of Pb, Ni, Cr, Fe in *Rubus plicatus* Weihe & Nees leaves (average ± standard deviation).

Protected area	Sample	Pb	Ni	Cr	Fe	Dry weight
Jiu Gorge	Bratcu Valley	0.015±0.1	0.009±1.1	0.026±0.1	6.72±1.2	1.003±0.02
	Meri stone quarry	0.079***±0.3	0.046***±1.4	0.065**±0.2	23.35***±1.1	0.68***±0.03
Iron Gates	Sirinia Valley	0.024±0.2	0.015±0.7	0.03±0.1	12.09±1.5	0.76±0.02
	Eșelnița stone quarry	0.096***±0.4	0.165***±0.6	0.228***±0.7	86.56***±2.1	0.62**±0.08

Note: p<0.01 – ***statistically very significant; p<0.05 – **distinctly significant; p<0.1 – *significant (s); p>0.1 ns– non-significant. The values measured at unpolluted areas were the reference (control).

Table 4. The total phenols content, total flavonoids, and antioxidant capacity (average ± standard deviation).

Natural protected area	Sample	TPC (mg GAE/ g)	FRAP (mmol TE/g)	TFC (mg QE/g)	DPPH (mmol TE/g)
Jiu Gorge	Bratcu Valley	85.48±3.89	0.36±0.007	84.72±8.45	326.08±8.97
	Meri stone quarry	75.71±1.13	0.37±0.001	189.97±5.32	346.95±1.45
	t -test	0.15	0.56	0.01	0.18
Iron Gates	Sirinia Valley	62.54±1.33	0.36±0.007	47.59±47.5910	337.74±3.47
	Eșelnița stone quarry	32.00±2.35	0.29±0.011	31.86±1.70	330.58±6.66
	t -test	0.010	0.024	0.057	0.355

Note: p<0.01 – statistically very significant; p<0.05 – distinctly significant; p<0.1 – significant (s); p>0.1 ns– non-significant. The values measured at unpolluted areas were the reference (control).

Discussion

Morpho-anatomy of the leaves

Lesions, necrosis, and insect attacks on shrub leaves signal the plants' suffering, even at a macroscopic level. Moreover, they suggest cellular and functional stress of the affected organisms. Some species, such as blackberry, manage to survive even in less favorable conditions. The blackberry succeeds in occupying different types of habitats through the adaptations it has, accumulated throughout evolution, such as hypostomatic leaves, tector hairs, and thorns, but also through the ability to adapt short term to environmental conditions, such as identified in the present study. One adaptation was manifested by the increase of the number of stomata on the leaves of plants located in the particulate matter (PM) pollution area to support the supply of gases in vital processes, such as respiration and photosynthesis. However, all types of air pollutants, especially PM, have potentially harmful impacts on morphological, physiological, and biochemical parameters, which can further reduce plant growth and development, e.g., they can cause direct chronic injury (chlorosis) and productivity losses (premature leaf death, reduced height growth) (Rai 2016). In contrast, many plants show no visible changes because, much more often, plant changes occur at the anatomical and biochemical level (Rai 2016).

In our case, the fact that the epidermal cells of the leaves were smaller in polluted areas compared to unpolluted ones, led to an increase in the density of stomata. This increase can also be explained by the fact that the leaves are covered by impurities, which cause stomata to be blocked, and their basic functions to suffer, which led to the adaptation to increasing their number, as already observed in many species of wild plants (Chaudhary and Rathore 2018). It is natural for the density of stomata to increase if the epidermal cell size decreases. PM can be deposited in stomatal openings and disrupt the intensity of respiration and transpiration or even enter stomatal pores and disrupt mesophyll function (Burkhardt and Grantz 2016; Grantz et al. 2018). Long-term changes in stomatal behavior (e.g., stomatal size and stomatal density) Long-term changes of stomatal behavior (e.g., stomatal size and stomatal density) determined by the environment may co-occur across species and genotypes (Reid et al. 2003). Over time, stomata developed adaptative mechanisms to respond to different environmental factors to balance the conflict between the two fundamental processes: photosynthesis and transpiration (Mansfield 1998). Air quality influences morphology (structure of stomata, number of stomata, number of leaves, leaf surface, etc.), along with biochemical influences (ascorbic acid, pigments, enzymes, proteins, and sugar content) and physiological influences (pH and relative water content) (Sharma et al. 2017; Kaur and Nagpal 2017). Moreover, there are plant species that, because they adapt to pollution, can also improve air quality (Łukowski et al. 2018). One such species from the genus *Rubus* is *R. ellipticus* Sm., recommended for the development of green belts due to its high tolerance to air pollution (Sharma et al. 2019).

We also measured cuticle thicknesses in all leaf categories, and the results showed that on the surface of leaves grown in a polluted environment, the cuticle layer was increased, which is another form of adaptation of the cuticle, along with its main role of protection against dehydration, a secondary function of physical protection, by self-cleaning, against dust or pathogens (Yeats and Rose 2013). Thus, the adaxial surface presented a layer of epicuticular wax with dimensions of 3050 μm - 3700 μm (for the leaves from the stone quarries), while in the control samples this layer was 1200 μm - 1670 μm. On the abaxial side, the cuticle was thicker, but much thicker in the leaves from the polluted areas (4170 μm - 4780 μm) compared to those from the unpolluted areas (2100 μm - 2200 μm). The structural aspects we identified confirm the morphologically highlighted damages. The two valleys, even if they are similar from the point of view of pollution, have different climatic conditions, but the fact that in both points of the IGNP the same trend was observed leads to the conclusion that other common environmental factors have influenced this growth.

We observed that the blackberry leaves had fewer hairs on the adaxial epidermis, compared to the abaxial one, which allowed the wind to blow the dust from this level, and prevent its accumulation, which would have led to even stronger shading of the leaf. Only larger dust particles remained on the adaxial side. The capacity of leaves to retain particles from the atmosphere depends on the particle's interactions and their surfaces (Moradi et al. 2017; Soheili et al. 2023), on the species or the shape of the leaf, the presence of hairs allowed a significantly higher accumulation PM (Leonard et al. 2016). Atmospheric dust induces stress on plants similar to drought. Leaves with high tector hair content can retain more dust, causing chlorosis and necrosis on the leaves. Fine particles up to 2.5 μm can penetrate inside the leaf tissues through stomata, leading to the degradation of chloroplasts and assimilating pigments (Soheili et al. 2023). However, in the polluted areas, both on the adaxial and abaxial sides, the density of the tector hairs was higher than in the leaves from the unpolluted areas, as a stress adaptation factor, but leaf tector hairs influence biophysical processes such as light reflectance (Ehleringer et al. 1976).

Assimilating pigments

A similar condition was recorded in terms of the amount of assimilating pigments, which is much higher in the case of plants grown in polluted areas, although air pollution leading to a decrease in the amount of chlorophyll was also reported (Prusty et al. 2005). It would seem that PM mainly decreases plant vigor through a shading effect, whereby accumulated PM absorbs and scatters light rays, preventing them from freely accessing chloroplasts. This is reflected in a decrease in photosynthetic efficiency (Przybysz et al. 2014; Saadullah et al. 2014) and an additional increase or decrease in leaf temperature (Eller 1977), due to albedo-type effects (Sharma et al. 2015). We believe that the shading caused by dust can also lead to an adaptation response of the individuals of some species through the quantitative increase of chlorophyll, just as it happens in shaded plants or transferred to the shade (Brand 1997). Chlorophyll *a* and chlorophyll *b* absorb slightly different wavelengths of light, sun leaves have a higher Chl *a + b* content and higher values for the ratio Chl *a/b* (Lichtenthaler et al. 1981). Plants that are shade-adapted will optimize their growth to suit the conditions, so while plants in the sun will tend to produce more chlorophyll *a*, those in the shade will produce more chlorophyll *b*. Leaves from the more shaded parts will contain more chlorophyll *b* compared to chlorophyll *a* (i.e., the Chl *a/b* ratio will be lower) (Lichtenthaler 2007). The change in the Chl *a/b* ratio is a simple parameter to reveal changes quickly and roughly in light-harvesting complexes (LHCs) (Chazaux et al. 2022). The Chl *a/b* ratio also indicates the quality of the chloroplasts (Lichtenthaler et al. 1981). Thus, the high quality of the granule membranes is indicated by a ratio of 2 whereas a higher value will indicate a degradation of the granule membranes (Danielsson and Albertsson 2008). Carotenoids are essential for photosynthesis and photoprotection and their derivatives are signaling molecules in response to environmental conditions (Sun et al. 2022).

The impact of PM can be far-reaching because their accumulation has significant effects not only on the physiological processes of the whole plant but also on organisms at higher trophic levels, such as folivore insects (Khan et al. 2013; Łukowski et al. 2018).

Heavy metals and antioxidants

It is demonstrated that the effects and toxicity of dust depend on the origin source and quantity (Łukowski et al. 2020). As the leaves are the primary receptors of pollutants (Rai 2016), they are generally used for analysis. Thus, leaves are heavy metal accumulators, just like in the case of Babić et al. (2022), where high accumulations of Pb in *Mentha piperita* L. plants were reported, especially in roots and leaves (up to 1.26 mg kg-1), less in stems, and also in leaves, chromium 0.23 mg kg^{-1}, cadmium 0.12 mg kg^{1,} and manganese 49.88 mg kg^{-1}. In our case, the only variable between the control and tested samples, from the perspective of heavy metal accumulation, is the quarrying activity, which is solely responsible for increasing the concentration of heavy metals in blackberry leaves. Lead, one of the non-essential heavy metals associated with poisoning, was in higher quantities in the Eșelnița quarry in the Iron Gates Natural Park compared to the Meri quarry in the Jiu Gorge National Park.

Absorption of ingested lead can induce, in adults, high blood pressure and cardiovascular disease, fetal neurodevelopmental effects, and reduced learning ability in children (WHO Report 2011). Provisional Tolerable Weekly Intake (PTWI) recommendations are 0.025 mg/kg (25 µg/kg) body weight, i.e., 1.75 mg/week (1750 µg/week) for a person weighing 70 kg (SCOOP 3.2.11. 2004). Maximum levels of lead in vegetable mg/kg, as defined in Commission Regulation (EC) No 466/2001 are between 0.027 mg/kg and 0.1 mg/kg, depending on the European state (SCOOP 3.2.11. 2004).

Heavy metals accumulated in some plants increase the level of antioxidant potential and total phenolic content (Márquez-García et al. 2012; Makuch-Pietraś et al. 2023), but in another species, the increase of heavy metal concentrations (Cu, Ni, Zn) led to the decrease in phenol concentrations (Kulbat-Warycha et al. 2020). The lowest amount of flavonoids was recorded in the Eșelnița quarry, possibly due to the high amount of Pb, which is identified here as the highest. Thus, in Ukraine (Konieczynski et al. 2021), probably due to the anthropogenic factor, the order of the amount of tested metals in mg/kg dry weight was: Cu < Mn < Zn < Fe. Total flavonoids were found in the range of 7.30–251.60 mg/g dry weight (Konieczynski et al. 2021). The relevant analyses were the level of Zn Mn, and TFC (Konieczynski et al. 2021).

Metals on the leaves associated with vehicle presence were chromium, copper, and manganese found in PM (Leonard et al. 2016).

When plants are exposed to pollutants, reactive oxygen species (ROS) increase, which first leads to oxidative stress and finally causes the cell death procedure (Shahid et al. 2014). Oxidative stress can increase antioxidant production (Williams et al. 2004). In our case, only the total content of flavonoids from the Meri quarry was higher. In plants, flavonoids can increase metal chelation, which leads to a decrease in levels of hydroxyl radicals (Mira et al. 2002; Williams et al. 2004). On the other hand, flavonoids function as a defense mechanism against herbivorous insects and mammals, which is why they contribute to resistance to diseases as constitutive antifungal agents or as phytoalexins in plants (Harborne and Williams 1992). We identified insect eggs on leaves from the Meri quarry. A similar situation was described with the leaf extracts collected both from the cultivated area of a former sodium factory, inactive for 25 years, or from the calamine waste area or the settling pond area formed as a result of material removed during the processing of processes of zinc ores and lead, where a significantly higher DPPH free radical scavenging activity was identified (Czaja et al. 2015).

Conclusions

Blackberry is a species that manages to adapt to the dust pollution of the quarries to survive, through an increased number of stomata, and higher amounts of assimilating pigments in leaves.

Stone quarries are a polluting factor for plant species, even for the most resistant ones, located in the immediate vicinity, consequently, the amount of heavy metals found in blackberry leaves is high. Lead, as a non-essential heavy metal, was identified in much higher quantities in blackberry leaves in the vicinity of stone quarries.

Dust pollution decreased the amounts of phenolics in the leaves of blackberry, a medicinal plant, and increased the total flavonoid content and antioxidant capacity.

To protect the human species from various sources of air pollution, buffer zones, and plant curtains are sometimes established. We also recommend the introduction of such procedures if the source of pollution is in a protected area to reduce the impact between the polluter and the plant and animal species of interest in the protected area. Quarries should be obliged to ensure a buffer space around them.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

All authors contributed to the study's conception and design. AP-V, DNP, DC, FNS, A-RD: material preparation and data collection; AP-V and DNP: morpho-anatomical and physiological analysis; SIV, OS and DNP: biochemical analysis; TOC and DNP: SEM micromorphology; DC: revised it critically for important intellectual content and approved the version to be published. AP-V wrote the first draft of the manuscript. All authors read, commented, and approved the previous and final versions of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main.

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Conservation In Practice

Building a global-oriented ecological civilization: Huzhou's actions and practice, China

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Abstract

Biodiversity, ecosystems and the services they provide are crucial to the sustainable development of cities, the health and well-being of residents and the maintenance of urban ecological security. The continued decline of global species biodiversity and ecosystem service function has seriously affected the sustainable development of regional social economy. The core of ecological civilization thought is sustainable development, and promoting sustainable development is also the path and means to achieve ecological civilization. As the significant birthplace of ecological civilization thought of "Lucid waters and lush mountains are invaluable assets", Huzhou has always been committed to creating an "important window" for building an ecological civilization and actively integrating biodiversity conservation into ecological conservation. Through unremitting efforts in recent decades, Huzhou has successfully achieved green sustainable development and reversed the trend of biodiversity loss. Thus, there is a need for a systematic review of successful initiatives in this region and identify the experiences and methods that can advance the sustainable development also in other parts of the world.

Key words: Biodiversity conservation, ecological civilization, Huzhou City, sustainability, sustainable development goals (SDGs)

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Introduction

The second phase of the 15th Conference of the Parties to the United Nations Convention on Biological Diversity (CBD COP15) was held in Montreal, Canada from December 7 to December 19, 2022. During the conference, Huzhou City, in Zhejiang Province, Eastern China (Fig. 1), not only won the title of the world's only "International Cooperation Demonstration Zone of Ecological Civilization" (State Council of the People's Republic of China 2022a, b) but also was recognized as "the Charming City of Biodiversity" and "the Natural Urban Platform". As the significant birthplace of ecological civilization thought of "Lucid waters and lush mountains are invaluable assets" (Zhai et al. 2019), Huzhou has always been committed to creating an "important window" for building an ecological civilization and actively integrating biodiversity conservation into ecological conservation. At the same time, Huzhou has been striving to achieve the goal of green and sustainable development and promoting the harmonious coexistence between human and nature. Therefore, it can be said that Huzhou has provided an action plan and practical experience for the world to realize the coordinated development of economy, society and ecological environment.

Biodiversity, ecosystems and the services they provide are crucial to the sustainable development of cities, the health and well-being of residents and the maintenance of urban ecological security (Naeem et al. 2012; Folke et al. 2016; Wood et al. 2018). Now, more than 50% of the world's population lives in urban areas (Benz et al. 2021), and rapid urbanization will lead to the loss and fragmentation of natural habitat to a certain extent (Li et al. 2022; Aguilera and González 2023), thus aggravating the risk of biodiversity loss and ecosystem degradation, which will pose a major threat to human survival and development (Cobbinah et al. 2015; Henle et al. 2016; Gao and Zhang 2020). As one of the world's most biologically diverse countries (Liu et al. 2013), China has formulated a national strategy and action plan for biodiversity conservation in the new era, included biodiversity conservation plans in important economic and social development plans, and integrated biodiversity conservation with the whole process of ecological civilization. At present, many cities in China are developing an ecological civilization and have achieved a certain degree of effectiveness. For example, in addition to Huzhou, there are five cities in China (Kunming, Yunnan Province; Chengdu, Sichuan Province; Nanyang, Henan Province; Jiaxing, Zhejiang Province; Shenzhen, Guangdong Province) that have won the title of "Biodiversity Charming City". It is reported that the selection for the honor took the Singapore Index on Cities' Biodiversity into consideration, recommended by the Secretariat of the Convention on Biological Diversity. However, the effectiveness of biodiversity conservation and sustainable development combined with building an ecological civilization is still not fully summarized and discussed.

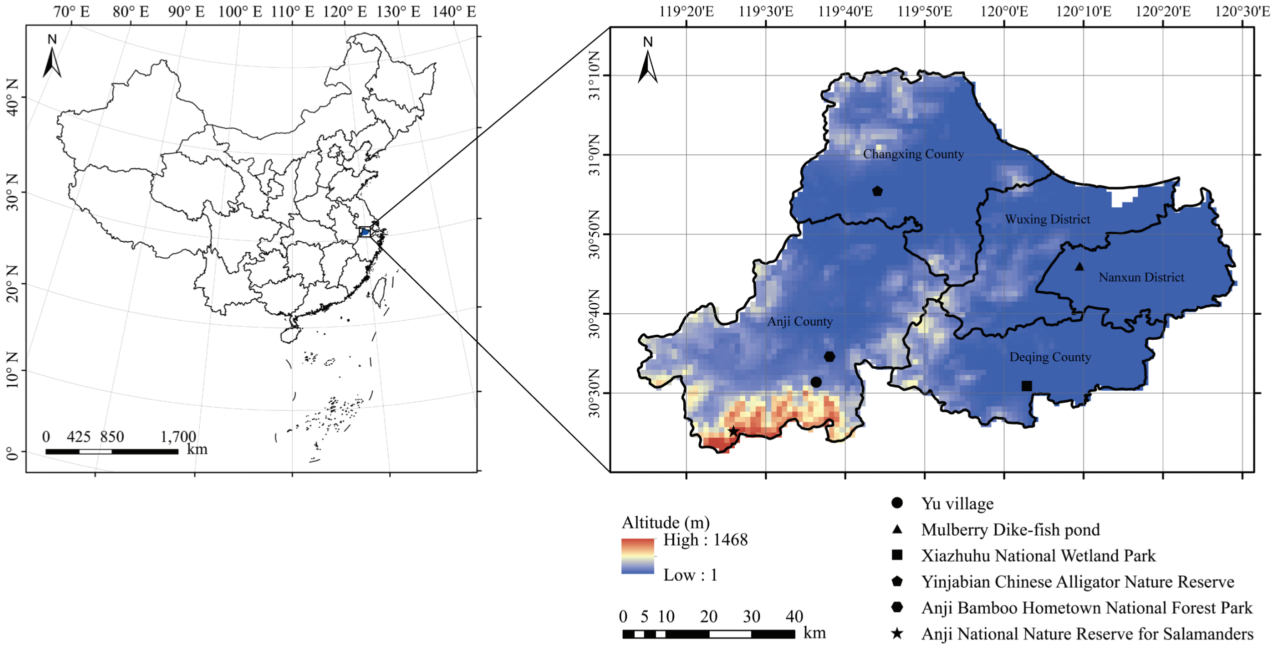

Figure 1. Location of the study area. (Left) Huzhou is located in the eastern part of China. (Right) The administrative divisions of Huzhou and the distribution location of typical cases of ecological civilization (the location of the center point).

For Huzhou, its 5820 km^2 of land is home to 3.41 million permanent residents (by the end of 2022). In fact, nighttime lighting data can be a good indicator of the level of urbanization and intensity of anthropogenic activities. According to the annual data distribution of nighttime lighting (Fig. 2), the urbanization process in Huzhou has been accelerating in the last decade (Pan et al. 2015). However, Huzhou has still made remarkable achievements in building an ecological civilization and biodiversity conservation (Huzhou People's Government 2022a). Therefore, it is essential to summarize and analyze the typical cases and achievements of Huzhou in promoting ecological civilization and biodiversity conservation in recent years. Meanwhile, it is of great significance to put forward how Huzhou should further promote ecological civilization and deepen international exchanges and cooperation in the new period, so as to leverage Huzhou's strengths and pilot experiences in realizing the 2030 goals of global biodiversity conservation and global sustainable development.

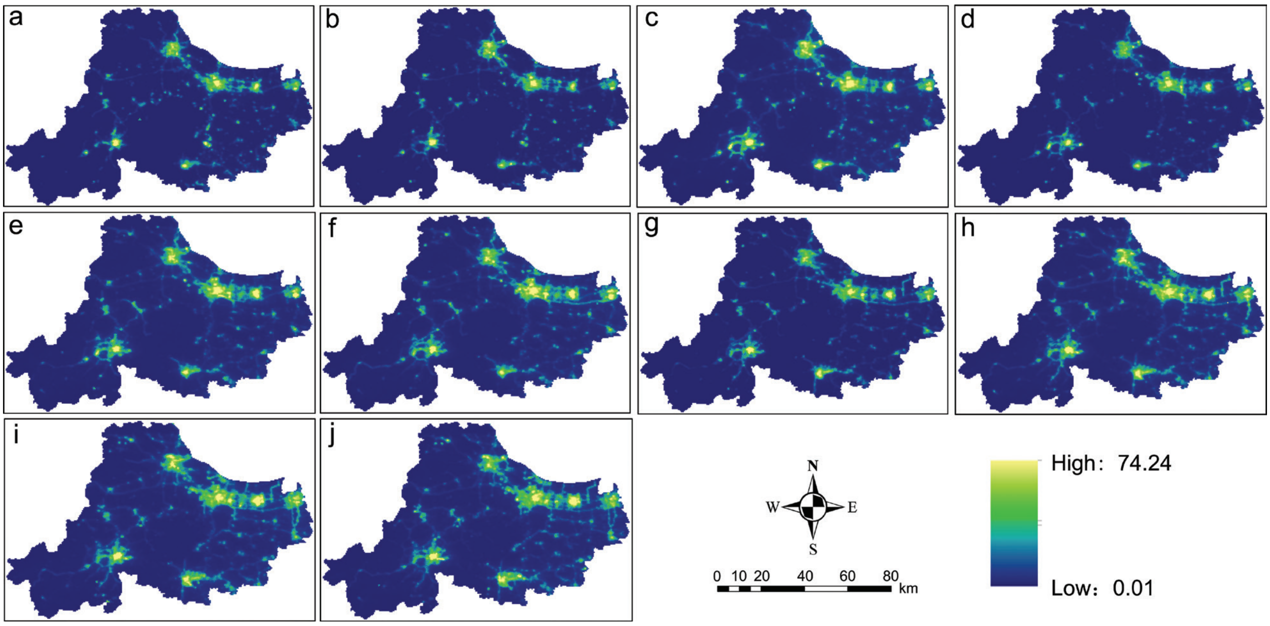

Figure 2. Changes of nighttime light data of Huzhou, 2012–2021. a-j represent the annual data distribution of nighttime lights in Huzhou from 2012 to 2021, respectively. Nighttime lighting data well reflects the degree of urbanization, intensity of human activities and the intensity of land development. The nighttime light data obtained from the Resource and Environment Science and Data Center (Xu 2022).

The concepts and a system of institutions for an ecological civilization in Huzhou of China

A civilization may thrive if its natural surroundings thrive, and will suffer if its natural surroundings suffer (Xi 2019). However, the earth's ecosystem is facing an unprecedented crisis, and the threat to global biodiversity continues to deteriorate, thus limiting global sustainable development. To effectively cope with the crisis, China has actively promoted ecological civilization, continuously enhanced the diversity, stability and sustainability of the ecosystem, and explored a unique road of biodiversity conservation and sustainable development.

Since 2012, China has emphasized ecological civilization as a long-term national strategy to promote sustainable development (Lu et al. 2017; Wu et al.

2019). Building an ecological civilization should focus on the harmonious coexistence between people and nature (Pan 2016; Wu et al. 2019). While human beings transform nature, they should also protect nature, and the harmonious and unified relationship between human and nature should be maintained (Ye 1984). In 2014, Huzhou was listed as the first prefecture-level ecological civilization demonstration zone (National Development and Reform Commission 2014). For more than a decade, Huzhou has always adhered to achieving harmony between people and nature, taking biodiversity conservation and sustainable development as essential components to ensure the progression of building an ecological civilization (Fig. 3). Specifically, Huzhou has promoted regional biodiversity conservation and sustainable development by incorporating the concepts of ecological sustainability and biodiversity conservation into the planning and management of the region's sustainable development. Furthermore, Huzhou has also used naturalness evaluation to guide regional biodiversity conservation and planning management (including strengthening the protection of animal and plant habitats, setting up and preserving biological corridors, etc.).

Sustainable development, being one of the most important development concepts in building an ecological civilization, has set its core goal of realizing the balanced development of ecology, economy and society, and striving to seek the

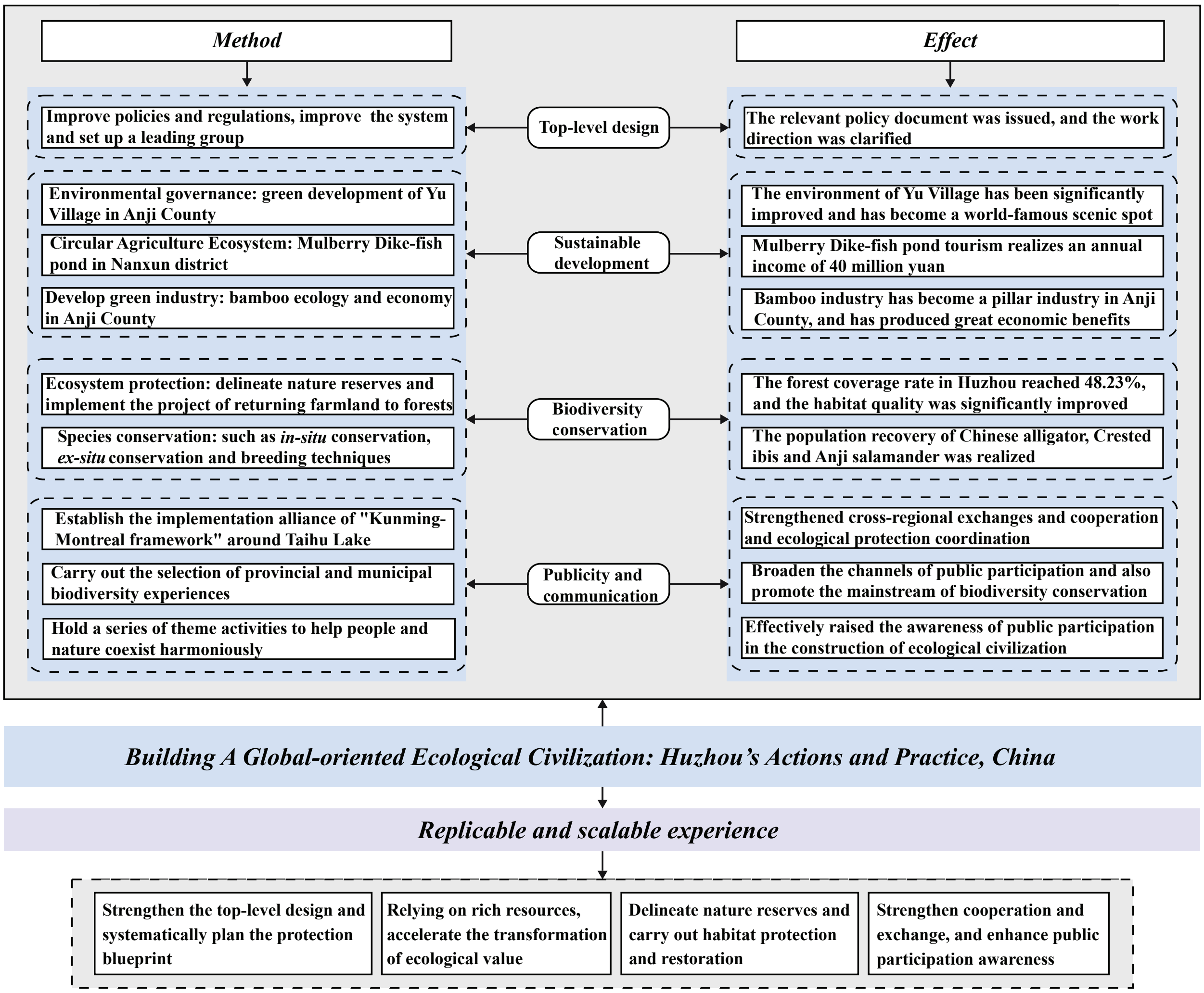

Figure 3. The framework of building an ecological civilization in Huzhou: method, effect and experience.

harmonious unity between maximizing the benefits from resource utilization and development and minimizing the impacts on natural environment. In addition, biodiversity conservation is intrinsically linked to the United Nations (UN) 2030 Sustainable Development Goals (SDGs), such as poverty reduction, food security and combating climate change. Therefore, protecting biodiversity also means promoting sustainable development. Simultaneously, biodiversity conservation is essential for the global realization of sustainable development goals. As an important foundation and carrier of enhancing ecological conservation (Yu 2022), biodiversity is an important evaluation index to measure the degree of developing ecological civilization (Ren and Guo 2021), and it plays an irreplaceable role in building an ecological civilization (Wang et al. 2020). Hence, scientific and effective biodiversity conservation needs to be carried out under the concept and framework of building an ecological civilization. In addition, to a great extent, the development of ecological civilization needs to be continuously promoted through biodiversity conservation and sustainable utilization (Fig. 4). Therefore, there is an inseparable internal relationship between biodiversity conservation and building an ecological civilization. Generally speaking, coordinating the concept and system of the harmonious development of people and nature is one of the important prerequisites for the success of building an ecological civilization in Huzhou.

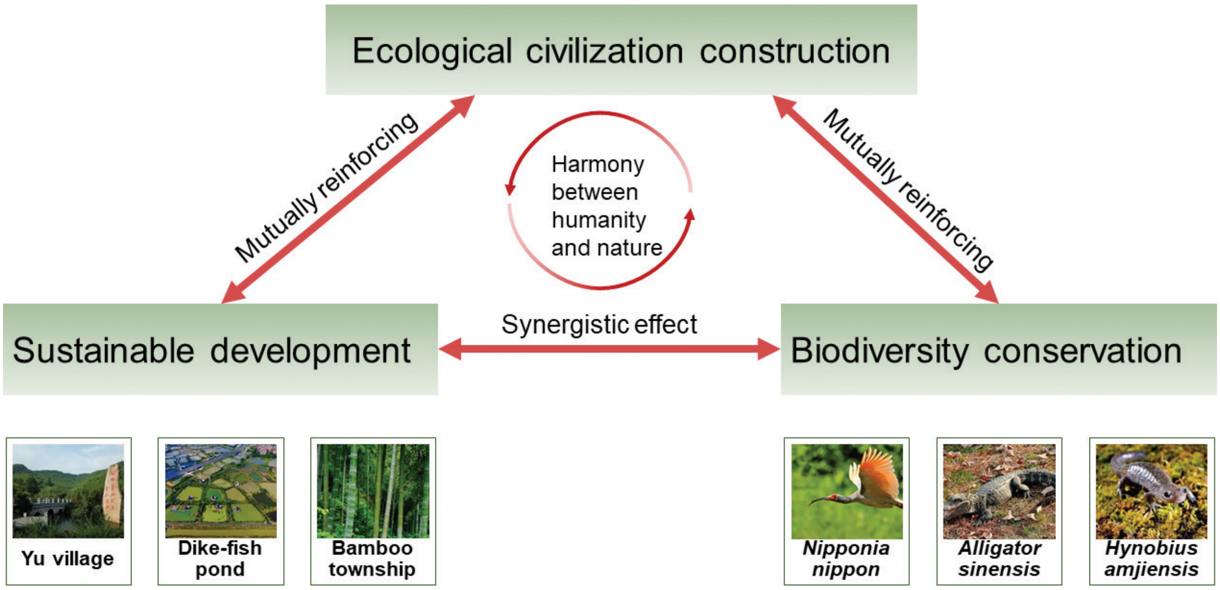

Figure 4. Sustainable development and biodiversity conservation promote the process of building an ecological civilization in Huzhou. In addition, typical cases of sustainable development include: green development of Yu Village in Anji County, Mulberry Dike-Fish Pond System in Nanxun district, bamboo ecology and bamboo economy in Anji County. Furthermore, typical cases of biodiversity conservation include: the population of *Nipponia nippon* in Deqing County, *Alligator sinensis* in Changxing County and *Hynobius amjiensis* in Anji County increased steadily.

Adhere to the priority of conserving resources, protection and natural recovery

In terms of resource conservation, Huzhou always adheres to green technology innovation and development (Ministry of Science and Technology of the People's Republic of China 2021), improves resource utilization, and reduces the discharge of production wastes. For example, the comprehensive utilization rate of crop straw in Huzhou has reached 97.49% (People's Daily Online 2022). In addition, Huzhou actively promotes the reform of the supply-side structure (Huzhou People's Government 2017), improves product quality, and extends product life. In the meantime, Huzhou also advocates that residents should keep a healthy lifestyle, prompt thrift and frugality, and reduce municipal solid waste (MSW) and waste discharge. For example, in 2021, the total amount of household waste in Huzhou was reduced by 3% compared to the previous year, and the recycling rate in urban and rural areas exceeded 60% (Huzhou People's Government 2022b). In terms of protection priority and natural restoration, Huzhou adheres to the concept of green development, strictly abides by the red line of ecological environment protection, leaving enough space and time for natural restoration. In addition, Huzhou insists on natural restoration, supplemented by artificial treatment, and changes post-treatment to pre-protection. In the process of construction and development, Huzhou has prioritized environmental protection, continuously strengthened the protection of forest resources, continuously promoted "land greening action", and increased the forest coverage area. By 2022, the forest coverage rate of Huzhou had reached 48.23% (Huzhou People's Government 2022c), achieving full coverage of provincial forest cities and towns, ranking the top in Zhejiang Province (Department of Forestry of Zhejiang Province 2022).

Establish a leading group for building an ecological civilization

To strengthen the overall planning and coordination of building an ecological civilization, Huzhou has set up the leading group office to carry out the substantive operations and promote the reform and innovation of building an ecological civilization. The leading group is headed by the main leaders of the municipal party committee and government, and regularly schedules the progress of projects for developing ecological civilization. In addition, Huzhou has also issued supporting measures to investigate the responsibility of government leaders for ecological environment damage, and to further strengthening the leadership's responsibility for the protection of ecological environment and resources.

Develop a system of institutions for an ecological civilization

Huzhou advocates the conservation and sustainable use of biodiversity in the whole process of building an ecological civilization, and actively promotes biodiversity conservation as a significant component of economic and social development planning. Furthermore, Huzhou also insists on taking the natural carrying capacity as the basis, accelerates the formation of green development model, advocates green and low-carbon life, and jointly promotes high-quality development and high-level biodiversity conservation. In terms of system development, Huzhou has successively issued a series of local policies and regulations, including the "Regulations on Building an Ecological Civilization Pilot Demonstration Zone in Huzhou" (Huzhou People's Government 2016), the "Action Plan for Ecological Restoration and Biodiversity Conservation of Important Water Systems in Huzhou (2021–2025)" (Huzhou People's Government 2022d) the "Implementation Opinions on Financial Support for Biodiversity Conservation" (Huzhou People's Government 2022e), and the "Action Plan for

Comprehensively Strengthening Biodiversity Conservation (2022–2025)”. In addition, Huzhou has also completed the preparation of the “Specification for Evaluation of Biodiversity Conservation and Sustainable Development Base”. The introduction of a series of policies will help to promote the mainstreaming of biodiversity conservation in Huzhou, and meanwhile, will help to promote people’s deeper understanding and participation in developing an ecological civilization. However, there are also various problems such as the disconnection between environmental policies and regional economic development policies. Hence, the complementarity and coordination between various environmental policies need to be strengthened. In this regard, Huzhou officially released the “Regulations on Promoting the Construction of Ecological Civilization Model City in Huzhou” on April 19, 2024 and it will be formally implemented on May 1, 2024. This regulation makes clear provisions for green low-carbon development, ecological protection and restoration, and the realization of the value of ecological products. It is worth noting that this regulation is also the first one in China to promote the construction of an ecological civilization model city.

Actions and effects of sustainable development in Huzhou

Huzhou plays an important role in ecological protection and ecological barrier in Taihu Lake Basin and Yangtze River Delta of China. Ecological advantage is the biggest advantage of Huzhou and also the most valuable resource of Huzhou. As a leading demonstration zone for building an ecological civilization in China, Huzhou has always adhered to the principle of the unity of biodiversity conservation and economic and social development, and has achieved remarkable results. In addition, Huzhou has also given full play to its own ecological advantages and embarked on a path of sustainable development featuring ecological beauty, industrial prosperity and people’s wealth. As the first city-level ecological civilization demonstration area in China, Huzhou unswervingly practices the concept that “Lucid waters and lush mountains are invaluable assets”, and deeply implements the strategy of “ecological poverty alleviation”. Furthermore, Huzhou has explored a new development path of mutual promotion between economic and social development and ecological environmental protection through various models, such as the development of characteristic biological resources, enterprise cooperation and co-construction, and cooperative trusteeship and sharing. Next, we will introduce three typical successful cases in the process of building an ecological civilization in Huzhou, and also show the world the beauty of green mountains, clean waters and the road of ecological prosperity.

Environmental governance: Green development of Yu Village in Anji County

Yu Village is a small mountain village in Anji County of Huzhou City, which is located in the northern mountain area of Zhejiang Province (Zhang et al. 2021). This is the earliest practical sample of beautiful rural construction in China, and also one of the earliest areas in Zhejiang to develop “farmhouse enjoyment”. However, before 2005, Yu Village was mainly engaged in limestone mining (Zhang et al. 2021). Although the annual net income of the village was more than 1–2 million Yuan, the ecological environment had been seriously damaged.

Sand and stones flew over the bamboo forest, and the water in the river turned into white mud. From bitter experience, the villagers of Yu Village decided to close the hillsides for afforestation and environmental protection. In 2003, Zhejiang Province started the project of “Demonstration of Thousands of Villages and Renovation of Ten Thousand Villages” in the whole province, which promoted the construction of rural living environment involving tens of millions of farmers. Since then, the industrial development of Yu Village has undergone a transformation. From 2003 to 2005, the village closed mines and cement plants in succession, began to change its development path, unswervingly practiced the concept that “Lucid waters and lush mountains are invaluable assets”, and integrated the concept of building an ecological civilization into the reform and development of small towns (Zhang et al. 2021). In the past 20 years, villagers have been committed to protecting green mountains and clean waters.

Through the construction of scenic spots, the development of homestays, farmhouses, and other tourism projects, Yu Village has established a new era of rural governance model and promoted the overall revitalization of the countryside. Ultimately, Yu Village in Anji County has changed from a village with serious ecological damage and environmental pollution to a beautiful village (Fig. 5), surrounded by green mountains and dense forests. It is reported that in 2022, the annual village collective economic income of Yu Village reached 13.05 million Yuan, the per capita net income of 64,000 Yuan, and the operating income exceeded 8 million yuan (People’s Daily Online 2023), which has been greatly improved compared with 20 years ago. At present, Yu Village has been recognized by the United Nations World Tourism Organization (UNWTO) as one of the world’s best tourist villages and has become a world-famous scenic spot (Fig. 5).

Figure 5. The comparison between Yu Village’s past and present. (Left) The ecological environment is seriously damaged and the air quality is poor. (Right) Beautiful scenery and excellent ecological environment.

Circular agriculture ecosystem: Mulberry Dike-Fish Pond System in Nanxun district

Huzhou, located in the south of China, has an important agricultural cultural heritage and a typical ecological breeding mode of agricultural circular economy (Wang et al. 2018). In November, 2017, Huzhou Nanxun’s “Mulberry Dike-Fish Pond System” was officially recognized as one of the Globally Important Agricultural Heritage Systems (GIAHS) by the Food and Agriculture Organization of the United Nations (FAO) (FAO 2017a). The Mulberry Dike-Fish Pond System is located in the west of Nanxun District, with a total area of about 6,900 hectares. It originated more than 2,500 years ago in the Spring and Autumn

Period and the Warring States Period (FAO 2017b). As an important agricultural cultural heritage and a typical ecological breeding mode of agricultural circular economy in China, it makes full use of the land and creates a composite agricultural production model. By combining mulberry planting, sericulture and fish farming, a mode of digging ponds for fish farming has been created, which uses pond sludge to fertilize mulberry, sericulture with mulberry leaves, feed fish with silkworm feces, and recycle agricultural ecosystem (FAO 2017b; Wang et al. 2018) (Fig. 6). In order to promote the better inheritance and utilization of traditional farming culture in green and high-quality development, Huzhou has always followed the principle of "protection priority, appropriate utilization, multi-participation, and benefit sharing", based on farming culture and ecological agriculture, combined the protection and development of "Mulberry Dike-Fish Pond System" with modern agricultural development, and assisted the construction of beautiful countryside and the building of an ecological civilization. Through the restoration and protection of Nanxun's original ecological "Mulberry Dike-Fish Pond System", it has become a new way for traditional aquaculture to develop in depth, promoting villagers to increase income and becoming a new tourist attraction (Fig. 6). In recent years, the Mulberry Dike-Fish Pond System has gradually become an important brand and a new tourist attraction of rural tourism in Huzhou. It can receive more than 1 million tourists and realize about 40 million Yuan of tourism income each year, leading the villagers to the road of ecological prosperity and common prosperity (Fig. 6).

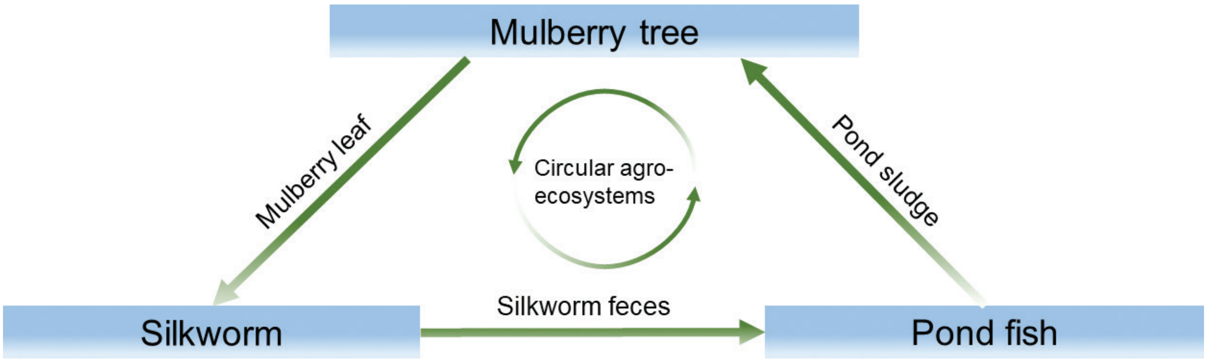

Figure 6. A schematic diagram of ecological cycle of Mulberry Dike-Fish Pond System in Huzhou.

Develop green industry: Bamboo ecology and bamboo economy in Anji County

Anji has a high-quality and beautiful ecological environment, for instance, 75% of the county's land is covered by forest. In addition, it has been considered as a model for achieving sustainable rural development through the bamboo industry (Flynn et al. 2017). There are more than 500 wild bamboo species in China, and more than 360 in Anji County, which is home to the largest bamboo variety in the world (Department of Forestry of Zhejiang Province 2020). In addition, there are 179 species of monopod bamboo, accounting for 71.6% of the total number of its species in the world (Flynn et al. 2017). The existing bamboo forest area of Anji is about 674 km^2 (Huang et al. 2022), accounting for 36% of the total area of Anji County (1886 km^2). Anji is rich in bamboo production, ranking first in China. In addition, the Bamboo Hometown National Forest Park is also located in Anji County, with a total area of 114.56 km^2 (Wang et al. 2022). Based on this, Anji relies on bamboo resources to promote the sustainable development of bamboo industry. Furthermore, Anji has also taken appropriate management interventions (such as improving the drought resistance of bamboo forests) to carry out ecological management of bamboo forests, maintain the health of bamboo forests, and promote the scientific utilization and ecological recovery of bamboo resources, which is of great significance for ensuring the ecological security and sustainable economic development of bamboo forests. Anji County adheres to the principle of industrial integration and green development, gives full play to its industrial advantages, takes into account the ecological, social, and economic benefits of the bamboo industry, and constantly promotes the development of bamboo tourism towards globalization, uniqueness, and high-quality, so as to maximize the value of ecological advantages. Furthermore, taking advantage of the bamboo industry, Anji has also vigorously developed various derivative industries (such as food, beverages, handicrafts, etc.) to form more than 700 varieties of products (Chen et al. 2011). In addition, Anji has also taken beautiful countryside of China as a big platform for the development of “bamboo tourism”, which has promoted the integrated development of the bamboo industry and other industries, and truly achieved the goal of increasing income with bamboo. Specifically, nearly 60% of Anji’s revenues come directly from the bamboo industry (Flynn et al. 2017). In addition, Anji has also innovatively promoted the reform of “bamboo forest carbon sink”, opened up the channels of bamboo forest carbon sink from production to collection, storage and trading, and realized that the carbon sink can be measured, mortgaged, traded and realized. At present, the bamboo industry has become a pillar industry and a key economic growth point in Anji (Chen et al. 2011). Therefore, the fast-growing bamboo not only opens a new path for Anji, but also finds a good balance between ecological civilization and economic development (Fig. 7).

Figure 7. Bamboo landscape and bamboo products of Anji Bamboo Township. (Left) Bamboo forest landscape and footpath. (Right) bamboo handicrafts.

Actions and effects of biodiversity conservation in Huzhou

Biodiversity is not only the basis of human survival and development, but also the blood and foundation of the life community on earth. Moreover, biodiversity conservation is one of the core issues of the United Nations Sustainable Development Goals (SDGs) (Reyers and Selig 2020). In 2020, Chinese State Council approved the construction of an innovation demonstration zone for the National Sustainable Development Agenda in Huzhou, aiming to provide practical experience for the implementation of the 2030 Agenda for Sustainable Development (State Council of the People's Republic of China 2022a, b). Biodiversity conservation is necessary for realizing China's new concept of an ecological civilization (Lu et al. 2017; Wu et al. 2019). Accordingly, Huzhou has always adhered to the principle of giving priority to protection and natural restoration, and followed the laws of nature. In order to achieve the goal of promoting the formation of a new pattern of harmonious coexistence between people and nature, Huzhou has also actively explored natural-based solutions (Nbs) to avoid excessive human intervention, effectively protected important ecosystems, biological species and genetic resources, and striven to be at the forefront of biodiversity conservation in the country and even in the world. For the past few years, Huzhou has continued to promote the construction of "Forest Huzhou", actively carried out land greening actions, and made efforts to improve the forest stock and forest "carbon fixation" capacity. By 2022, the forest coverage rate of Huzhou had reached 47.55%, and two national forest parks have been built. In addition, Huzhou also attaches great importance to strengthening the construction and management of nature reserves. At present, two national nature reserves and three national wetland parks have been built, with a wetland protection rate of more than 54%. As of 2022, there are more than 2,200 species of wild higher plants, nearly 600 species of wild vertebrates and nearly 2,500 species of insects in Huzhou. Furthermore, Huzhou is not only rich in biodiversity resources, but also has many rare and endangered species. In the following, the Crested ibis, Chinese alligator and Anji salamander will be taken as examples to introduce the achievements of Huzhou in protecting these rare and endangered species, hoping to provide reference and inspiration for global biodiversity conservation.

Ex-situ conservation: Population reconstruction of Crested ibis

Crested ibis (*Nipponia nippon*) is a globally endangered (EN) bird, endemic to East Asia, and a national key protected wild animal (National Forestry and Grassland Administration and Ministry of Agriculture and Rural Affairs 2021; IUCN 2023) (Fig. 8). Due to the loss of habitat, the wild Crested ibis was seriously endangered and almost extinct in the mid-20th century. In 1981, the last seven Crested ibis in the world were found in Yangxian County of Shaanxi Province. Meanwhile, Huzhou was once the home of Crested ibis, but environmental degradation and habitat destruction led to the extinction of Crested ibis in the late 1950s. In order to rebuild the wild Crested ibis population, at the end of 2007, the Crested ibis group reconstruction and wild release project applied by Deqing County was declared to be approved. In addition, the Xiazhuhu National Wetland Park in Deqing County has been selected as a conservation area for crested ibis due to its unique ecological environment. By taking manual assistance measures and conducting physiological research, the problem of the low fertilization rate was solved well, and the environmental adaptability and reproduction ability of Crested ibis were finally improved. Based on the latest monitoring data, the population size of Crested ibis in Deqing of Huzhou has increased from five pairs in 2008 to 669 individuals in 2022, including 287 wild individuals (National Forestry and Grassland Administration 2022).

Figure 8. Crested ibis were released to the wild.

In-situ conservation: Population reconstruction of Chinese alligator

Chinese alligator (*Alligator sinensis*) is a critically endangered (CR) species, and a national key protected wild animal (National Forestry and Grassland Administration and Ministry of Agriculture and Rural Affairs 2021; IUCN 2023) (Fig. 9). Chinese alligators were once distributed in vast areas of China (Wang et al. 2021). However, due to climate change, human hunting and habitat destruction, the number of Chinese alligators has decreased sharply (Wang et al. 2021). At present, they are only distributed in the middle and lower reaches of the Yangtze River. Most of them live in Changxing Chinese Alligator Provincial Nature Reserve and Anhui Chinese Alligator National Nature Reserve. In 1979, Yinjiabian Chinese Alligator Nature Reserve was established in Changxing and upgraded to a provincial nature reserve in 2007. Since then, the reserve has also carried out a lot of scientific research and practical work on the breeding and conservation of Chinese alligators. In order to ensure the reproduction of the Chinese alligator population, there were three major problems in the early stage of the establishment of the reserve: (i) Adult Chinese alligators swallowed dozens of young crocodiles at one time when they are hungry; (ii) The winter cold resulted in a survival rate of hibernating young crocodiles of only about 30%; (iii) Fierce fighting among adult Chinese alligators often led to the death of young crocodiles by crushing their eggs. Therefore, the reserve actively raised funds, built breeding ponds, adult ponds, sub-adult ponds and overwintering ponds for young crocodiles, and purchased and completed the electronic chip marking of adult Chinese alligators, so that managers could accurately record the reproductive behavior of females and males. After more than 40 years of development, the number of Chinese alligators has increased from 11 in early 1979 to more than 9,000 in the reserve (Xinhuanet Zhejiang 2022).

Figure 9. Chinese alligator in the Yinjiabian Chinese Alligator Provincial Nature Reserve in Changxing, Zhejiang province.

In-situ conservation: Population increase of Anji salamander

Anji salamander (*Hynobius amjiensis*) is a globally endangered (EN) species. This species is an amphibian unique to China and also a national key protected wild animal (Fig. 10). Anji salamander is mainly found in the peat layer under the cover of alpine *Sphagnum palustre* at altitudes above 1300 m (Yu et al. 2022). In recent years, due to the deterioration of the ecological environment, global warming, increased human interference and other reasons, the living space of Anji salamander is gradually shrinking. In addition, their population renewal ability is relatively weak, and their population continuity is faced with huge survival pressure, even putting it on the verge of extinction. Based on the Longwangshan Provincial Nature Reserve established in 1985 (mainly for protecting deciduous broad-leaved forest), the Anji National Nature Reserve for Salamanders was officially established in 2017, focusing on the protection of Anji salamander, while protecting other rare and endangered animals and plants (such as *Muntiacus crinifrons*, *Neofelis nebulosi*, and *Shaniodendron subaequale*). By constructing artificial breeding population, monitoring and protecting the survival and breeding habitat, the population of Anji salamander has been restored. Anji salamander was first discovered in 1992. The wild population and survival rate of Anji salamander have increased in recent years due to conservation efforts. The latest monitoring data shows that today, after 30 years of protection, their wild population has exceeded 500 (China Central Television 2022). In addition, their survival rate has increased from less than 5% 20 years ago to 70% now (China Network 2022).

Figure 10. Anji salamander and its artificial simulated habitat.

Publicity and communication practice of building an ecological civilization in Huzhou City

Strengthen cooperation and exchange: establish the implementation alliance of “Kunming-Montreal framework” around Taihu Lake

Huzhou took the opportunity of building an “International Cooperation Demonstration Zone of Ecological Civilization”, and actively established the implementation alliance of “Kunming-Montreal framework” around Taihu Lake (Fig. 11). This also indicates that the four cities around Taihu Lake (Huzhou, Wuxi, Suzhou and Changzhou) will make concerted efforts to jointly protect and govern the area, and jointly draw a beautiful picture of the harmonious coexistence of man and nature around Taihu Lake. In addition, Huzhou officially established the Huzhou Center of Biodiversity Conservation in 2023, and actively carried out international exchanges, brand communication, personnel training, etc., with a view to telling the international community a good story about building an ecological civilization.

Promote the construction and selection of biodiversity experience site

In order to build a demonstration window for biodiversity conservation, Huzhou fully taps local characteristic resources, actively builds a unique biodiversity experience site and a natural education base, and highlights the diversified functions of the base such as ecological protection and popular science education (Fig. 11). Up to now, Huzhou has selected 2 provincial and 16 municipal biodiversity experience sites, which is of great significance for broadening public participation channels and promoting the building of ecological civilization.

Hold a series of thematic publicity activities

In order to strengthen the publicity of biodiversity to the public, Huzhou has made full use of “International Biodiversity Day”, “World Environment Day”, and “National Ecological Day” to carry out thematic activities such as popular science lectures as well as publicity and education (Fig. 11), so as to unblock the ways and channels for the public to obtain knowledge related to ecological civilization, and improve the awareness and enthusiasm of the public for participating in building an ecological civilization.

Figure 11. Publicity activities held by Huzhou in the process of building an ecological civilization. (Upper left) The practical alliance of “Kunming-Montreal framework” around Taihu Lake was established. (Lower left) Theme activities of “International Biodiversity Day”. (Right) Popular science education activities in biodiversity experience areas.

Enlightenment and summary of building an ecological civilization in Huzhou City

Insights into how other cities achieve the same goal

Currently, human beings are facing many global ecological problems such as climate change, biodiversity loss, and environmental pollution, which pose serious challenges to human survival and development. In the context of the United Nations Sustainable Development Goals (SDGs), Huzhou has made significant contributions to global biodiversity conservation and green sustainable development through a series of ecological civilization building activities. Specifically, Huzhou has carried out a series of specific actions, such as launching the bamboo forest carbon sink reform, exploring Gross Ecosystem Product (GEP) accounting and standardizing the operation of the “two mountains” cooperative (Huzhou People’s Government 2023). These activities will help to improve global biodiversity conservation, promote green development and realize the United Nations Sustainable Development Goals (SDGs). There is no doubt that the case and method of building an ecological civilization in Huzhou can provide profound insights for other cities to achieve the same goal.

The case study of Huzhou we have chosen promotes the ecological civilization and sustainable development globally through practical actions such as green and low-carbon development, resource recycling, and biodiversity conservation. The reason why Huzhou has made many achievements in ecological civilization and sustainable development is due to top-level design and policy support, which has resolved the contradiction between ecological environmental protection and economic development well (Fig. 3). If other regions in the world want to achieve green and sustainable development like Huzhou, it is very important for the government to formulate a top-level design that is actively suitable for local development and promote its implementation. In addition to the guidance of government departments, it is also very important to improve public awareness and participation and enhance their ecological conscience (Trochet and Schmeller 2013). In addition, Huzhou abides by nature’s laws, explores solutions predicated on protecting nature, and avoid excessive human intervention in nature. According to the data released by Huzhou Natural Resources and Planning Bureau in 2023, the area of nature reserves in Huzhou currently accounts for more than 5.7% of the city’s land area. Therefore, actively delineating nature reserves and carrying out habitat protection and restoration are also among the ways to replicate the successful experience of Huzhou. In this regard, the integration of biodiversity conservation in spatial planning will provide more basis for the green development of cities, and will also be beneficial to urban planners in other cities in China and around the world. Finally, while actively relying on existing resources to transform ecological value, other cities also need to promote rational use of resources and reduce waste, and improve public awareness and participation, which also plays a very important role in promoting the construction of ecological civilization to achieve sustainable development. Significantly, Huzhou has always prioritized environmental conservation and restoration of nature. Therefore, for Chinese and global policy makers, it is important to change their mindset, actively transform ecological advantages into development advantages, and promote the development of the green energy industry through “government-enterprise cooperation” to promote a virtuous cycle of conservation and development.

Mission and responsibility of Huzhou in the new era

In recent years, Huzhou has achieved remarkable results in the process of building an ecological civilization and biodiversity conservation. This time, Huzhou was recognized by COP15 as the “International Cooperation Demonstration Zone of Building an Ecological Civilization”, which also gave Huzhou a new mission and task. The Kunming-Montreal Global Biodiversity Framework, adopted during the second phase of COP15, proposed to promote sustainable development and protect biodiversity as the core objectives. Biodiversity conservation is not only an important part of building an ecological civilization, but also related to the survival, development, and future of human beings. In the next step, under the guidance of the Ministry of Ecology and Environment, Huzhou will closely communicate with the secretariat of the United Nations Convention on Biological Diversity, to study and formulate future-oriented local action plans for biodiversity conservation and ecological civilization around the implementation of the Post-2020 Global Biodiversity Framework. To be more specific, Huzhou will continue to increase the protection of important ecosystems, species and biogenetic resources in the future, and strive to create a new pattern of harmonious coexistence between humans and nature. In addition, Huzhou will also strive to build a world-renowned demonstration zone for international cooperation in ecological civilization, and strive to explore the best cases and demonstration samples for the systematic protection and utilization of biodiversity.

The Kunming-Montreal Global Biodiversity Framework notes that achieving the global targets of sustainable development and biodiversity conservation by 2030 requires concerted actions and cooperation between governments. Therefore, it is necessary to strengthen international exchanges and cooperation and constantly promote the improvement of institutional mechanisms. In the future, Huzhou will also draw a blueprint for its future from the perspective of building an ecological civilization and the harmonious coexistence between human and nature, actively carry out global action, global response and global cooperation, and promote the improvement of a fair, reasonable and win-win global biodiversity governance system. Furthermore, Huzhou will also continue to follow the sustainable development path of ecological beauty, green industry and prosperity of the world. In addition, Huzhou should not only promote the green transformation of its own economic and social development, but also actively contribute to the global low-carbon transformation and contribute successful experience to the green development of the world. Specifically, Huzhou will further increase exploration and practice, and strive to form more learnable, replicable and promotable Huzhou experience in the aspects of institutional mechanism innovation, pilot demonstration construction, standard application and promotion, etc., and make efforts to create a number of replicable green development demonstration cases for China and the world. Generally speaking, Huzhou will always be committed to making greater contributions to the joint construction of a community of the earth’s life and a clean and beautiful world, and strive to realize the beautiful vision of coexistence between human and nature.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization, P.Y. and J.W, investigation, P.Y. and J.W, data curation, P.Y. and J.W, writing—original draft preparation, P.Y, writing—review and editing, P.Y. and X.Z, visualization, P.Y. and X.Z, supervision, J.W, project administration, J.W, funding acquisition, J.W. All authors have read and agreed to the published version of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text.

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