Nature Conservation 29: 1–26 (2018)
doi: 10.3897/natureconservation.29.24856
http://natureconservation.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Nature Conservation
Launched to accelerate biodiversity conservation

Effects of set-aside management on certain elements of soil biota and early stage organic matter decomposition in a High Nature Value Area, Hungary

Zsolt Tóth[1,2], Elisabeth Hornung[1], András Báldi[3]

1 *Department of Ecology, Institute for Biology, University of Veterinary Medicine Budapest, Rottenbiller u. 50, H-1077 Budapest, Hungary* **2** *Doctoral School of Environmental Sciences, Faculty of Agricultural and Environmental Sciences, Szent István University, Páter K. u. 1, H-2100 Gödöllő, Hungary* **3** *Lendület Ecosystem Services Research Group, Institute for Ecology and Botany, MTA Centre for Ecological Research Alkotmány u. 2-4, H-2163 Vácrátót, Hungary*

Corresponding author: *Zsolt Tóth* (toth.zsolt@univet.hu)

Academic editor: *D. McCracken* | Received 6 March 2018 | Accepted 8 August 2018 | Published 29 August 2018

http://zoobank.org/0C5CC2C8-2409-4C09-A11C-8099859D6A11

Citation: Tóth Z, Hornung E, Báldi A (2018) Effects of set-aside management on certain elements of soil biota and early stage organic matter decomposition in a High Nature Value Area, Hungary. Nature Conservation 29: 1–26. https://doi.org/10.3897/natureconservation.29.24856

Abstract

Agricultural intensification is one of the greatest threats to soil biota and function. In contrast, set-aside still remains a management practice in certain agri-environmental schemes. In Hungary, the establishment of sown set-aside fields is a requirement of agri-environmental schemes in High Nature Value Areas. We tested the effects of set-aside management on soil biota (bacteria, microarthropods, woodlice and millipedes), soil properties and organic matter decomposition after an initial establishment period of two years. Cereal – set-aside field pairs, semi-natural grasslands and cereal fields were sampled in the Heves Plain High Nature Value Area in Eastern Hungary, in May 2014. Topsoil samples were taken from each site for physical, chemical, microbial analyses and for extraction of soil microarthropods. Macrodecomposers were sampled by pitfall traps for two weeks. The biological quality of soil was estimated by the integrated QBS index ("Qualità Biologica del Suolo", meaning "Biological Quality of Soil") based on diversity of soil microarthropods. To follow early stage organic matter decomposition, we used tea bags filled with a site-independent, universal plant material (*Aspalathus linearis*, average mass 1.26 ± 0.03 g). Tea bags were retrieved after 1 month to estimate the rate of mass loss. We found significant differences between habitat types regarding several soil physical and chemical parameters (soil pH, K and Na content). The study showed positive effects of set-aside management on soil biodiversity, especially for microarthropods and isopods. However, we did not experience similar trends in relation to soil bacteria and millipedes. There was higher intensity of organic matter decomposition in soils of set-aside fields and semi-natural grasslands (remaining mass on average: 74.17% and 76.6%, respectively) compared to cereal fields (average remaining mass: 81.3%). Out of the biotic components, only the biological quality of soil significantly influenced (even if marginally) plant tissue decomposition. Our results highlight the importance of set-aside fields as shelter habitats for soil biota, especially for arthropods. Set-aside fields that are out of a crop rotation for 2 years could be a valuable option for maintaining soil biodiversity, as these fields may simultaneously conserve elements of above- and below-ground diversity.

Keywords

agri-environmental schemes, agrobiodiversity, detritivores, soil biological quality, tea bag method

Introduction

The European Union (EU) is one of the most intensive agricultural regions per unit of surface area in the world (Monfreda et al. 2008). Agriculture is a dominant form of land management in Europe, with 40% of the total land area of the EU 28 used for crop production and for grasslands (European Commission 2013).

Agroecosystems and agricultural landscapes provide important soil related ecosystem services, i.e. the maintenance of soil fertility and structural properties, filtering and providing reservoir for water, nutrient cycling and climate regulation (Dominati et al. 2010). Production and maintenance of healthy soils in agricultural areas are therefore key elements in the development of sustainable agriculture. The importance of soil communities (microbiota, meso- and macrofauna) contributing to a very diverse range of biochemical and biophysical processes has long been recognised (Barrios 2007). However, the decomposer subsystem and soil related ecosystem services are still poorly understood (Bardgett and Wardle 2010). Several studies have demonstrated the importance of soil fauna in the maintenance of soil fertility (e.g. Brussaard et al. 2007). Soil microarthropods [springtails (Collembola), mites (Acari), proturans (Protura), diplurans (Diplura), pauropods (Pauropoda), symphylans (Symphyla) etc.] millipedes (Diplopoda) and terrestrial isopods (Isopoda: Oniscidea) have essential roles in litter decomposition, nutrient mineralisation and the improvement of soil properties (Culliney 2013). In addition to earthworms, these organisms are responsible for the first steps in the decomposition processes by fragmentation and inoculation of dead plant material (Lavelle and Spain 2001). They promote litter breakdown through their feeding and burrowing activities that support microbial decomposition (Lavelle and Spain 2001). Given their importance in nutrient cycling, the lack of knowledge on how agricultural practices affect these taxa and their functions is striking. The soil biological activity that can be measured e.g. by litter decomposition rate, depends largely on the diversity of soil organisms (Hättenschwiler et al. 2005) and is the result of complex interactions (Scheu 2002).

Numerous studies have shown that agricultural intensification represents a major threat to soil biodiversity and to the provision of ecosystem services (e.g. Altieri 1999).

Local land use, microclimate, pH, landscape diversity and habitat structure influence the species richness and abundance of soil detritivores (Hopkin and Read 1992, Hopkin 1997, Warburg 1987). Plant species richness and plant community structure greatly affect the above-ground microclimate which has indirect effects on soil biota and on decomposition dynamics of substrates through their chemistry, physiology, rhizodeposition and the quantity, quality and diversity of litter (Dudgeon et al. 1990, Smith and Bradford 2003, Hättenschwiler et al. 2005, Tripathi et al. 2013).

The establishment of semi-natural habitats (grassy strips, sown or naturally regenerated set-aside fields, hedgerows, treelines etc.) in agricultural landscapes is a common practice to enrich habitat diversity or to connect isolated habitats (e.g. Critchley et al. 2004, Smith et al. 2008, Kovács-Hostyánszki et al. 2011, Morris et al. 2011). These green patches in arable landscapes support high biodiversity and provide suitable environmental conditions for several plant and animal species (Altieri 1999, Kovács-Hostyánszki et al. 2011, Tóth et al. 2016). The presence of set-aside fields contributes to a productive and ecologically balanced soil environment through improving soil properties necessary for plant health (activation of soil biology, addition of organic matter, N fixation, microclimate modification etc.). Moreover, these habitats may have important impacts on the adjacent cropping systems through spillover (e.g. Blitzer et al. 2012).

European agricultural policy has long relied on agri-environmental schemes (AES) to alleviate the negative environmental impacts of agricultural intensification (Batáry et al. 2015). The first set-aside scheme was introduced by the EU in 1988 to reduce of production surplus. However, despite the positive environmental effects, set-aside management was abolished in most EU countries because of increasing production demands (Rowe et al. 2009). In countries where set-aside still remains a management practice, it serves as an essential component of agri-environment schemes (Batáry et al. 2015). However, the effectiveness of such measures on soil biodiversity and function is still questionable.

Insight in conservation management in Central and Eastern European countries could be particularly valuable, as their agrobiodiversity is still high compared to Western Europe (Báldi et al. 2013). In Hungary, rotational set-aside management has been present as part of the national agri-environment scheme since 2002 (Ángyán et al. 2003). The maximum period of setting aside a given arable field is three years. Set-aside fields are generally sown with a seed mixture of grass and leguminous species (Ángyán et al. 2003).

The present study aimed to test the following hypotheses:

(i) set-aside management has profound effects on soil physical and chemical properties,
(ii) set-aside fields and semi-natural grasslands provide more favourable conditions for studied soil organisms compared to cereal fields,
(iii) plant tissue decomposition is higher in set-aside fields and semi-natural grasslands
(iv) decomposition rate is positively correlated with measures of soil biodiversity.

Methods

Study site

The study was conducted in the region of North-eastern Hungary (Heves County) in 2014 (see map in Suppl. material 5). About 72% of the land was under agricultural management (ca. 60% arable and 12% grasslands) (Bükk National Park 2018). The study area can be characterised by a continental climate with extreme high temperature and low precipitation in summer. The study sites belong to the Heves Plain High Nature Value Area (HHNVA), which was established in the framework of the zonal action schemes of the National Agri-Environmental Programme in 2002 and covers around 40 000 hectares (Ángyán et al. 2003). The grasslands were extensively mown or grazed, mainly by cattle and sheep and no chemicals were applied. The most dominant species were Kentucky bluegrass (*Poa pratensis*), Pseudovina (*Festuca pseudovina*) and meadow foxtail (*Alopecurus pratensis*). Establishment of set-aside fields was part of the arable farming action plan. The main crops were cereals, sunflower and oilseed rape. Farmers' fields had to be managed by regular crop rotation during the 5-year long contract period: cereal 20–25%, alfalfa 20–30%, oilseed rape and other crops (pea, sunflower, corn etc.) 25–30%, set-aside 20–25%. Fields could be taken out of production for 1–3 years. The set-aside fields were sown with a three component seed mixture comprised of two parts grass (e.g. *Festuca pratensis*, *Festuca arundinacea*, *Poa pratensis*, *Dactilys glomerata*) and one part leguminous species (usually *Medicago sativa*) after the last harvest, in the autumn. Vegetation was mown once a year, after the 15th of June, leaving the cut vegetation on site.

Study design

Within the study area, two-year-old set-aside fields (Sa) were chosen, each with an adjacent cereal field (CSa) with seven replicates (Figure 1). Six semi-natural grasslands (G) and six cereal fields without set-asides (C) were also assigned as controls for comparisons. All cereal fields involved in the study were managed similarly, fertilised with about 90 kg nitrogen/ha/year and sown with winter wheat (*Triticum aestivum*) and, in one case, barley (*Hordeum vulgare*). Grasslands were managed extensively, without fertiliser application and grazed or mown once per year. The mean area (± SE) of the study sites was 30.21 ± 3.93 ha. The paired set-aside and cereal fields were of similar size and relief (difference in the field area within pairs: mean ± SE 8.14 ± 1.85 ha).

Soil sampling and analyses

Soil was sampled randomly by taking five soil cores from 0–15 cm depth in May 2014. Before soil analyses, soil cores corresponding to each site were pooled to obtain a composite sample. Physical and chemical analyses of soils were carried out on air-dried

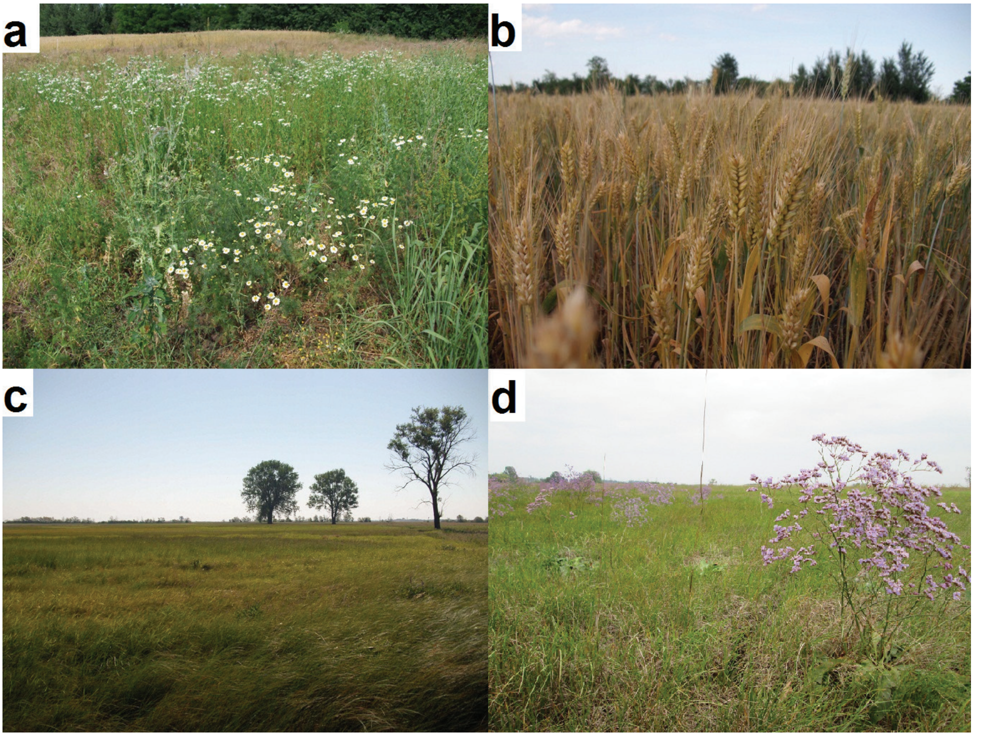

Figure 1. The main habitat types investigated in the Heves Plain High Nature Value Area, Hungary (**a** 2-year-old set-aside field **b** cereal field and **c–d** semi-natural grassland with a typical plant species, *Limonium gmelinii*).

samples from which crop residues, root fragments and rocks larger than 2 mm had been removed (MSZ 21470-2 1981). Soil plasticity, determined by the heaviness index according to Arany (K_A), concluded the soil physical condition and texture (MSZ 08-0205 1978). Soil pH was measured in 1 M KCl suspensions for 12 h after mixing (MSZ 08-0206-2 1978). Soil organic matter (SOM%) was determined using MSZ (Hungarian Standard) 08-0452 (1980). Total nitrite-nitrate nitrogen (NO_2-NO_3 N) was measured using a modified Kjeldahl method (MSZ 20135 1999). Plant-available phosphorus (P), potassium (K), sodium (Na) and magnesium (Mg) concentrations were extracted using AL (ammonium-lactate) (MSZ 20135 1999) and measured using inductively coupled plasma-atomic emission spectrometry (ICP-Thermo Jarrell Ash ICAP 61E). The total water soluble salt content was determined by MSZ (Hungarian Standard) 08-0206-2 (1978).

Microbial analyses: soil bacteria

To determine the effects of set-aside management on the bacterial community structure, composite soil samples were taken from each field in May 2014. Soil sampling locations (five subsamples per field) were randomly chosen from the upper surface (0–5 cm). DNA was isolated from samples with NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany). The quality of nucleic acids was assessed with 1% agarose gel electrophoresis stained with ethidium bromide. Nucleic acid quantification was undertaken with a Qubit 2.0 Fluorometer (Thermo Fischer Scientific, Waltham, MA, USA) throughout the study.

For bacterial community fingerprinting with the terminal restriction fragment length polymorphism (T-RFLP) method, 16S rDNA fragments were amplified with a 5' VIC-labelled 27F primer (VIC-5'-AGAGTTTGATCMTGGCTCAG-3') and a 518R primer (5'-ATTACCGCGGCTGCTGG-3'). Polymerase chain reactions (PCRs) were undertaken with 5 µl of DreamTaq PCR buffer, 0.3 µM of each primer, 0.2 mM of each dNTP, 30 ng DNA sample, 1 U DreamTaq DNA Polymerase (Thermo Fischer Scientific) and nuclease-free water up to the final reaction volume of 50 µl. Amplification conditions were as follows: 95 °C for 3 minutes, then 32 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s, then a final extension at 72 °C for 7 minutes. To obtain molecular fingerprints after the amplification, 16S rDNA amplicons were digested with the restriction enzyme, *Alu*I (AG↓CT) (Thermo Fischer Scientific) as described by Révész et al. (2006). Fragments were separated on a Model 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), whereas primary evaluation of electropherograms was carried out with GeneMapper 4.0 software (Applied Biosystems). T-RF peaks with a peak height below 100 relative fluorescence units or with a peak abundance contribution below 1% were excluded from further analysis. For consensus T-RFLP profiles, duplicate electropherograms from each sample were aligned with each other by the T-Align programme (Smith et al. 2005) with a 0.5 bp confidence interval. Subsequently, the consensus profiles were aligned with T-Align programme for all samples; this step helps to eliminate background noise and to separate T-RFs properly that differ in one to two bases only in terms of their length. In the resulting data matrix, relative peak area ratio was calculated by dividing each individual T-RF peak area by the total peak area of each profile. Subsequently, this matrix was used for statistical analysis of the T-RFLP data.

Microarthropods sampling and QBS index

Soil microarthropods were collected by taking undisturbed soil cores (8 cm in diameter, 400 cm^3, six per plot) to a depth of 15 cm at 0, 10 and 20 m from the field edge along a transect. In semi-natural grasslands, soil samples were taken at a distance of 10 m from each other and 2–300 m from the field edge.

Soil fauna was extracted using the Berlese-Tullgren funnel method. During a 12-day extraction period, microarthropods were collected and stored in vials containing 70% ethanol. All animals were counted under a dissecting microscope. Then they were classified into taxonomic groups and the QBS index ("Qualità Biologica del Suolo", meaning "Biological Quality of Soil") was calculated according to Parisi et al. (2005). This is an integrated soil biological quality index based on eco-morphological types of edaphic microarthropods. The QBS index is the sum of the EMI (eco-morphological index) scores that increases with the degree of microarthropods' adaptation to soil environment (Parisi et al. 2005). Its concept is that high soil quality is associated with the number of microarthropod groups well-adapted to soil habitat. The strength of this indicator is its sensitivity to land use change and to short term variations in management practices. However, it is less sensitive to large variations in some soil parameters, such as SOM (Parisi et al. 2005).

Macrodetritivore sampling: Diplopoda and Isopoda

Macrodecomposers were sampled by pitfall traps for two weeks in May 2014. Traps were set along a 20 m transect 0, 5, 10 and 20 m from the field edge. In semi-natural grasslands, traps were placed at a distance of 10 m from each other and 2–300 m from the field edge. We applied funnel traps filled with ethylene glycol. They were sunk directly into the soil and covered with plastic roofs to shield from rain. Pitfall traps were returned to the laboratory and, after sorting for subsequent species identification, the samples were preserved in 70% ethanol. Millipedes (Diplopoda) and isopods (Isopoda: Oniscidea) were identified to species level. For identification of millipedes, the keys of Schubart (1934) and Korsós (2015) and for isopods, the key of Gruner (1966) were used.

Plant tissue decomposition: tea bag method

To follow microbial degradation of organic matter, the novel litter quality independent tea bag method was used (Keuskamp et al. 2013). In each plot, four pyramid-shaped, synthetic tea bags (mesh size: 280–300 μm) filled with rooibos (*Aspalathus linearis*; 1.26 ± 0.03 g) were placed at 3–5 cm depth under the soil surface in May 2014. A total of 104 tea bags were buried (four bags per plot × 26 plots). For further information about the tea and chemical descriptions, see Keuskamp et al. (2013). Before field application, tea bags were prepared by the protocol developed in the GLUSEEN project (http://www.gluseen.org/protocols/preparing-teabags/) to eliminate water soluble materials (e.g. simple sugars and phenols). This is important in order to exclude abiotic mass loss from precipitation-induced leaching. Tea bags were retrieved after 1 month. After gently removing adhered soil from the outside of tea bags in the laboratory, they were soaked under tap water to eliminate soil particles that had passed through the mesh. All samples were air dried at room temperature and then in a climate cabin at 36 °C until they reached a constant weight. Dried samples were used to measure changes in the mass of organic matter through time and to estimate the rate of decomposition.

Statistical analyses

All statistical analyses were performed in R 3.3.1 (R Development Core Team 2016), using the R packages 'lme4' (Bates et al. 2015), 'mvabund' (Wang et al. 2012) and 'vegan' (Oksanen et al. 2017). Non-metric multidimensional scaling (NMDS) was carried out with the software PAST 3.10 (Hammer et al. 2001).

Outliers were identified and removed prior to data analysis. After fitting the full models for each dependent variable, we used Akaike Information Criterion (AIC) to select the most parsimonious model. The lack of spatial independence of the paired set-aside and cereal fields was treated by application of a random factor ('location'). Since there was significant intercorrelation between soil characteristics and habitat type, their effects were tested in separate models. Assumptions of normality and homoscedasticity of the residuals were verified visually using diagnostic plots. Statistical significance was determined at the level: $\alpha = 0.05$.

Soil physical and chemical properties

The effects of land use on soil physical and chemical properties were tested by linear mixed-effects model (LMM), with 'habitat type' as explanatory variable and 'location' as random factor.

Soil bacteria

Alpha diversity metrics (Shannon diversity [H'] and Evenness [J'] indices based on T-RFLP abundance data) were calculated to estimate the diversity of bacterial communities. We used LMMs to determine the effects of habitat type, soil physical and chemical properties on bacterial alpha diversity. A PERMANOVA (Bray-Curtis index, permutation = 999) was conducted to assess differences in the bacterial communities by habitat type.

Soil arthropods

To characterise soil arthropod communities, QBS index, species richness (number of species in the sample) and abundance (number of individuals in the sample) were used. The LMMs were used to examine the effects of abiotic soil properties and habitat type on faunal richness and abundance. The influence of abiotic soil properties and habitat types on the species composition of isopod and millipede assemblages was tested by generalised linear mixed models (GLMMs) with the multivariate approach. As our data showed a negative binomial distribution, we thus used the 'manyglm' method (family = negative binomial). Then we conducted NMDS ordinations using the Bray-

Curtis dissimilarity index to visualise patterns of species composition of macrodetritivore assemblages. In the latter case, species with low relative abundance (*Trachelipus nodulosus*: 0.43%, *Porcellionides pruinosus*: 0.58%) were excluded from the analysis.

Plant tissue decomposition

Rates of decomposition were estimated with a single exponential decay model (Olson 1963):

$$M_t / M_0 = e^{-kt}, (1)$$

where M_0 is the initial dry mass, M_t is the residual dry mass at time t and k is the decay constant.

The effects of habitat type and abiotic soil properties were tested by a LMM.

Soil biodiversity – plant tissue decomposition linkage

A soil biodiversity index was calculated from the average of all standardised soil community characteristics (bacterial diversity, QBS index, macrofauna species richness and abundance) and used as a general indicator (Wagg et al. 2014). To reveal the relationship between soil biodiversity (and its biotic components) and the decomposition rate of plant residue, LMMs were also used.

Results

Soil physical and chemical properties

We experienced significant differences in soil pH, K_2O and sodium (Na) content amongst habitat types. Soil pH ranged from 4.42 to 6.86 in the different habitat types. It had the lowest value in semi-natural grasslands (G) followed by set-aside (Sa) and cereal fields (C and CSa) (Table 1). Soil K_2O was significantly higher in cereal fields (C and CSa) compared to set-aside fields and semi-natural grasslands. Moreover, there were significant differences in soil sodium content between grasslands and other habitat types (Table 1).

Soil biota

Soil bacteria

The Shannon and Evennes indices showed relatively high variability amongst habitat types, with values ranging from 1.37 to 3.03 and from 0.28 to 0.71, respectively. Only

Table 1. Basic properties of soil samples taken from the 0–15 cm depth (mean ± SE). Letters indicate significant differences amongst the means at $p < 0.05$. Abbreviations – SOM: soil organic matter, C: cereal fields, CSa: cereal fields adjacent to set-asides, Sa: set-aside fields, G: semi-natural grasslands.

	Habitat types			
	C	**CSa**	**Sa**	**G**
pH	6.09±0.22 **(a)**	5.54±0.22 **(ab)**	5.29±0.20 **(bc)**	4.92±0.13 **(c)**
K_A	44.83±1.23 **(a)**	47.14±2.77 **(a)**	43.57±0.94 **(a)**	47.17±1.44 **(a)**
Salt (m/m %)	0.04±0.01 **(a)**	0.03±0.00 **(a)**	0.04±0.01 **(a)**	0.05±0.01 **(a)**
$CaCO_3$ (m/m %)	0.32±0.29 **(a)**	0.19±0.17 **(a)**	0 **(a)**	0 **(a)**
SOM (m/m %)	3.57±0.43 **(a)**	3.29±0.21 **(a)**	3.52±0.28 **(a)**	4.02±0.29 **(a)**
NO_2-NO_3 N (mg kg^{-1})	13.04±6.67 **(a)**	7.88±1.04 **(a)**	23.27±5.39 **(a)**	35.67±16.49 **(a)**
P_2O_5 (mg kg^{-1})	300±57.79 **(a)**	165.71±23.61 **(a)**	136.86±43.21 **(a)**	233.75±119.06 **(a)**
K_2O (mg kg^{-1})	682±99.12 **(a)**	677.14±71.93 **(a)**	461±65.2 **(b)**	378±32.76 **(b)**
Na (mg kg^{-1})	182.92±66.35 **(b)**	97.37±13.77 **(b)**	72.14±13.35 **(b)**	500.5±96.61 **(a)**
Mg (mg kg^{-1})	565.33±81.85 **(a)**	542.14±70.63 **(a)**	605.57±68.48 **(a)**	775.17±118.34 **(a)**
SO_4-S (mg kg^{-1})	41.5±6.64 **(a)**	38.79±4.36 **(a)**	42.39±4.40 **(a)**	58.2±7.84 **(a)**

Table 2. Effects of habitat type and soil physicochemical properties on microarthropods and macrodetritivores. $CaCO_3$ and Mg variables are not included in the table, since they had significant effects on none of the dependent variables. Abbreviations – QBS: soil biological quality index, SR: species richness, SOM%: soil organic matter %, K_A: soil plasticity index according to Arany; +: positive effect, –: negative effect, NS: not significant, ***: $p \leq 0.001$, **: $p \leq 0.01$, *: $p \leq 0.05$, ˙: $p \leq 0.1$

	Microarthropods			**Macrodetritivores**				
				Isopoda		**Diplopoda**		
	diversity (QBS)	**abundance**	**composition**	**diversity (SR)**	**abundance**	**diversity (SR)**	**abundance**	**composition**
habitat	**	*	NS	*	**	NS	*	**
pH	– ***	+ ***	NS	– *	NS	NS	NS	NS
SOM %	+ *	NS	˙	NS	NS	NS	NS	NS
K_A	+ **	NS	NS	NS	NS	NS	NS	*
salt	NS	– ***	NS	NS	NS	NS	NS	˙
K_2O	NS	– ***	NS	NS	– ˙	NS	NS	NS
NO_2-NO_3 N	NS	+ ***	NS	NS	– *	NS	NS	NS
SO_4-S	+ **	NS	NS	NS	NS	NS	NS	NS
Na	– *	– **	NS	NS	NS	NS	NS	NS

Evennes index was significantly influenced by the studied environmental variables: it decreased with the SOM% ($t = -2.47$, $p = 0.05$), whereas increased with soil Na content ($t = 3.28$, $p = 0.022$) (Table 2). In this study, habitat type did not significantly affect bacterial alpha diversity (see Table 2) and community composition ($F_{PERMANOVA} = 1.2951$, $p = 0.122$).

Soil microarthropods

In total, 14385 specimens belonging to 19 taxa of microarthropods were sampled (Suppl. materials 1–2). The QBS index varied from 29 to 128 and showed significant differences amongst habitat types (Table 2, Suppl. materials 1–2). The highest values were found in semi-natural grasslands, while cereal fields without set-asides were characterised by the lowest QBS (Figure 2). Abundance of microarthropods was significantly affected by habitat type: it was the highest in set-aside fields compared to the other habitats (Figure 2). Nevertheless, all samples were dominated by mites, particularly oribatids (70.38% of the total microarthropods collected). Soil pH and Na content had negative, while soil plasticity and SO_4-S content had positive effects on the QBS index. There was a positive relationship between soil pH, NO_2-NO_3 N content and abundance of microarthropods. We found that the number of microarthropods decreased with total soluble salt concentration and with the amount of soil K_2O and Na (Table 2).

Soil macrodetritivores

In total, 1391 individuals of 8 macrodecomposer species were identified from samples collected by the pitfall traps, including 783 individuals of four isopod species (*Armadillidium vulgare*, Latreille, 1804; *Porcellionides pruinosus*, Brandt, 1833; *Trachelipus rathkii*, Brandt, 1833; *Trachelipus nodulosus*, C. Koch, 1838) and 608 individuals of four millipede species (*Brachydesmus superus*, Latzel, 1884*; Brachyiulus bagnalli*, Brölemann, 1924; *Iulus terrestris*, Linnaeus, 1758; *Megaphyllum unilineatum*, C. Koch, 1838) (Suppl. materials 3–4). The most abundant species were *Armadillidium vulgare* (89.27%) and *Iulus terrestris* (59.38%) from isopod and millipede species respectively. The total abundance of the studied macrodecomposers was highest in semi-natural grasslands, with 645 individuals of isopods and 379 individuals of millipedes. The lowest abundance of detritivores was recorded within cereal fields for isopods (4 individuals of total) and cereal fields adjacent to set-asides for millipedes (30 individuals of total). In total, 98 isopod and 37 millipede specimens were collected in set-aside fields.

In the present study, species richness was significantly affected by the studied environmental variables only in the case of isopods. We experienced significant effects of habitat type and soil pH on isopod species number increasing with soil acidity ($z = -2.236$, $p = 0.022$) (Table 2). Semi-natural grasslands were characterised by the highest species richness, while cereal fields without set-asides proved to be the most species-poor habitats (Figure 2). There were significant differences in abundance of iso- and diplopods amongst habitat types. Isopod individual numbers were the highest in semi-natural grasslands and the lowest in cereal fields without set-asides, respectively. By contrast, millipedes were the most abundant in the latter habitats (Figure 2). Soil K_2O and N had negative effects on the abundance of woodlice (Table 2).

Species composition of iso- and diplopod assemblages was affected by habitat type, salt concentration and soil plasticity (Table 2, Figure 3). *Brachyiulus bagnalli* mostly occurred in cereal fields ($Dev = 19.903$, $p = 0.009$), while *A. vulgare* preferred semi-

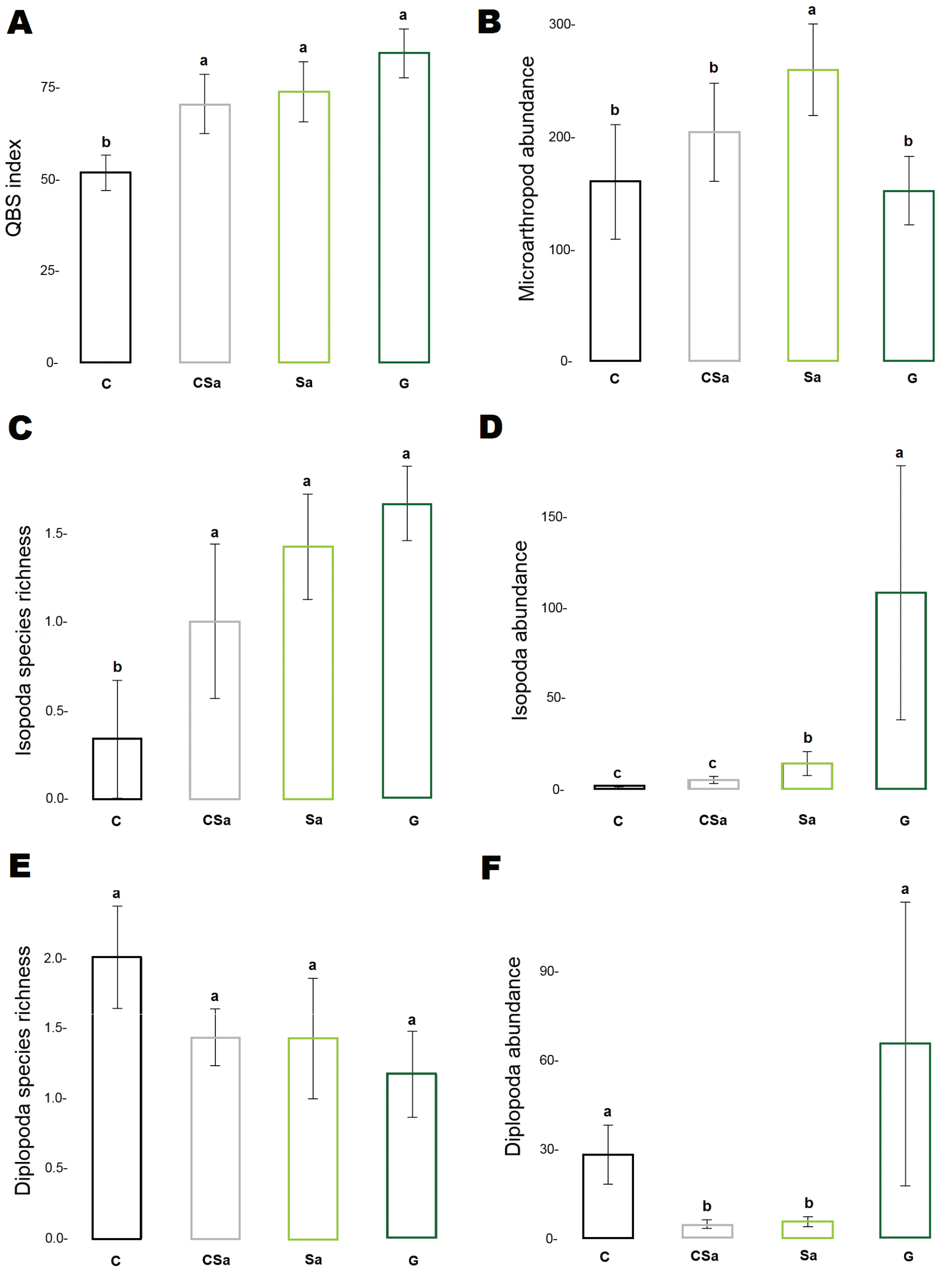

Figure 2. Diversity (expressed in species richness and QBS index) and abundance of microarthropods (**A–B**), isopods (**C–D**) and millipedes (**E–F**) in different habitat types. Error bars represent means and SE. Please note the different scaling for the Y axes. Letters indicate significant differences amongst the means. Abbreviations – C: cereal fields, CSa: cereal fields adjacent to set-asides, Sa: set-aside fields, G: semi-natural grasslands.

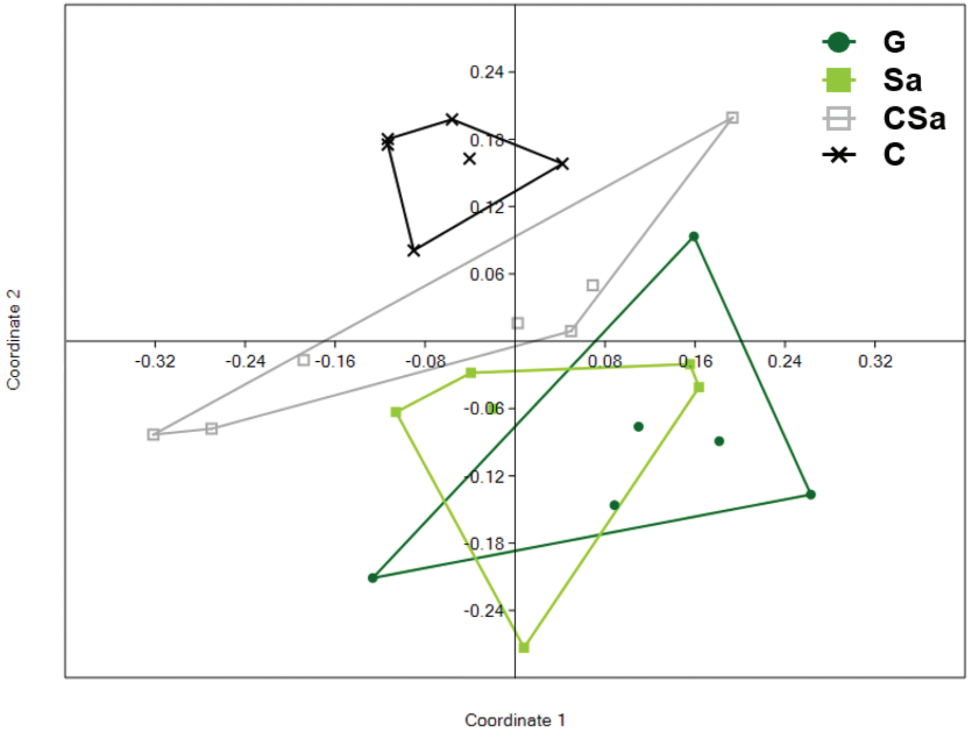

Figure 3. NDMS plot on species composition of macrodecomposer assemblages as related to habitat types. See Figure 2 for abbreviations.

natural grasslands characterised by soils with high salt concentration (Dev = 10.426, p = 0.03). *Brachydesmus superus* was observed in sites with higher soil plasticity index (Dev = 15.422, p = 0.008).

Plant tissue decomposition and its relationship with soil biodiversity

On average, 22.41% of organic matter was decayed during a month. Mass loss was significantly different between habitat types (F = 10.8618, p < 0.001). We experienced the highest decomposition in set-asides (remaining mass: 74.17%, on average) while the lowest in cereal fields (remaining mass: 81.3%, on average). The decomposition rate was negatively influenced by SOM content (F= 12.3966, p= 0.002). However, soil pH had positive effects on the intensity of mass loss (F = 5.3119, p = 0.033). Organic matter decomposition did not change with soil biodiversity (t = 1.2589, p = 0.255). Nevertheless, we found marginally significant QBS index – decay rate relationship (t = 2.1076, p = 0.08) (Figure 4).

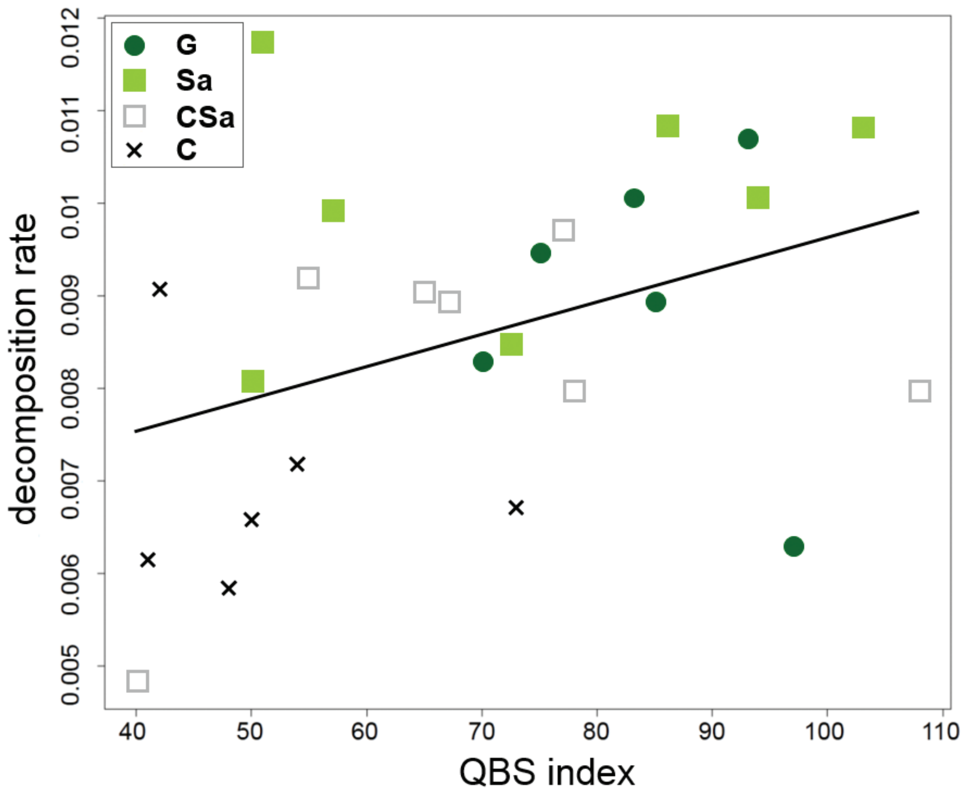

Figure 4. The relationship between decomposition rate of organic matter (g day^{-1}) and biological quality of soils (expressed in QBS index) based on the linear mixed-effects model. See Figure 2 for abbreviations.

Discussion

Soil physical and chemical properties

The general consensus in literature is that agricultural practices, particularly soil cultivation and manuring, can lead to drastic changes in soil physical and chemical properties (e.g. Bronick and Lal 2005, De Paul Obade and Lal 2014). Consistent with our first hypothesis, we found significant differences amongst habitat types in case of soil pH, K_2O and Na content, confirming the close relationships between agricultural management and abiotic soil conditions. The significantly lower soil pH in cereal fields compared to semi-natural grasslands and set-asides is probably due to previous soil correction by liming practice, while the relatively high K_2O content of soil experienced in former habitats could be the result of fertilisation. The extremely high amount of Na in soils of semi-natural grasslands is probably due to the nature of the typical saline soil type in the region (Stefanovits et al. 1999).

Soil biota

Soil bacteria

Contrary to our expectations, we found no significant differences amongst habitat types regarding bacterial alpha diversity and community composition. The majority of studies showed that microbial species richness increases both with plant diversity and reduction of anthropogenic disturbances (e.g. Swift et al. 1996), which mainly characterises set-aside fields and semi-natural grasslands. In addition to vegetation characteristics, soil type and land use are of particular importance in influencing microbial diversity (e.g. Garbeva et al. 2004a,b). Most of the cases (84%), reviewed by Allison and Martiny (2008), reported the sensitivity of microbial communities to N, P, K fertilisation. O'Brien et al. (2016) experienced higher microbial α diversity in fertilised fields. However, there are also contrary experiences (in accordance with our results): several previous reports did not reveal a correlation between plant species richness and bacterial diversity, emphasising rather the importance of soil properties (e.g. Fierer and Jackson 2006, Zul et al. 2007). In addition, agricultural treatments such as fertilisation, have no consistent effects on the diversity of bacterial communities as evidenced by, inter alia, the results of Fierer and co-workers (Fierer et al. 2012).

Out of the α diversity indices, the evenness of the bacterial communities was significantly influenced by SOM and sodium contents of soils. This corresponds with findings in which significant effects of soil pH, organic matter content, moisture and nutrient availability on microbial community structure have been reported (e.g. Fierer et al. 2012, Kuramae et al. 2012). The evenness index increased with soil sodium content which mainly characterised semi-natural grasslands. This could be attributed to the better quality of these undisturbed habitats supporting more stable and balanced bacterial communities.

Soil microarthropods

The more diverse and complex vegetation creates more favourable microclimatic conditions for soil microarthropods (Adejuyigbe et al. 1999): the structure of vegetation largely determines the moisture and temperature conditions of the soil, which has a serious effect on the studied animal groups (e.g. Hopkin 1997, Tsiafouli et al. 2005). It provides a better quality food source for microarthropods, predominantly determined by the C / N ratio of dead plant material (Seastedt 1984). Furthermore, these habitats positively affect the presence of soil animals providing refuges for them due to the lower degree of human disturbance resulting from agricultural activities (Barbercheck et al. 2008).

The higher microarthropod diversity and abundance in cereal fields adjacent to set-asides compared to cereals without set-aside fields are probably attributable to the spillover effect of set-aside fields: several studies have proved the positive role of semi-natural habitat patches as propagule sources, affecting favourably the adjacent areas as well (e.g. Blitzer et al. 2012). In addition to the above-mentioned environmental factors, the soil physical and chemical properties are of great importance for soil arthropods: soil pH, salt concentration, organic matter and nutrient content proved to be significant factors. Soil pH had a variable effect on the QBS index and the number of microarthropods: the former showed a positive relationship with pH, while the latter increased with soil acidity. Although most of the soil arthropods do not prefer acidic soils (Swift et al. 1979), the abundance of certain taxa (e.g. Oribatida) decreases with soil pH (e.g. Maraun and Scheu 2000). This might explain the higher microarthropod numbers found in soils with lower pH: most of the samples were dominated by mites (mainly oribatids). We experienced positive effects of soil organic matter and nitrogen content on microarthropods. The first is supported by the results of several studies (e.g. Edwards and Lofty 1969). This is likely to be closely related to the fact that soil organic matter serves as an energy and nutrient source for them (Swift et al. 1979). The observed positive relationship between soil nitrogen content and microarthropod diversity was probably due to their food preference. Although there were no significant differences between habitats, the nitrogen supply of soils of set-aside fields and grasslands was generally higher, which might be largely caused by the N-rich vegetation. The role of legumes, which characterised these habitats, is also essential in this respect as they make a significant contribution to atmospheric N fixation. Therefore, they increase soil fertility and return high quality litter to soil organisms (Mulder et al. 2002). The chemical composition of dead plant material is particularly important for the detritivore arthropods, remarkably affecting their diversity and abundance. It is well-known that most of them prefer N-rich detritus (e.g. Seastedt 1984), which might be found mainly in set-aside fields and semi-natural grasslands. Potassium also proved to be a significant soil nutrient: its increase resulted in unfavourable change in soil biological quality (expressed in the QBS index). Since there were significantly higher K_2O values in cereal fields compared to set-asides and grasslands, presumably fertilisation remains in the background. We can find examples of beneficial and adverse effects of fertilisation on soil microarthropods supporting the relevance of this issue (Bardgett and Cook 1998). The relatively high total soluble salt and Na concentrations refer unfavourable soil conditions not tolerated by the majority of soil organisms. Therefore, the negative effects of these soil parameters on soil arthropods are not surprising. The higher number of individuals found in clay soils (with higher K_A values) may be attributed to beneficial soil conditions (e.g. soil moisture, nutrient and organic matter). Nevertheless, it is important to emphasise that heavily-bound soils can lead to opposing changes, as limited pore space impedes movement of the soil microarthropods (O'Lear and Blair 1999).

Soil macrodetritivores

Species richness of isopods and millipedes reflected the regional species pool. All millipede and isopod species found in the sampling sites are rather common in the Hungarian Great Plain (Korsós and Hornung, unpublished results). In human modified habitats, such as agroecosystems, a wide range of millipede species generally occurs in relatively low species richness, but in high density (Golovatch and Kime 2009). Except for *Leptoiulus cibdellus* and *Porcellionides pruinosus*, the observed species were almost the same as found by Tóth et al. (2016). The latter can be regarded as synanthropic: appears in all kinds of anthropogenic habitats in Hungary (Vilisics et al. 2007, Hornung et al. 2008). In the present study, it occurred only in cereal fields similarly to a millipede species, *Brachydesmus superus*. This is not surprising as both species have broad tolerance to anthropogenic disturbance (Schubart 1934, Schmalfuss 2003). The significant effects of abiotic soil properties on isopods shown by LMM are probably related to habitat type. The beneficial effect of grasslands and set-aside fields on isopods might be mainly due to the more favourable microclimatic conditions, the better quality food source and the lesser anthropogenic disturbance (Tóth et al. 2016). However, we did not find such a clear habitat preference in the case of millipedes. In the present study, the habitat type was almost irrelevant regarding species richness. Nevertheless, it had significant effect on their abundance: cereal fields without set-asides proved to be the most favourable habitats with almost the same values as semi-natural grasslands. Differing habitat preference of isopods and millipedes can be explained by physiological attributes: millipedes are less sensitive to microclimatic effects being more drought resistant (Morón-Ríos et al. 2010) than isopods. Soil temperature and moisture content are the main abiotic background factors influencing the presence and abundance of the animals in question, especially that of terrestrial isopods. Their exoskeleton is permeable to water and so the desiccation threat restricts their occurrence to habitats with higher humidity and suitable shelter sites (e.g. Warburg 1987, Hopkin and Read 1992).

We identified that habitat type, salt concentration and soil plasticity (expressed in K_A) were the main factors influencing the species composition of the macrodecomposer assemblages (Figure 2). *Brachyiulus bagnalli* mainly occurred in cereal fields that can be explained by its habitat preference: it favours disturbed, open habitats, tolerating a wide range of drought and human presence (Schubart 1934, Korsós 1992). In contrast, *A. vulgare* preferred grasslands and its occurence was connected to soils with higher salt content, indicating a relatively high salinity tolerance. *Brachydesmus superus* was significantly influenced by soil plasticity. There may be several explanations for this phenomenon: for example, it strongly affects the soil moisture regime, soil chemistry, overall substrate availability and the movement of millipedes.

Plant tissue decomposition and its relationship with biodiversity

Plant tissue decomposition was the highest in set-aside fields and semi-natural grasslands during the studied period. There was also a significant difference in mass loss of organic matter between cereal fields with and without set-asides. We experienced the lowest degree of decomposition in the latter habitats.

It has long been proven that characteristics of ecosystems (e.g. physical, chemical and biological properties) basically determine their functionalities (Wiens 1972). Concerning the carbon cycle, the carbon sink and source ecosystems can be distinguished. Almost all natural habitats, characterised by a high amount of plant biomass, such as enhanced primary production and moderate carbon emissions, belong to the former category. By contrast, anthropogenic conversion of natural habitats results in degraded ecosystems becoming carbon sources (Bardgett and Wardle 2010). Consequently, more intensive decomposition processes and, thus, higher carbon emissions, could be expected in soils of cereal fields compared to grasslands and set-asides. However, we found higher organic matter decay in soils of the less disturbed habitats, which suggests greater rates of soil respiration. Nevertheless, it is important to emphasise that it is not possible to draw far-reaching conclusions from this one-month period of the examination. The dynamics of organic matter decomposition shows great spatial and temporal variation (Swift et al. 1979). At least a one-year period would have been necessary to seek a better picture, but agricultural activities in the sampling areas did not allow this.

In addition to the habitat type, two abiotic soil properties (soil pH and SOM%) also significantly affected the decomposition rates. The greater biodegradation observed in the more alkaline soils is probably attributed to the fact that the acidic soil pH is not favourable to the majority of soil organisms, which can lead to reduced organic matter decomposition. The negative relationship between SOM content and decay rates could infer less intensive mineralisation and immobilisation than humification, resulting in a higher SOM level due to the gradual accumulation of soil organic matter.

There was no significant correlation between soil biodiversity and organic matter decomposition in our research, despite the positive trend between the two variables. Reasons for the lack of significance may be related to one-off sampling, short period of the organic matter decomposition test, forced skip of key groups of decomposer organisms (fungi, earthworms) etc. In the case of microbes, we could estimate the diversity only of soil bacteria, although it is possible that habitats with acidic soils were dominated by fungi. This is also supported by the fact that soils with low pH and higher organic matter content – such as semi-natural grasslands and set-aside fields in our study – generally have a fungal-dominated food web (Bardgett and Wardle 2010). However, agricultural practices (fertilisation, grazing, ploughing etc.) generally lead to a shift from fungal-based soil food webs to more bacterial-based soil food webs (Bardgett and Wardle 2010). Ideally, therefore, both microbial groups should be taken into account as their role in breakdown of organic matter may differ depending on habitat type.

Out of the biotic components, only soil biological quality (expressed in the QBS index) significantly influenced (even if marginally) plant tissue decomposition. The positive effects of microarthropods on decay of organic matter have already been demonstrated in a number of studies (e.g. Crossley and Hoglund 1962). According to Seastedt's (1984) estimate, soil microarthropods consume 20–30% of the total annual litter, thus directly and indirectly promoting the breakdown of detritus. This is in line with the results of De Graaff and co-workers (2015) who found a decline in decomposition rates with the decrease of soil faunal diversity, while the reduction of microbial diversity has not affected the decay intensity.

Conclusions

Our results indicate that set-aside management under agri-environment schemes has profound effects not only on certain soil physical and chemical properties, but on soil biodiversity and function as well. The present study highlights the importance of set-aside fields particularly for the conservation to surface dwelling invertebrates. Set-aside fields function as semi-natural habitats providing favourable conditions especially for micro- and macroarthropods, supporting the regeneration of soil biological resources. Set-aside fields that are not part of a crop rotation for at least 2 years could be a valuable option for establishing ecological focus areas under the Common Agricultural Policy (CAP) in the EU, as these fields may help to conserve soil biodiversity.

However further research is required to look for the optimum management regimes for all soil-related organisms supporting the most abundant and diverse soil biota, particularly in relation to the establishment methods of set-aside or other semi-natural habitat types in agricultural landscapes.

Finally, we emphasise that evaluation of agri-environmental schemes, regarding soil biodiversity and function, is of high practical and theoretical importance. For example, data on soil biota, plant tissue decomposition and/or their relationship are essential to better understand mechanisms influencing biogeochemical cycles. Therefore, the biological and functional aspects of soil need to be better taken into account in a national or/and European soil monitoring scheme.

Acknowledgements

The authors thank the Bükk National Park Directorate and the landowners for permission to work on their fields. We are grateful to András Táncsics and Balázs Kriszt (Szent István University) for their contribution in microbial analyses. We would like to thank Zoltán Elek and Gergely Boros (MTA Centre for Ecological Research) for their assistance in field works and for advice. This research was financially supported by the LIBERATION EU FP7 project [FP7 KBBE 311781] and the 12190-4/2017/FEKUTSTRAT grant of the Hungarian Ministry of Human Capacities.

References

Adejuyigbe C, Tian G, Adeoye G (1999) Soil microarthropod populations under natural and planted fallows in southwestern Nigeria. Agroforestry Systems 47(1/3): 263–272. https://doi.org/10.1023/A:1006236001299

Allison SD, Martiny JBH (2008) Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences of the United States 105(Supplement 1): 11512–11519. https://doi.org/10.1073/pnas.0801925105

Altieri MA (1999) The ecological role of biodiversity in agroecosystems. Agriculture, Ecosystems & Environment 74(1/3): 19–31. https://doi.org/10.1016/S0167-8809(99)00028-6

Ángyán J, Tardy J, Vajnáné Madarassy A (Eds) (2003) Védett és érzékeny természeti területek mezőgazdálkodásának alapjai (Agriculture of Protected and Environmentally Sensitive Areas). Mezőgazda Kiadó, Budapest.

Barrios E (2007) Soil biota, ecosystem services and land productivity. Ecological Economics 64(2): 269–285. https://doi.org/10.1016/j.ecolecon.2007.03.004

Bardgett RD, Cook CR (1998) Functional aspects of soil animal diversity in agricultural grasslands. Applied Soil Ecology 10(3): 263–276. https://doi.org/10.1016/S0929-1393(98)00125-5

Bardgett RD, Wardle DA (2010) Above-Ground-Belowground Linkages. Biotic Interactions, Ecosystem Processes and Global Change. Oxford University Press, New York.

Batáry P, Dicks LV, Kleijn D, Sutherland WJ (2015) The role of agri-environment schemes in conservation and environmental management. Conservation Biology 29(4): 1006–1016. https://doi.org/10.1111/cobi.12536

Barbercheck ME, Neher DA, Anas O, El-Allaf SM, Weicht TR (2008) Response of soil invertebrates to disturbance across three resource regions in North Carolina. Environmental Monitoring and Assessment 152(1/4): 283–298. https://doi.org/10.1007/s10661-008-0315-5

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67(1): 1–48. https://doi.org/10.18637/jss.v067.i01

Báldi A, Batáry P, Kleijn D (2013) Effects of grazing and biogeographic regions on grassland biodiversity in Hungary - analysing assemblages of 1200 species. Agriculture, Ecosystems & Environment 166: 28–34. https://doi.org/10.1016/j.agee.2012.03.005

Blitzer EJ, Dormann CF, Holzschuh A, Klein AM, Rand TA, Tscharntke T (2012) Spillover of functionally important organisms between managed and natural habitats. Agriculture, Ecosystems & Environment 146(1): 34–43. https://doi.org/10.1016/j.agee.2011.09.005

Bronick CJ, Lal R (2005) Soil structure and management: A review. Geoderma 124(1/2): 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005

Brussaard L, de Ruiter PC, Brown GG (2007) Soil biodiversity for agricultural sustainability. Agriculture, Ecosystems & Environment 121(3): 233–244. https://doi.org/10.1016/j.agee.2006.12.013

Bükk National Park (2018) Hevesi Füves Puszták Tájvédelmi Körzet (Heves Steppe Grasslands Landscape Protection Area). http://bnpi.hu/oldal/hevesi-fuves-pusztak-tk-55.html [accessed 26 February 2018]

Critchley CNR, Allen DS, Fowbert JA, Mole AC, Gundrey AL (2004) Habitat establishment on arable land: Assessment of an agri-enviroment scheme in England, UK. Biological Conservation 119(4): 429–442. https://doi.org/10.1016/j.biocon.2004.01.004

Crossley DA Jr, Hoglund MP (1962) A litter-bag method for the study of microarthropods inhabiting leaf litter. Ecology 43(3): 571–573. https://doi.org/10.2307/1933396

Culliney TW (2013) Role of Arthropods in maintaining soil fertility. Agriculture 3(4): 629–659. https://doi.org/10.3390/agriculture3040629

De Graaff M-A, Adkins J, Kardol P, Throop HL (2015) A meta-analysis of soil biodiversity impacts on the carbon cycle. Soil (Göttingen) 1(1): 257–271. https://doi.org/10.5194/soil-1-257-2015

De Paul Obade V, Lal R (2014) Soil quality evaluation under different land management practices. Environmental Earth Sciences 72(11): 4531–4549. https://doi.org/10.1007/s12665-014-3353-z

Dominati E, Patterson M, Mackay A (2010) A framework for classifying and quantifying the natural capital and ecosystem services of soils. Ecological Economics 69(9): 1858–1868. https://doi.org/10.1016/j.ecolecon.2010.05.002

Dudgeon D, Ma HHT, Lam PKS (1990) Differential palatability of leaf litter to four sympatric isopods in a Hong Kong forest. Oecologia 84(3): 398–403. https://doi.org/10.1007/BF00329766

Edwards CA, Lofty JR (1969) The influence of agricultural practice on soil microarthropod populations. In: Sheals JG (Ed.) The soil ecosystem. Systemic Association, London, 237–247.

European Commission (2013) Agriculture, Forestry and Fishery Statistics Pocketbooks 2013 Edition. Publications Office of the European Union, Luxembourg.

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States 103(3): 626–631. https://doi.org/10.1073/pnas.0507535103

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6(5): 1007–1017. https://doi.org/10.1038/ismej.2011.159

Garbeva P, Van Veen JA, Van Elsas JD (2004a) Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppression. Annual Review of Phytopathology 42(1): 243–270. https://doi.org/10.1146/annurev.phyto.42.012604.135455

Garbeva P, Voesenek K, Van Elsas JD (2004b) Quantitative detection and diversity of the pyrrolnitrin biosynthesis locus in soil under different treatments. Soil Biology & Biochemistry 36(9): 1453–1463. https://doi.org/10.1016/j.soilbio.2004.03.009

Golovatch SI, Kime RD (2009) Millipede (Diplopoda) distributions: A review. Soil Organisms 81: 565–597. http://www.senckenberg.de/files/content/forschung/publikationen/soilorganisms/volume_81_3/24_golovatch.pdf

Gruner H (1966) Die Tierwelt Deutschlands. 53. Teil. Krebstiere oder Crustacea. V. Isopoda, 2. Lieferung. Gustav Fischer Verlag, Jena.

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics software package for education and data analysis. Palaeontologia Electronica 4: 1–9. http://palaeo-electronica.org/2001_1/past/past.pdf

Hättenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology Evolution and Systematics 36(1): 191–218. https://doi.org/10.1146/annurev.ecolsys.36.112904.151932

Hopkin SP, Read HJ (1992) The biology of Millipedes. Oxford University Press, Oxford.

Hopkin SP (1997) Biology of the Springtails. University Press, Oxford.

Hornung E, Vilisics F, Sólymos P (2008) Low α- and high β-diversity in terrestrial isopod assemblages in the Transdanubian region of Hungary. In: Zimmer M, Charfi-Cheikhrouha F, Taiti S (Eds) Proceedings of the International Symposium of Terrestrial Isopod Biology, ISTIB-07, Tunis (Tunisia) 28-31 March 2007. Shaker Verlag, Aachen, 1–11.

Keuskamp JA, Dingemans BJJ, Lehtinen T, Sarneel JM, Hefting MM (2013) Tea Bag Index: A novel approach to collect uniform decomposition data across ecosystems. Methods in Ecology and Evolution 4(11): 1070–1075. https://doi.org/10.1111/2041-210X.12097

Korsós Z (1992) Millipedes from anthropogenic habitats in Hungary (Diplopoda). Berichte des naturewissenschaftlich-medizinischen Vereins in Innsbruck 10: 237–241. http://www.zobodat.at/pdf/BERI_S10_0237-0241.pdf

Korsós Z (2015) Magyarország ikerszelvényesei. Illusztrációtáblák és adatlapok a fajok meghatározásához (Diplopods of Hungary. Illustrations and data sheets to species identification). Hungarian Natural History Museum, Budapest.

Kovács-Hostyánszki A, Kőrösi Á, Orci KM, Batáry P, Báldi A (2011) Set-aside promotes insect and plant diversity in a Central European country. Agriculture, Ecosystems & Environment 141(3/4): 296–301. https://doi.org/10.1016/j.agee.2011.03.004

Kuramae EE, Yergeau E, Wong LC, Pijl AS, Van Veen JA, Kowalchuk GA (2012) Soil characteristics more strongly influence soil bacterial communities than land-use type. FEMS Microbiology Ecology 79(1): 12–24. https://doi.org/10.1111/j.1574-6941.2011.01192.x

Lavelle P, Spain AV (2001) Soil Ecology. Kluwer Scientific, Amsterdam.

Maraun M, Scheu S (2000) The structure of oribatid mite communities (Acari, Oribatida): Patterns, mechanisms and implications for future research. Ecography 23(3): 374–382. https://doi.org/10.1111/j.1600-0587.2000.tb00294.x

Monfreda C, Ramankutty N, Foley JA (2008) Farming the planet: 2. Geographic distribution of crop areas, yields, physiological types, and net primary production in the year 2000. Global Biogeochemical Cycles 22(1): GB1022. https://doi.org/10.1029/2007GB002947

Morón-Ríos A, Rodríguez MÁ, Pérez-Camacho L, Rebollo S (2010) Effects of seasonal grazing and precipitation regime on the soil macroinvertebrates of a Mediterranean old-field. European Journal of Soil Biology 46(2): 91–96. https://doi.org/10.1016/j.ejsobi.2009.12.008

Morris AJ, Hegarty J, Báldi A, Robijns T (2011) Setting aside farmland in Europe: The wider context. Agriculture, Ecosystems & Environment 143(1): 1–2. https://doi.org/10.1016/j.agee.2011.07.013

MSZ (Hungarian Standard) 08-0205 (1978) Determination of physical and hydrophysical properties of soils.

MSZ (Hungarian Standard) 08-0206-2 (1978) Evaluation of some chemical properties of the soil. Laboratory tests. (pH value, phenolphtaleine alkalinity expressed in soda, all water soluble salts, hydrolite (yˇ1^-value) and exchanging acidity (yˇ2^- value).

MSZ (Hungarian Standard) 08-0452 (1980) Use of high-capacity analyser systems for soils analyses. Quantitative determination of the organic carbon content of the soil on Contiflo analyzer system.

MSZ (Hungarian Standard) 21470-2 (1981) Environmental protection. Preparation of soil sample. Determination of electrical conduction, humidity and pH.

MSZ (Hungarian Standard) 20135 (1999) Determination of the soluble nutrient element content of the soil.

Mulder CPH, Jumpponen A, Högberg P, Huss-Danell K (2002) How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. Oecologia 133(3): 412–421. https://doi.org/10.1007/s00442-002-1043-0

O'Brien SL, Gibbons SM, Owens SM, Hampton-Marcell J, Johnston ER, Jastrow JD, Gilbert JA, Meyer F, Antonopoulos DA (2016) Spatial scale drives patterns in soil bacterial diversity. Environmental Microbiology 18(6): 2039–2051. https://doi.org/10.1111/1462-2920.13231

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Sólymos P, Stevens MHH, Wagner H (2017) Vegan: Community Ecology Package. http://CRAN.R-project.org/package=vegan [accessed 26 February 2018]

O'Lear HA, Blair JM (1999) Responses of soil microarthropods to changes in soil water availability in tallgrass prairie. Biology and Fertility of Soils 29(2): 207–217. https://doi.org/10.1007/s003740050546

Olson JS (1963) Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44(2): 322–331. https://doi.org/10.2307/1932179

Parisi V, Menta C, Gardi C, Jacomini C, Mozzanica E (2005) Microarthropod communities as a tool to assess soil quality and biodiversity: A new approach in Italy. Agriculture, Ecosystems & Environment 105(1/2): 323–333. https://doi.org/10.1016/j.agee.2004.02.002

R Development Core Team (2016) R: A language and environment for statistical computing – R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org

Révész S, Sipos R, Kende A, Rikker T, Romsics C, Mészáros É, Mohr A, Táncsics A, Márialigeti K (2006) Bacterial community changes in TCE biodegradation detected in microcosm experiments. International Biodeterioration & Biodegradation 58(3-4): 239–247. https://doi.org/10.1016/j.ibiod.2006.06.018

Rowe RL, Street NR, Taylor G (2009) Identifying potential environmental impacts of large-scale deployment of dedicated bioenergy crops in the UK. Renewable & Sustainable Energy Reviews 13(1): 260–279. https://doi.org/10.1016/j.rser.2007.07.008

Scheu S (2002) The soil food web: Structure and perspectives. European Journal of Soil Biology 38(1): 11–20. https://doi.org/10.1016/S1164-5563(01)01117-7

Schmalfuss H (2003) World catalog of terrestrial isopods (Isopoda: Oniscidea). Stuttgarter Beitrage zur Naturkunde. Serie A, Biologie 654: 1–341.

Schubart O (1934) Tausendfüssler oder Myriapoda I: Diplopoda. In: Dahl F (Ed.) Die Tierwelt Deutschlands und der angränzenden Meeresteile, Teil 28. Gustav Fischer Verlag, Jena.

Seastedt TR (1984) The role of microarthropods in decomposition and mineralization processes. Annual Review of Entomology 29(1): 25–46. https://doi.org/10.1146/annurev.en.29.010184.000325

Smith CJ, Danilowicz BS, Clear AK, Costello FJ, Wilson B, Meijer WG (2005) T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. FEMS Microbiology Ecology 54(3): 375–380. https://doi.org/10.1016/j.femsec.2005.05.002

Smith J, Potts S, Eggleton P (2008) The value of sown grass margins for enhancing soil macrofaunal biodiversity in arable systems. Agriculture, Ecosystems & Environment 127(1-2): 119–125. https://doi.org/10.1016/j.agee.2008.03.008

Smith VC, Bradford MA (2003) Litter quality impacts on grassland litter decomposition are differently dependent on soil fauna across time. Applied Soil Ecology 24(2): 197–203. https://doi.org/10.1016/S0929-1393(03)00094-5

Stefanovits P, Filep G, Füleky G (Eds) (1999) Talajtan (Soil Science). Mezőgazda Kiadó, Budapest.

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. University of California Press, Berkeley.

Swift MJ, Vandermeer J, Ramakrishnan PS, Anderson JM, Ong CK, Hawkins BA (1996) Biodiversity and agroecosystem function. In: Cushman JH, Mooney HA, Medina E, Sala OE, Schulze ED (Eds) Functional Roles of Biodiversity: A Global Perspective. Wiley, Chichester, 261–298.

Tóth Z, Hornung E, Báldi A, Kovács-Hostyánszki A (2016) Effects of set-aside management on soil macrodecomposers in Hungary. Applied Soil Ecology 99: 89–97. https://doi.org/10.1016/j.apsoil.2015.11.003

Tripathi G, Deora R, Singh G (2013) The influence of litter quality and micro-habitat on litter decomposition and soil properties in a silvopasture system. Acta Oecologica 50: 40–50. https://doi.org/10.1016/j.actao.2013.01.013

Tsiafouli MA, Kallimanis AS, Katana E, Stamou GP, Sgardelis SP (2005) Responses of soil microarthropods to experimental short-term manipulations of soil moisture. Applied Soil Ecology 29(1): 17–26. https://doi.org/10.1016/j.apsoil.2004.10.002

Vilisics F, Sólymos P, Hornung E (2007) Study on habitat features and associated terrestrial isopod species. In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe II: Proceedings of the 8th Central European Workshop on Soil Zoology, České Budějovice (Czech Republic), 20–22 April 2005. Academy of Sciences of the Czech Republic, České Budějovice, 195–199.

Wagg C, Bender SF, Widmer F, van der Heijden MGA (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences of the United States 111(14): 5266–5270. https://doi.org/10.1073/pnas.1320054111

Wang Y, Naumann U, Wright S, Warton DI (2012) mvabund: An R package for model-based analysis of multivariate data. Methods in Ecology and Evolution 3(3): 471–474. https://doi.org/10.1111/j.2041-210X.2012.00190.x

Warburg MR (1987) Isopods and their terrestrial environment. Advances in Ecological Research 17: 187–242. https://doi.org/10.1016/S0065-2504(08)60246-9

Wiens JA (1972) Ecosystem structure and function. Oregon State University Press (Corvallis).

Zul D, Denzel S, Kotz A, Overmann J (2007) Effects of plant biomass, plant diversity, and water content on bacterial communities in soil lysimeters: Implications for the determinants of bacterial diversity. Applied and Environmental Microbiology 73(21): 6916–6929. https://doi.org/10.1128/AEM.01533-07

Supplementary material 1

Table S1

Authors: Zsolt Tóth, Elisabeth Hornung, András Báldi

Data type: occurence

Link: https://doi.org/10.3897/natureconservation.29.24856.suppl1

Supplementary material 2

Table S2

Authors: Zsolt Tóth, Elisabeth Hornung, András Báldi

Data type: occurence

Link: https://doi.org/10.3897/natureconservation.29.24856.suppl2

Supplementary material 3

Table S3

Authors: Zsolt Tóth, Elisabeth Hornung, András Báldi

Data type: occurence

Link: https://doi.org/10.3897/natureconservation.29.24856.suppl3

Supplementary material 4

Table S4

Authors: Zsolt Tóth, Elisabeth Hornung, András Báldi

Data type: occurence

Link: https://doi.org/10.3897/natureconservation.29.24856.suppl4

Supplementary material 5

Map file

Authors: Zsolt Tóth, Elisabeth Hornung, András Báldi

Data type: KML file

Link: https://doi.org/10.3897/natureconservation.29.24856.suppl5

Nature Conservation 29: 27–38 (2018)
doi: 10.3897/natureconservation.29.26221
http://natureconservation.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Nature Conservation
Launched to accelerate biodiversity conservation

Cladocera remains and vegetation types to assess the state of oxbows

István Gyulai[1], Csilla Lakatos[2], János Tamás Kundrát[2], Zsuzsanna Balogh[2], Edina Simon[2], Béla Tóthmérész[3]

1 *Department of Hydrobiology, University of Debrecen, Debrecen, Egyetem tér 1, H-4032 Hungary* **2** *Department of Ecology, University of Debrecen, Debrecen, Egyetem tér 1, H-4032 Hungary* **3** *MTA-DE Biodiversity and Ecosystem Services Research Group, Debrecen, Egyetem tér 1, H-4032 Hungary*

Corresponding author: *Edina Simon* (edina.simon@gmail.com)

Academic editor: *M. Pinna* | Received 24 April 2018 | Accepted 29 September 2018 | Published 18 September 2018

http://zoobank.org/98E1D00F-2827-4ADF-B64B-FAA1C80E414

Citation: Gyulai I, Lakatos C, Kundrát JT, Balogh Z, Simon E, Tóthmérész B (2018) Cladocera remains and vegetation types to assess the state of oxbows. Nature Conservation 29: 27–39. https://doi.org/10.3897/natureconservation.29.26221

Abstract

We assessed the usefulness of Cladocera remains for establishing the ecological status of oxbows and also tested the association of Cladocera species with various vegetation types. Cladocera remains were collected from the surface sediment of four habitat types (tangled vegetation, open water, reeds and tunnels) and 15 physical and chemical parameters of surface water were studied. In the surface sediment samples, we identified 32 Cladocera taxa. There was a significant difference in the number of species amongst habitat types as per ANOVA. The benthic and plant associated Cladocera communities of reeds, tangled vegetation, open water and tunnels were clearly separated from each other by NMDS ordination. CCA showed that habitat types had characteristic Cladocera species: *Pleuroxus* species were frequent in the tangled vegetation habitat, while *Chydorus* species were frequent in the open water. Remarkably, in reeds, *Bosmina* species were frequent, although these species are usually common in open water. Specimens of the *Alona* genus were found everywhere. Our findings suggest that the remains of Cladocera species may be useful indicators to assess and monitor the structure of freshwater lakes.

Keywords

Water-Sediment chemistry, Macrophytes, Zooplankton Indicator species

Introduction

There are several organic and inorganic remains in sediments which reflect the history of oxbows. In lake sediments, some of the most common animal remains are those of Cladocera which derive from both water and sediment (Kurek et al. 2010). The taxonomic structure of Cladocera remaining in sediment cores indicates past changes in the environment, such as eutrophication (Visconti et al. 2008, Nevalainen and Luoto 2013), acidification (Jeziorski et al. 2008) and changes in the water level (Korponai et al. 2016). Cladocera communities play an important role in a lake's food web because of their intermediate position, which means that Cladocera species have significant effects on the ecology status and water quality of lakes (Jeppesen et al. 2011, Zhi et al. 2012). Due to this position in the food web, Cladocera are sensitive indicators of environmental changes (Kurek et al. 2010).

Earlier studies indicated that the boundary zone between macrophyte beds and open water is particularly important as a refuge for cladocerans (Lauridsen et al. 1996; Davidson et al. 2010). At the same time, Cladocera communities vary with macrophyte bed size and open water which is an important daytime refuge for potentially migrating pelagic cladocerans (Lauridsen et al. 1996).

In this study, we tested the effect of habitat types of oxbows on Cladocera communities in the Upper Tisza Region, in northeast Hungary. We also studied the correlation between the water chemical parameters of Cladocera species. We hypothesised that there were Cladocera species characteristic of typical oxbow habitats and that they are useful indicators for assessing and monitoring the structure and ecological state of lakes.

Methods

Study sites

Many oxbows were formed during the 19th century with the controlling of the River Tisza. To assure shipping and flood-control, more than 100 meanders were cut. As a result, many artificial oxbow lakes were formed along the River Tisza (Babka et al. 2011, Balogh et al. 2016, 2017, Kundrát et al. 2017). In the Upper-Tisza region, there are more than 40 oxbows. This region is mainly cultivated using traditional agricultural systems with meadows, orchards and some cereal cultivation. The characteristic land use of this countryside has changed considerably during the last 200 years (Varga et al. 2013).

The oxbows studied were in the Upper Tisza region, near the town of Vásárosnamény in Hungary. The following oxbows were studied: Keskeny Holt-Tisza (48°9'5.64"N, 22°20'9.30"E), Foltos-kerti Holt-Tisza (48°5'47.58"N, 22°23'47.64"E) and Patkó Holt-Tisza (48°6'27.66"N, 22°23'1.56"E). In each oxbow, four sampling points were chosen to represent typical habitat types: tangled vegetation, open water, reeds and tunnels. In the tangled habitat, *Ceratophyllum demersum* (about 90%) and *Potamogeton natans* (about 10%) were the most abundant plant species. In the open water habitats, there were no aquatic plants. In the reed habitat, the main aquatic plant species was *Phragmites australis*. The tunnel habitats were at least 1.2 m deep and wide open surfaces with little vegetation towards the sides were typical (*Ceratophyllum demersum*).

Cladocera identification

For Cladocera identification, surface sediment subsamples (1 cm^3) were treated with 100 ml 10% KOH (Normapur, VWR) solution and heated at 100 °C for about 1 hour. Hydrofluoric acid (HF) (38%, VWR) was used to remove the inorganic material following identification. Then we added safranine O (ALFA (AESAR)) to the sample to stain the remains. We prepared quantitative slides by pipetting 100 µl of each subsample on to a microscope slide and then examined it under a microscope (B–183, OPTIKA Microscopes, Italy) at magnifications of 100 and 400; about 200 Cladocera remains were counted from each sample. Usually two slides are sufficient for identifying at least 200 remains, which is the recommended number for counting (Kurek et al. 2010). The identification was based on Bledzki and Rybak (2016) and Szeroczyńska and Sarmaja-Korjonen (2007).

Water analyses

Surface water samples were collected in plastic bottles and parallel measurements were performed at the study sites. Water depth, transparency, temperature, conductivity (WTW cond. 340i) and pH (WTW pH 315i) were measured. Samples were stored at 4 °C until the laboratory process. In the laboratory, the content of suspended solids, chlorophyll-a, Chemical Oxygen Demand (COD) and the concentrations of carbon dioxide, ammonium-nitrogen, nitrite-nitrogen, nitrate-nitrogen and orthophosphate were measured. Laboratory analyses of water samples were based on APHA (2000) and Nollet and De Gelder (2011).

Sediment analyses

To determinate the organic matter content of surface sediment, the loss on ignition method was used. After drying at 105 °C, 0.2 g samples were cremated at 550 °C for 4 h in a muffle furnace (Nabertherm L5/C6, Germany). The loss on ignition was calculated with the following equation: LOI550 = 100*(DW105–DW550)/WS, where LOI550 was the percentage loss on ignition at 550 °C, DW105 was the dry weight of samples at 150 °C and DW550 was the weight of the sample at 550 °C (Heiri et al. 2001, Matthews 2014). To determine the content of calcium carbonate in the surface sediment, the samples were burnt at 950 °C for 4 h. After cooling, we measured them with analytic scales. The calculation of the loss on ignition was conducted with the following equipment: LOI950 = 100*(DW550–DW950)/WS, where LOI950 is the percentage of loss on ignition at 950 °C and DW950 is the weight of the sample after heating at 950 °C (Heiri et al. 2001, Matthews 2014).

Statistical analyses

The benthic and plant associated Cladocera communities were studied, based on vegetation types, by non-metric multidimensional scaling (NMDS) ordination. CCA was used to display the correlation between water chemistry and the Cladocera community (Lepš and Šmilauer 2003). One-way ANOVA was used to test the effect of habitat types on Cladocera diversity and water chemistry. In the case of significant differences, Tukey's Multiple Comparison test was used (Abbott 2016).

Results

Cladocera diversity

In total, we counted 1324 Cladocera specimens in the samples; altogether, we identified 32 taxa (Table 1). There was a significant difference in the number of Cladocera species amongst the vegetation types by ANOVA ($F_{3,8} = 4.744$, $P = 0.034$) (Fig. 1). A significantly higher number of Cladocera species was found in the open water than in the reed vegetation type ($P < 0.05$). There was no significant difference in the number of Cladocera individuals amongst the vegetation types (oxbows: $F_{3,8} = 0.500$, $P = 0.693$ Fig. 2).

The benthic and plant associated Cladocera communities of reeds, tangled vegetation, open water and tunnels were clearly separated from each other by NMDS ordination. The communities of benthic Cladocera in tangled vegetation, open water and tunnels were similar to each other (Fig. 3). A similar result was found in the cases of plant associated Cladocera communities when using NMDS ordination (Fig. 4).

Water physico-chemistry and sediment chemistry differences amongst vegetation types

There were no significant differences in the water physico-chemistry parameters studied (depth: F = 1.234, p = 0.359; visibility: F = 0.591, P = 0.638; temperature: F = 0.164, P = 0.918; pH: F = 2.433, P = 0.140; conductivity: F = 0.029, P = 0.993; suspended solids: F = 1.038, P = 0.427; CO_2: F = 2.519, P = 0.132; COD: F = 0.004, P = 1.000; NH_4^+: F = 1.406, P = 0.310; NO_3^-: F = 0.696, P = 0.580; Chlorophyll-a: F

Table 1. Summary of Cladocera species and individual numbers based on the oxbows and vegetation types studied.

	Habitat affinity	Keskeny Holt-Tisza				Foltos-kerti Holt-Tisza				Patkó Holt-Tisza			
		tangled veg-etationn	open	reeds	tunnel	tangled veg-etationn	open	reeds	tunnel	tangled veg-etationn	open	reeds	tunnel
A. affinis	reeds	125	50	0	0	10	7	17	0	3	3	8	13
A. elongatus	sediment	25	0	0	0	0	0	0	0	0	0	0	0
A. emarginatus	tangled vegetation	0	0	0	0	0	0	33	0	0	0	0	0
A. excisa	tangled vegetation/ reeds	0	0	0	50	5	0	0	0	0	18	2	0
A. exigua	tangled vegetation	75	0	0	0	5	0	67	0	3	0	0	0
A. guttata	tangled vegetation/ reeds	150	50	0	17	10	0	67	0	40	4	0	0
A. harpae	plants	100	0	0	0	0	7	33	0	0	9	0	0
A. intermedia	sediment	0	250	25	17	10	7	83	22	48	7	0	25
A. nana	plants	25	50	0	17	0	0	0	0	0	0	0	13
A. quadrangularis	sediment/plant	75	100	25	0	0	0	0	67	5	0	0	41
A. rectangula	sediment	225	400	0	0	35	54	183	0	113	3	0	16
B. coregoni	open water	650	1050	1050	900	40	39	167	700	0	3	2	44
B. longirostris	plants/open water	2075	4600	2350	683	300	196	1133	344	0	1	2	53
B. longispina	open water	0	150	75	133	5	0	67	0	0	0	0	0
C. fennicus	sediment	0	0	0	0	0	4	17	0	0	0	0	0
C. gibbus	sediment	0	0	0	0	0	0	17	0	0	0	0	0
C. rectirostris	plants	0	0	25	33	0	0	0	0	0	0	0	6
C. sphaericus	sediment	175	200	25	50	25	18	150	11	25	53	2	38
D. longispina	open water	0	0	0	0	60	0	0	0	0	0	0	13
D. rostrata	sediment	25	50	0	50	0	0	17	0	0	0	0	0
E. lamellatus	sediment/plant	0	0	25	0	0	0	0	0	0	0	0	0
G. testudinaria	plants	150	150	0	0	10	7	17	0	0	0	0	0
K. latissima	plants	0	0	0	0	0	0	0	0	0	3	0	0
L. acanthocercoides	sediment/plant	0	0	0	0	0	0	0	0	0	0	0	6
L. leydigi	sediment	0	50	25	33	0	0	0	0	0	0	2	6
M. dispar	sediment	0	0	0	0	0	0	0	0	0	0	2	0
O. tenuicaudis	tangled vegetation/ reeds	0	0	0	0	0	0	17	0	10	0	0	0
P. laevis	plants	0	0	0	50	0	7	17	0	0	0	0	9
P. trigonellus	sediment/plant	25	50	0	17	10	11	33	0	3	0	0	0
P. truncatus	tangled vegetation/ reeds	0	0	0	0	0	0	17	0	13	0	0	0
P. uncinatus	sediment	0	0	0	0	0	0	0	0	0	1	0	0
S. crystallina	plants/open water	0	0	0	17	0	0	0	0	0	0	0	0

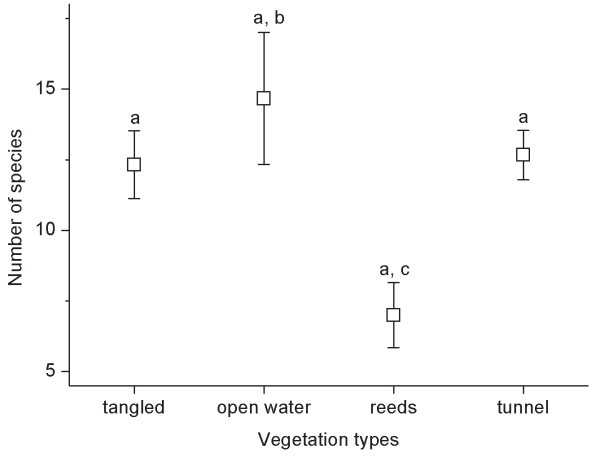

Figure 1. Number of Cladocera species by vegetation type.

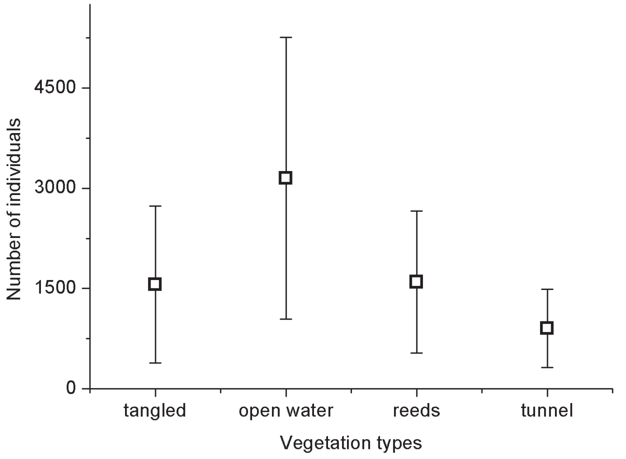

Figure 2. Number of Cladocera individuals by vegetation type.

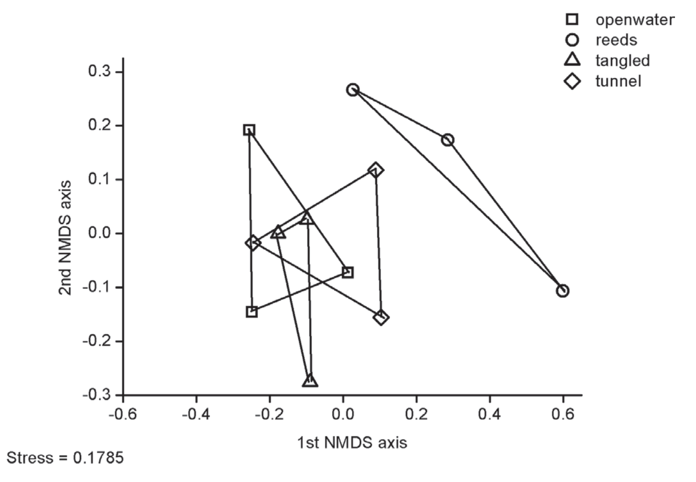

Figure 3. NMDS ordination of the benthic Cladocera remains of vegetation types (stress=0.1785).

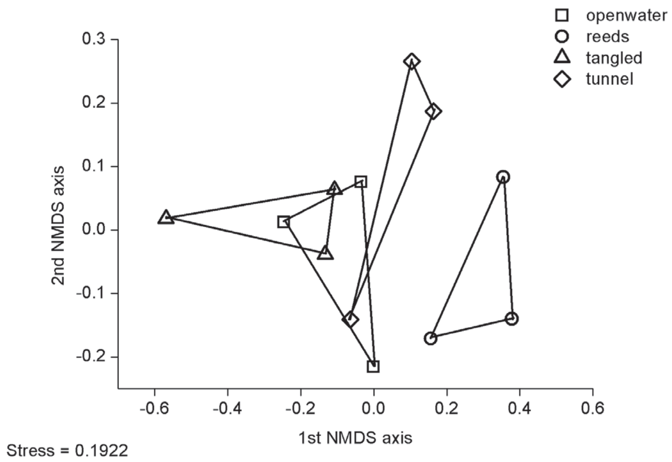

Figure 4. NMDS ordination of the plant associated Cladocera remains of vegetation types (stress=0.1922).

= 2.823, P = 0.107) amongst vegetation types (Table 2). Similar to the water physico-chemical parameters, significant differences were not found amongst vegetation types based on the organic matter content (F = 3.159, P = 0.086), nor on the calcium carbonate content (F = 0.134, P = 0.937) of sediment (Table 2).

Correlation between sediment and water chemistry and Cladocera communities

Based on the CCA ordination, our results show positive correlations between the organic matter and the calcium carbonate content of sediment and *A. elongatus*, *A. rectangular*, *L. leydigi* and *A.quadrangularis* species (Fig. 5). In the cases of water chem-

Table 2. Physical and chemical parameters of surface water and sediment (mean ± SD) according to vegetation type.

Parameters	Vegetation type			
	tangled vegetation	**open water**	**reeds**	**tunnel**
Water				
depth, cm	59 ± 20	80 ± 20	135 ± 35	75 ± 20
visibility, cm	49 ± 21	54 ± 7	73 ± 23	47 ± 4
temperature, °C	12 ± 1	11 ± 1	11 ± 1	11 ± 1
pH	8.3 ±0.3	8.6 ± 0.2	8.5 ± 0.3	7.9 ± 0.1
conductivity, µS cm^{-1}	334 ± 78	346 ± 73	343 ± 73	370 ± 920
suspended solid, mgl^{-1}	8 ± 4	10 ± 2	12 ± 8	3 ± 1
CO_2,mgl^{-1}	19 ± 5	10 ± 2	24 ± 3	15 ± 4
COD, mgl^{-1}	6 ± 5	4 ± 2	5 ± 3	6 ± 4
NH_4^+, mgl^{-1}	3 ± 1	1 ± 1	2 ± 1	1 ± 1
NO_3^-, mgl^{-1}	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
Chlorophyll-a, mgl^{-1}	6 ± 2	9 ± 3	15 ± 7	4 ± 1
Sediment				
organic matter, %	4.1 ± 0.4	3.4 ± 0.2	2.4 ± 1.0	2.9 ± 0.8
$CaCO_3$, %	0.7 ± 0.2	0.8 ± 0.3	0.7 ± 0.5	0.6 ± 0.2

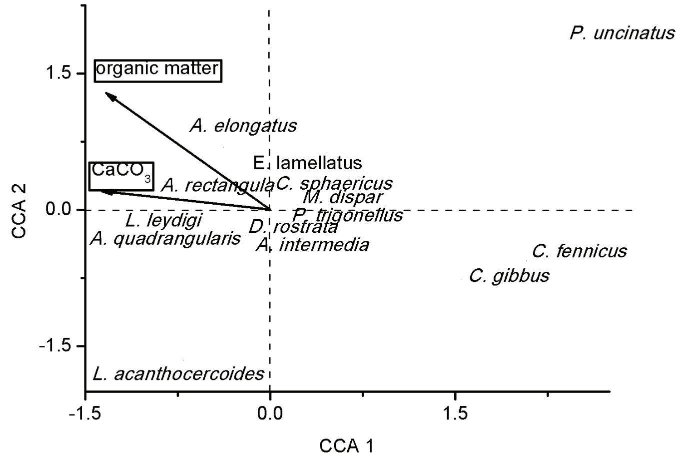

Figure 5. CCA ordination of benthic Cladocera taxa and sediment parameters.

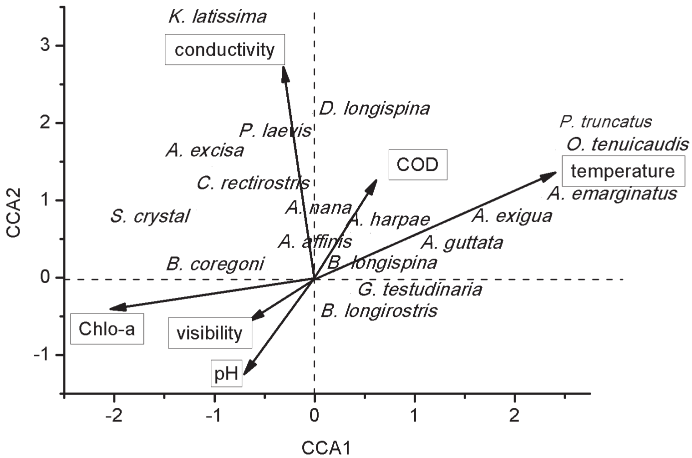

Figure 6. CCA ordination of Cladocera taxa and water chemical parameters.

istry, data positive correlations were found between conductivity and *K. latissima* species, between chemical oxygen demand and *A. nana*, *A. harpae* and *A. exigua* species and between temperature and *P. truncatus*, *O. tenuicaudis* and *A. emarginatus* (Fig. 6).

Discussion

Our study demonstrated the usefulness of Cladocera remains in the assessment of the ecological status of oxbows. Similarly to our study, Gulyás and Forró (1999) and Korhola and Rautio (2001) also demonstrated a correlation between habitat types and Cladocera species. The CCA results corroborated the habitat preferences reported by Gulyás and Forró (1999) and Korhola and Rautio (2001). At the tunnel of the Keskeny Holt-Tisza oxbow, we found the kind of Cladocera species which usually live in vegetation zones. In the tangled vegetation of the Keskeny Holt-Tisza oxbow, we found *Alonella exigua*, *Acroperus harpae* and *Alona guttata* which were typical tangled vegetation species; however, *Acroperus elongatus* and *Alona affinis* were mostly living in the biotechton of vegetation. In the tangled vegetation of the Foltos-kerti Holt-Tisza, *Alonella excisa* was a typical tangled vegetation species. We also found *Daphnia longispina* remains there; this species is characteristically an open water and/or generalist species. In tunnels, *Alona quadrangularis* was a benthic species as reported by literature. The Cladocera species we found in the tangled vegetation of the Patkó Holt-Tisza oxbow usually lived in tangled vegetation and in sediment. In the open water, we found the kind of species which usually live in sediment and in vegetation and not usually in open water. Probably these species are able to adapt quickly to the modified environmental conditions caused by human disturbance (i.e. the intense utilisation of the oxbow for recreational fishing).

There were significant differences amongst oxbows and the habitat types based on water chemistry parameters. Similar to earlier studies (Lukács et al. 2009, 2011), we found that aquatic plants influenced the water chemistry parameters. Lukács et al. [(2011) demonstrated that the amount of chlorophyll-a was very high in sweet grass beds communities, but a small amount of chlorophyll-a was found in chestnut and water lily beds. Our findings also demonstrated that there was a strong interaction between water chemistry parameters and reed habitats.

We found that temperature was in a positive correlation with the number of Cladocera individuals. Nevalainen and Luoto (2010) also reported that many Cladocera species are sensitive to seasonal temperature changes. Zawisza et al. (2016) and Wojewódka et al. (2016) reported that several studies described strong correlations between pH, conductivity and Cladocera taxa. Bjerring et al. (2009) found negative correlations between temperature and chlorophyll-a and several Cladocera taxa. We found a positive correlation between conductivity and Cladocera taxa, while a negative correlation was found between pH and Cladocera taxa.

Conclusions

Our results show that Cladocera taxa are usually associated with characteristic habitat types; however, human disturbance can change the habitat association of these species by changing the local environment conditions. Based on our results, Cladocera are useful indicators for assessing and monitoring the structure of freshwater lakes.

Acknowledgements

Csilla Lakatos and István Gyulai were supported by the TÁMOP 4.2.1/B-09/1/KONV-2010-0007 and TÁMOP- 274 4.2.2/B-10/1-2010-0024 projects of the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program'. E. Simon was supported by the TÁMOP-4.2.2.A-11/1/KONV. Research was supported by OTKA K 116639, KH 126481 and KH 126477 grants.

References

Abbott ML (2016) Using Statistics in the Social and Health Sciences with SPSS and Excel. John Wiley & Sons.

American Public Health Association (APHA) (2000) Standard methods for the examination of water and wastewater. Washington (DC).

Babka B, Futó I, Szabó S (2011) Clustering oxbow lakes in the Upper-Tisza Region on the basis of stable isotope measurements. Journal of Hydrology (Amsterdam) 410(1–2): 105–113. https://doi.org/10.1016/j.jhydrol.2011.09.026

Balogh Zs, Harangi S, Gyulai I, Braun M, Hubay K, Tóthmérész B, Simon E (2017) Exploring river pollution based on sediment analysis in the Upper Tisza region (Hungary). Environmental Science and Pollution Research International 24(5): 4851–4859. https://doi.org/10.1007/s11356-016-8225-5

Balogh Zs, Harangi S, Kundrat JT, Gyulai I, Tóthmérész B, Simon E (2016) Effects of anthropogenic activities on the elemental concentration in surface sediment of oxbows. Water, Air, and Soil Pollution 227(1): 13–21. https://doi.org/10.1007/s11270-015-2714-x

Bjerring R, Becares E, Declerck S, Gross EM, Hansson LA, Kairesalo T, Nykanen M, Halkiewicz A, Kornijow R, Conde-Porcuna JM, Seferlis M, Nöges T, Moss B, Amsinck SL, Odgaard BV, Jeppesen E (2009) Subfossil Cladocera in relation to contemporary environmental variables in 54 Pan-European lakes. Freshwater Biology 54(11): 2401–2417. https://doi.org/10.1111/j.1365-2427.2009.02252.x

Bledzki LA, Rybak JI (2016) Freshwater Crustacean zooplankton of Europe. Springer.

Davidson TA, Sayer CD, Perrow M, Bramm M, Jeppesen E (2010) The simultaneous inference of zooplanktivorous fish and macrophyte density from sub-fossil cladoceran assemblages: A multivariate regression tree approach. Freshwater Biology 55(3): 546–564. https://doi.org/10.1111/j.1365-2427.2008.02124.x

Gulyás P, Forró L (1999) A guide for the identification of Cladocera occurring in Hungary. KGI, Budapest. [In Hungarian]

Heiri O, Lotter AF, Lemcke G (2001) Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. Journal of Paleolimnology 25(1): 101–110. https://doi.org/10.1023/A:1008119611481

Jeppesen E, Nõges P, Davidson TA, Haberman J, Nõges T, Blank K, Lauridsen TL, Søndergaard M, Sayer C, Laugaste R, Johansson LS, Bjerring R, Amsinck SL (2011) Zooplankton as indicators in lakes: A scientific-based plea for including zooplankton in the ecological quality assessment of lakes according to the European Water Framework Directive (WFD). Hydrobiologia 676(1): 279–297. https://doi.org/10.1007/s10750-011-0831-0

Jeziorski A, Yan ND, Paterson AM, Desellas AM, Turner MA, Jeffries DS, Keller B, Weeber RC, Mcnicol DK, Palmer ME, McIver K, Arseneau K, Ginn BK, Cumming BF, Smol

JP (2008) The widespread threat of calcium decline in fresh waters. Science 322(5906): 1374–1377. https://doi.org/10.1126/science.1164949

Korhola A, Rautio M (2001) Cladocera and other Branchiopod Crustaceans. In: Tracking Environmental Change Using Lake Sediments. Smol JP, Birks HJB, Last WM (Eds) Kluwer Academic Publishers, Dordrecht, The Netherland, 5–41. https://doi.org/10.1007/0-306-47671-1_2

Korponai J, Gyulai I, Braun M, Kövér C, Papp I, Forró L (2016) Reconstruction of flood events in an oxbow lake (Marótzugi-Holt-Tisza, NE Hungary) by using subfossil cladoceran remains and sediments. Advances in Oceanography and Limnology 7: 131–141. https://doi.org/10.4081/aiol.2016.6168

Kundrát JT, Balogh Zs, Harangi S, Tóthmérész B, Simon E (2017) Assessment of anthropogenic, seasonal and aquatic vegetation effects on the contamination level of oxbows. Community Ecology 18(3): 237–243. https://doi.org/10.1556/168.2017.18.3.2

Kurek J, Korosi JB, Jeziorski A, Smol JP (2010) Establishing reliable minimum count sizes for cladoceran subfossils sampled from lake sediments. Journal of Paleolimnology 44(2): 603–612. https://doi.org/10.1007/s10933-010-9440-6

Lauridsen TL, Pedersen LJ, Jeppesen E, Sønergaard M (1996) The importance of macrophyte bed size for cladoceran composition and horizontal migration in a shallow lake. Journal of Plankton Research 18(12): 2283–2294. https://doi.org/10.1093/plankt/18.12.2283

Lukács BA, Dévai G, Tóthmérész B (2009) Aquatic macrophytes as bioindicators of water chemistry in nutrient rich backwaters along the Upper-Tisza river (in Hungary). Phytocoenologia 39: 287–293. https://doi.org/10.1127/0340–269X/2009/0039–0287

Lukács BA, Dévai G, Tóthmérész B (2011) Small scale macrophyte-environment relationship in an oxbow-lake of the Upper-Tisza valley (Hungary). Community Ecology 12(2): 259–263. https://doi.org/10.1556/ComEc.12.2011.2.15

Matthews JA (2014) Encyclopedia of Environmental Change. SAGE.

Nevalainen L, Luoto TP (2013) Limnological deterioration forces community and penotypic changes in cladocera: Tracking eutrophication of mallusjärvi, a lake in southern Finland. Boreal Environment Research 18: 209–222.

Nevalainen L, Luoto TP (2010) Temperature sensitivity of gamogenesis in littoral cladocerans and its ecological implications. Journal of Limnology 69(1): 120–125. https://doi.org/10.4081/jlimnol.2010.120

Nollet LM, De Gelder LSP (2011) Handbook of water analysis. CRC Press.

Szeroczyńska K, Sarmaja-Korjonen K (2007) Atlas of Subfossil Cladocera from Central and Northern Europe. Friends of the Lower Vistula Society, Świecie, Poland.

Varga K, Dévai G, Tóthmérész B (2013) Land use history of a floodplain area during the last 200 years in the Upper-Tisza region (Hungary). Regional Environmental Change 13(5): 1109–1118. https://doi.org/10.1007/s10113-013-0424-8

Visconti A, Manca M, De Bernardi R (2008) Eutrophication-like response to climate warming: An analysis of Lago Maggiore (N. Italy) zooplankton in contrasting years. Journal of Limnology 67(2): 87–92. https://doi.org/10.4081/jlimnol.2008.87

Wojewódka M, Zawisza E, Cohuo S, Macario-González L, Schwalb A, Zawiska I, Pérez L (2016) Ecology of Cladocera species from Central America based on subfossil assemblages. Advances in Oceanography and Limnology 7: 151–162. https://doi.org/10.4081/aiol.2016.6266

Zawisza E, Zawiska I, Correa-Metrio A (2016) Cladocera Community Composition as a function of physicochemical and morphological parameters of Dystrophic Lakes in NE Poland. Wetlands 36(6): 1131–1142. https://doi.org/10.1007/s13157-016-0832-x

Zhi W, Zhiyong Z, Junqian Z, Yingying Z, Haiqing L, Shaohua Y (2012) Large-scale utilization of water hyacinth for nutrient removal in Lake Dianchi in China: The effects on the water quality, macrozoobenthos and zooplankton. Chemosphere 89(10): 1255–1261. https://doi.org/10.1016/j.chemosphere.2012.08.001

Nature Conservation 29: 39–56 (2018)
doi: 10.3897/natureconservation.29.26876
http://natureconservation.pensoft.net

CONSERVATION IN PRACTICE

A peer-reviewed open-access journal
Nature Conservation
Launched to accelerate biodiversity conservation

Using field-based entomological research to promote awareness about forest ecosystem conservation

Greg P. A. Lamarre[1,3], Yohan Juin[2], Emmanuel Lapied[3,4], Philippe Le Gall[5], Akihiro Nakamura[6]

1 *Institute of Entomology, Biology Centre, Czech Academy of Sciences, Ceske Budejovice, Czech Republic* **2** *Department of Sciences, Guebre-Mariam French High school, Addis-Abeba, Ethiopia* **3** *Taxonomia Foundation, 7 rue Beccaria, 75012 Paris, France* **4** *Norwegian University of Life Sciences (NMBU), Isotope Laboratory, Fougnerbakken 3, 1430 Ås, Norway* **5** *UMR Evolution, Genomes, Comportement & Ecologie, CNRS-IRD, University of Paris-Sud, Paris-Saclay, Gif-sur-Yvette, France* **6** *CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China*

Corresponding author: *Greg P.A. Lamarre* (greglamarre973@gmail.com)

Academic editor: *M. Samways* | Received 22 May 2018 | Accepted 29 August 2018 | Published 18 September 2018

http://zoobank.org/CD1C6297-64B1-4218-B8D6-F1F86A14826D

Citation: Lamarre GPA, Juin Y, Lapied E, Le Gall P, Nakamura A (2018) Using field-based entomological research to promote awareness about forest ecosystem conservation. Nature Conservation 29: 39–56. https://doi.org/10.3897/natureconservation.29.26876

Abstract

Interactions between plants, insect herbivores and associated predators represent the majority of terrestrial biodiversity. Insects are vital food sources for many other organisms and provide important ecosystem functions and services including pollination, waste removal and biological control. We propose a complete and reproducible education programme to guide students to understand the importance of managing and conserving forest ecosystems in their region through the study of insect ecology and natural history. Our programme involved lectures, workshops and field surveys of insects with a group of 60 high school students in Eastern Africa (Ethiopia). It addresses the key stages of an entomological research project including: 1) general entomological knowledge and understanding the role of insects in terrestrial diversity and in ecosystem functioning and services; (2) the proposal of simple research questions including hypothesis development and evaluation using scientific literature, 3) fieldwork using different types of light traps; 4) sorting and identification of the insect orders using simple diagnostic keys and illustrated plates; 5) analysing and interpreting the results and 6) demonstrating findings to peers and a public audience. Identifying insects, exploring their natural history and understanding their functions in the field bring the students towards a better understanding and awareness of the importance of forest ecosystem conservation.

Keywords
Conservation awareness, forest ecosystem, Ethiopia, biological education, Des Insectes et des Hommes, public outreach

Introduction

Insects and plants have undergone a co-evolutionary arms race for over 200 million years (Labandeira et al. 1994; Van Eldijk et al. 2018). Plant-insect interactions represent almost 75% of terrestrial biodiversity composed of host plants, herbivore insects and their predators (Price 2002), which are mostly other arthropods (May 2010). Insects are central components of terrestrial biodiversity. It is therefore essential that the public is educated to understand and appreciate the multiple roles of insects in ecosystem functioning and services. Given the diversity of insects and complex nature of ecosystem functioning, however, this appreciation can be seen as an abstract notion to comprehend for many public audiences.

The importance of insects and the ecosystem functions they provide are best-illustrated by their fascinating natural history. This facilitates the use of insects as a model for an introduction to ecology and public outreach towards ecosystem conservation and management. For example, insects are soil engineers. Excluding decomposer and detritivorous insects from a forest ecosystem leads to the accumulation of unrecycled organic matter (Beynon et al. 2015). Such a build-up of organic matter can have severe consequences for nutrient cycling and other ecosystem functions. The interactions between herbivore insects and their host plants, which are under constant selective pressure, are one of the main forces driving plant species coexistence in hyperdiverse tropical rainforests (Fine et al. 2004; Lamarre et al. 2012a). The role of insects in pollination is also crucial for structuring plant species coexistence and this biotic interplay allowed many flowering plants to evolve bright colours and incredible shapes as reproductive strategies to attract insects. Some insects, such as wild bees, are beneficial to food production, as they pollinate the large majority of crops (Picanço et al. 2017), while others are agricultural pests. At the top of the food web, insect predators and parasitoids regulate populations of herbivores and other agricultural pests. This makes understanding and maintaining insect diversity vital to global food security. Our agricultural practices are expected to evolve towards agroecology which promotes natural populations of beneficial insects such as pollinators and pest controlling predators and parasitoids (FAO 2014). A better understanding of insects and their functions allow us to realise sustainable practices for our agroecosystems adapted for fluctuating climates.

Despite its global importance, entomology as a discipline has been slowly disappearing along with a lack of formal training and the rarity of insect taxonomists in universities (Leather 2009, Wilson 2017) and in conservation studies (Clark and May 2002). Leather (2009) pointed out that:

"*Entomologists are like endangered mammals such as tigers and polar bears in that they and their habitats are on the verge of extinction and this is likely to have a profound negative effect on science in general*".

The statement is generally shared amongst the scientific communities where basic biodiversity research is being neglected (Leather and Quicke 2009, Leather 2015; Wilson 2017 and references therein). Advances in entomological knowledge will be crucial for tackling future challenges such as climate changes and other global-scale anthropogenic disturbances leading to the loss of biodiversity and associated ecosystem functions. A large-scale loss of insect diversity has been reported by Hallmann and colleagues (2017) who showed a decline of over 75% in flying insect biomass within protected areas of Germany. Ongoing disturbances such as climate change are predicted to increase the impacts of insect pests and vectors on food production, human livelihoods and health (DeLucia et al. 2008; Jeffs and Lewis 2013). Such observations stimulated numerous media reactions, but, due to a lack of information and education on biodiversity and insect taxonomy, the issues behind these findings remain difficult to fully apprehend.

Here we recommend the use of insects and field study in biological education programmes to increase the understanding of the value of biodiversity. In our field study, the action of observing and inspecting live insects, in the middle of the forest at night for the first time, is unique in creating a memorable experience of nature (Borsos et al. 2018). Our education initiative utilises similar teaching methods to the British Bioblitz programme – i.e. a complete field course on insects that are easy to observe and collect in their natural habitat. We report the feasibility of the novel "Des Insectes et des Hommes" ("Insects and Humans") education programme in Ethiopia (Eastern Africa) as a case in point. To our knowledge, no biological education programme has been yet proposed using a scientific framework in the study of insect communities in the French education system.

The programme addresses every step of entomological research projects and is comparable to a typical undergraduate entomology programme. These activities include: (1) acquisition of general entomological knowledge and understanding the role of insects in terrestrial ecosystem functioning and services; (2) proposal for research questions including hypothesis development and evaluation using scientific literature; (3) fieldwork using light traps as the main collecting technique; (4) sorting and the identification of the 10 main orders of insects using illustrated plates and entomological supports; (5) database preparation, data collection and analysis and interpretation of the results; (6) communicating their findings to the public audience via oral presentation and written reports.

The general philosophy of our field course is governed by the need for investigating the identity (taxonomy), exploring natural history and understanding the functions (ecology) of insects in an observable way in the field. We envisage that this process will develop behavioural changes in the participants and presentation audiences, such as greater curiosity towards insects and their habitats and a motivation to protect them (conservation). The proposed educational programme also provides a basic understanding of natural forest ecosystems at both global and local scales, fostering local community awareness of forest habitat disturbances and the need for conservation in their region.

Methods

Study site and target participants

The French High school Guebre-Mariam in Addis-Abeba (Ethiopia) hosted the project and provided facilities for the lectures, the lab work and the student oral presentations. The target groups are primarily high school students, but this does not exclude the course being applied to other lower and higher education students as well as the wider public (Matthews and Flage 1997). We encouraged the targeting of those who did not take entomological or ecological courses. Here the case study consisted of two classes of students aged between 15 and 19 (mean age of 16.4 years) corresponding to the first year of high school in the French education system (i.e. "Seconde" in French, year 11 in UK and 10th grade in USA). Our programme was fully integrated into the biology major programme ("Sciences et Vie et de la Terre"). A total of 57 students from the two classes (3 were absent) representing 10 different nationalities were randomly allocated to six groups prior to the initiation of this programme (3 groups per class). We decided that an average of nine students per group was an adequate size for efficient interactions amongst students and teachers.

Fieldwork occurred in the Oromiya region within the Menagesha National Park, located at Suba village, 50 km east from Addis-Abeba. The protected forest represents nearly 10000 ha of altitudinal subtropical forests including 2500 ha of pristine forest and 1000 ha of plantations in the surroundings (Demissew 1988). The natural forest is dominated by endemic trees characteristic of the altitudinal highlands of Ethiopia such as the native African juniper (*Juniperus procera*), the Kousso tree (*Hagenia abyssinica*) and the endangered fern pine (*Podocarpus falcatus*). The protected area is part of an old and complex geological formation on the southwest facing slopes of the extinct Wechecha volcano (3,385 m). The project site received almost 1100 mm of precipitation from June to September with an average temperature of 11 °C. This pristine mountain forest remnant also hosts many native and endemic fauna and is thus an important refugia for endangered species, such as the two endemic mammals, the Menelik Bushbuck (*Tragelaphus scriptus meneliki*) and the White-footed mouse (*Stenocephalemys albipes*). Few participants in our course had previously visited this exceptional national park even though it is the last native primary rainforest in the vicinity of Addis-Abeba.

Implementation procedure

We led a complete and reproducible education programme using the study of insect to guide the students towards understanding the importance of conserving and managing forest ecosystems in their region. The programme ran for one entire school week with approximately 8 hours of activities per day (Monday to Friday, 18–22 April 2016) which included a total of 5–6 hours fieldwork at night. Five sequences are presented chronologically below to describe the programme activities. Finally, we discussed the education implications of our course by summarising feedback from the students and teachers 18 months after the programme was concluded.

Sequence #1: Introduction to insect ecology (Day 1, lectures ~ 4h). The first sequence consisted of a general introduction to insect diversity and ecology (Figure 1). Two lectures of 2 hours were given by GPAL in an amphitheatre and served to introduce insects as key components of terrestrial biodiversity, focusing on their extreme abundance and species richness. The lecturer also addressed the importance of insects in providing ecosystem functions and services. We explained the principles of co-evolutionary theory with some examples of anti-herbivore traits such as trichomes on the leaves of plants easily visible in the field. We also taught that herbivorous insects tend to be specialised to feeding on a few evolutionary related host plant species (Novotny and Basset 2005). We discussed the ecological and evolutionary factors that determine the degree to which a herbivore is associated with one or multiple plants. Specialisation is a crucial notion to comprehend because it is related to patterns of association, coexistence and diversification of insect and plant assemblages. GPAL introduced the most dominant insect orders likely to be collected by the students and their range of microhabitats in the forest (Figure 1). Prior to identification of insects to order level (Seq: #3 and #4), the lecture also highlighted the ecological functions of insects in forest ecosystems (e.g. pollinators, scavengers, decomposers, predators). In conjunction with their fascinating natural histories, this context was needed to understand the importance of studying insect functional diversity (see Lamarre et al. 2016).

Finally, we presented the daily schedule of the programme to all participants and formed the field groups before organising the equipment. The participants were encouraged by teachers to ask practical as well as scientific questions about the programme. As a mandatory step prior to any scientific project, we recommended a literature review. In our case, students reviewed online biodiversity studies pertinent to Ethiopian forest ecosystems. We introduced students to an online scientific literature search engine, in this case Google Scholar, which was previously unknown to most students. However, we found neither a local insect list nor any entomofaunal knowledge for our study region (with the exception of information associated with human related vector mosquitoes and flies, but see Rougeot 1977). Such an education project therefore has the potential to produce a basic insect species list (e.g. an inventory) and is an effective form of citizen science (Campanaro et al. 2017, Scheuch et al. 2018) similar to the Bioblitz project in the UK. Detailed inventories, generated from our field course, can serve as a baseline study on the insect communities found in Menagesha National Park.

Sequence #2: Workshop for building research questions (day 1, lab activities ~2h). We allocated student groups to two classrooms to keep the number of students manageable during the workshop. Instructors, GPAL and YJ, led the workshop discussion. In each class, in concert with the students that reached some specific interests

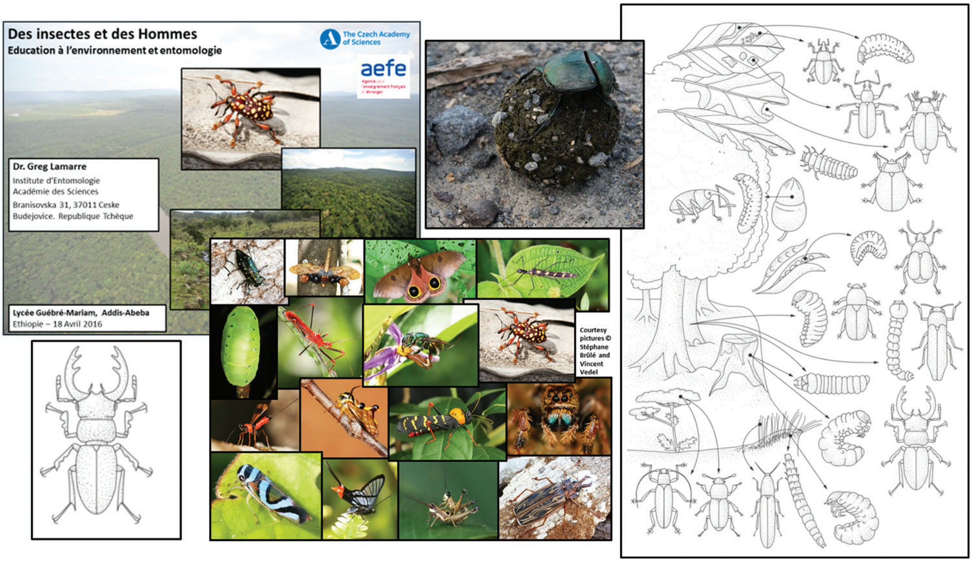

Figure 1. Slide, plates and pictures used for the teaching of the role of insect in global diversity and ecosystem functioning (Seq#1). It includes the PowerPoint presentation for lectures; an illustration of a dung beetle to present their crucial role as soil engineers; an illustrated plate of the multiple arthropods observed in tropical rainforests and finally the myriad insects inhabiting one unique tree associated with different niches (Evans, 1977).

in particular insect group, we proposed a series of simple research questions on insect community composition and structure (community ecology). These questions were related to the previous lectures and were feasible to investigate within the duration of the fieldwork (i.e. generating a sufficient dataset) and the timeline of the project (Table 1). Instructors needed to prepare a list of questions prior to the workshop to facilitate discussion and, if necessary, modify questions in the context of the local area, forest structure, compositions and weather conditions.

During this workshop, one group decided to investigate the three most dominant insect orders found in the national park to generate an illustrated leaflet detailing the charismatic and dominant insects for future visitors. GPAL briefly lectured in Sequence #1 about other insect sampling techniques that might be employed in forest ecosystems and the importance of trap choice in targeting specific insect functional groups (Lamarre et al. 2012b). We introduced two different types of light traps in order to study and compare the number of collected individuals and assemblage composition of the target insect orders. Students from two groups compared the differences in efficiency between the two light trap techniques amongst different groups of insects, allowing them to understand the insect flight activity and seasonality. We discussed the origin and differences in attraction of insect groups to lights (phototactic responses). Sampling efficiency was compared in terms of capturing the dominant, representative species of the local habitats and in terms of capturing species of the target group (e.g. moths). We discussed with the students how to structure a dataset (order × sample × trap) we will generate from the light trapping (Table 1). For some groups, we devel-

Table 1. Scientific questions proposed and examined by each of the six groups during the programme Des Insectes et des Hommes with the themes and topics relevant to the questions.

Groups	No students	Questions (FR)	Questions (ENG)	Themes and relevant topics related to lectures	Proposed by
Group 1	12	Quels sont les différents types de pièges utilisés pour réaliser un échantillonnage standardisé d'une communauté d'insecte?	Which insect traps do we use to sample a standardised insect community?	Methodology (Insect sampling), complementarity of traps for distinct taxonomic and/or functional groups, Inventory and monitoring techniques (day-night)	Instructors
Group 2	10	Comment, après la collecte, trier les insectes?	How, after sampling, do entomologists sort their insect collection?	Methodology (procedure after sampling), entomology (sorting, organising, labelling, identification), museum (conditioning and transport, fate in collection)	Students
Group 3	10	Quelles sont les différences de captures d'insectes entre les deux types de pièges lumineux?	What are the differences in insect community structure (abundance-based per order) between the two different light traps?	Physiology (contrasting responses to light intensity, phototaxis), range of light attraction amongst forest habitats (spatial independence between traps), ecology (insect diversity of Ethiopian forest)	Instructors
Group 4	8	Quels sont les trois ordres dominants d'insectes de la forêt de Menagesha?	What are the three most dominant insect orders in the Menagesha National Park?	Conservation biology, biodiversity study (species list, field inventory for protected area), ecology, (community structure and composition in subtropical altitudinal forest)	Students
Group 5	8	Par quel type de piège lumineux les lépidoptères sont-ils le plus attirés ?	What is the most efficient light trapping technique to capture a moth community?	Conservation biology (biological indicator), methodology (efficiency), long-term monitoring (umbrella species, Lepidoptera), sampling bias	Instructors
Group 6	9	Quelles sont les différences d'abondance de capture de Coléoptères et de Lépidoptères entre le 19 avril et le 20 avril ?	What is the relative abundance in Lepidoptera and Coleoptera before and after rainy events?	Ecology, climate change (responses of contrasting functional groups under rainy event), plant-insect interactions (food sources for herbivore insects with host-plant producing new leaves early rainy season)	Instructors/ students
Overall	57				

oped a posteriori research question during the fieldwork. For instance, a couple of hours of rainfall during the second night of trapping created the opportunity to study the effect of weather variations on insect activities and, ultimately, the assemblage of captured insects. Students also studied the differences in abundance of Coleoptera (beetles) and Lepidoptera (moths), the two most dominant taxa, between clear and rainy nights. This, however, relies on a random event (rainfall) and was therefore not reproducible. Additional questions would have also been possible on butterfly species in the genus *Papilio* and *Graphium*, for example, to investigate the feeding behaviour of these two genera and identify the plants which they visited for pollen (see Jemal and

Getu 2018). Butterflies are excellent models for exploring and to disseminating ideas on feeding ecology, trophic interactions and food webs, especially in species-specific assemblages of plants in threatened montane forests. We need to acknowledge, however, that the choice of habitats, their diversity and the extent to which students will sample with sufficient intensity are not reproducible. Finally, we recommended the educators to remain flexible in terms of questions or objectives proposed to the students and to consider simple "scientifically safe" questions related to lectures (Seq#1) relevant to the topics (entomology, ecology and conservation) and feasible during a short programme.

Sequence #3: Fieldwork using light traps (first class, day 2; and second class, day 3). Prior to the students' arrival, we first explained the survey protocols (including the safety procedures) to the national park service and rangers. Each student from the two classes performed one entire day and a part of the night in the field. We set up two types of light traps in the forest understorey for two nights at around 1 km from the camp in the afternoon (~2 hours taken for trap installation, day 2). The first light trap consisted of a 2.5 m × 1.5 m white sheet attached between two tree trunks using ropes. We suspended one 250 W mercury vapour bulb, powered by a generator, in the upper centre of the white sheet to attract nocturnal flying insects (Figure 2). A small camp (with a few students) was established near the trap to protect the equipment from water damage. At least 500 m from the first light-trap, we set up a portable light trapping device. This second light trap illuminated an 80 cm cylindrical white sheet using a 30 cm actinic tube of 12 W black light (Figure 2). This type of actinic lamp powered by a small-sized battery could also be used alone for the programme, as mercury vapour lamps, ballasts and generators are not always accessible.

During the day, we also introduced the use of aerial fruit traps and pitfall traps for collecting the butterflies, wasps, ants, beetles and spiders commonly found in the understorey and in the canopy (Suppl. material 1: Figure S1). The students, however, ultimately only collected data from the light-traps, as this technique yielded the highest abundance and diversity of insects in the limited sampling period available. Light trapping has a unique advantage amongst other sampling techniques, as this allowed students to observe closely the behaviour of live insects as well as interesting interactions, such as predation and competition. Light trapping also provides a unique opportunity to introduce some morphological traits characterising a distinct insect order and their ecology (but insects need to be immobile on the sheet). For example, the scaled wings are a diagnostic feature of adult Lepidoptera and the proboscis is related to the function of the organism in the ecosystem (pollination).

Fieldwork is often considered as the exciting and adventurous part for field biologists. We shared our experiences of working in tropical countries with the students before dinner (1800 h) and prepared to reach the light trapping sites with headlamps. Before commencing night-time fieldwork, the instructors conducted the safety briefing (e.g. with regards to venomous insects, generator cables and cold weather), explained the schedule and, with students, organised the equipment such as collecting and killing jars (for safety reasons, we used nail polish instead of cyanides), forceps and sample labels. When arriving at light traps, most participants were quick to show an interest in

Figure 2. Pictures from the light-trapping session with two classes of the French High school Guebre-Mariam of Addis- Abeba (Seq#3). The students developed specific entomological skills from the different methods and recognised the choice of the trap needed to target a given insect. The students worked at night and respected safety procedure with toxic products (see killing jars) and finally were able to collect live insects manually. The second light trap technique (i.e. actinic lamp) is shown on the two left pictures while the manual light trap is shown on the four right-side pictures.

knowing the names of collected insect specimens. The illustrated plates (Figure 3), used for identifying the insects, were first presented prior to the collection and used while sampling. Once the groups were fully prepared, GPAL demonstrated how to sample the insects on the sheet to individual students. Students then captured insects on the sheet directly using plastic jars or forceps (Figure 2). At least one instructor supervised one light trap during the entire duration of the fieldwork. The instructors ensured that each group collected arthropods representing the majority of the focal taxonomic groups found on the white sheet, reported the time, date, type of light trapping and weather conditions in their notebook before switching to the second light trap. The instructors also ensured that each group collected insect samples at least twice from each of the two light traps. Between light trapping events, one out of the three groups moved towards the camp in order to start sorting, labelling and organising the insect specimens. We sampled insects until 2300 h or 2400 h. Instructors and some volunteering students then organised and transported the equipment back to the camp and cleaned the site.

Sequence #4: Sorting and identification of the main orders of insects (day 4, lab activities ~6h). One of the objectives of the programme was for the students to be able to identify the major insect orders observed in situ and to take a closer look at insect morphological and functional traits under the microscope. For this purpose, we created coloured plates illustrating examples of insects belonging to each order and simple taxonomic identification keys (Figure 3), which helped students recognise diagnostic

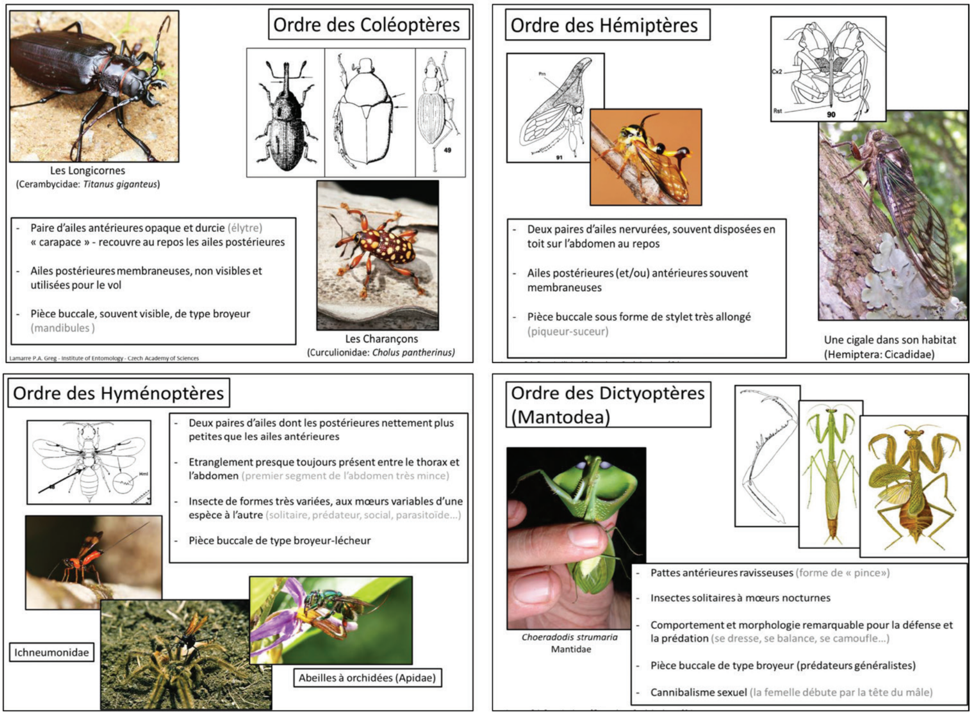

Figure 3. Illustrated plates of some insect orders created for the programme, used to provide the scientific terminology and enabled students to apply and use knowledge connected to the taxonomy of a given insect (Seq#3, #4). Plates are available upon request to the corresponding author.

features of each insect order (see Delvare and Aberlenc 1989). By using these plates, most student groups were able to count and identify their specimens on a petri dish to order (Figure 4). We validated the students' insect identifications and asked them to explain which morphological features were used to identify and sort insects (whilst relating insect traits to an ecosystem function). They finally put individuals belonging to the same order into the same falcon tubes (50 ml) with 70% ethanol to preserve them along with a corresponding label (order, type of trap, location and date). For moth specimens, we explained how to preserve the specimens in a glassine envelope.

All individual specimens from light trapping were counted and identified to order level. Collected specimens included Lepidoptera (moths), Coleoptera (beetles), Hemiptera (true bugs), Orthoptera (grasshoppers), Hymenoptera (bees, wasps, ants), Diptera (flies, mosquitoes), Mantodea (praying-mantises), Phasmatodea (stick insects) and also other arthropods (e.g. spiders). We observed that most of the difficulties in identification were in distinguishing Diptera from Hymenoptera, in some cases one pair of wings was detached or absent. Instructors were able to work interactively with the students to resolve their identification problems and pointed out additional useful morphological traits (e.g. buccal appendices in addition to wing pattern). The use of drawings in notebooks coupled with a microscope camera helped students to learn

Figure 4. Pictures of the laboratory activities including the organisation, sorting and counting of the collected specimen using a systematic procedure under microscope (Seq#4).

the diagnostic characteristics effectively. We also recommended the use of a projector, which offers a direct visualisation of the differences in insect morphology illustrated by high-resolution pictures or diagrams. Lepidoptera, Coleoptera and Hemiptera were the most successfully identified taxa. Some large-sized Hemiptera in Coreidae, Reduviidae and Pentatomidae families were sometimes confused with beetles. Mantodea and Phasmatodea were well distinguished from other orders after the first observation. Separating the different orders in the field (after observation and collection) and the lab (under microscope) received positive feedback by the students and was generally perceived as less challenging than the identification of specimens at higher taxonomic level (family or genus). However, confirmation of students' identifications by instructors was needed. This often generated valuable discussions within a small group of students on the process of discriminating key morphological features amongst insect orders and on functional attributes related to ecosystem services (e.g. pollen-carrying apparatus in bees).

Sequence #5, #6: How to present your data and report your findings? (day 5, lab activities ~5h). We introduced Microsoft Excel software and gave instructions on correct data formatting procedures (Excel was projected on a screen from the teacher's computer). First, one student per group volunteered to enter the insect data. It consisted of the abundances (the number of individuals) of insect orders, the abundances per sampling nights for each insect order or, for some student groups, the abundances associated with the total number of individuals of each order collected between rainy and dry nights from the two type of traps. Each group composed one Excel spreadsheet. Second, GPAL and YJ visited each group to show students how to generate graphical diagrams of their data in Excel. Most of the students chose either bar plots or Venn diagrams to present their results (Figure 4). Using a projector in the classroom, GPAL presented the procedure of constructing a statistical linear model illustrated by a simple correlation plot. Students were asked to think collectively on the interpretation and the presentation of their results and discussion. Instructors ensured that each group work towards their previously devised research objectives when preparing their presentations. If research questions were not sufficiently addressed with their data, instructors provided more guidance and support. Each group worked on a Microsoft Power Point presentation that consisted of 8–10 slides with simple graphics and fieldwork pictures, sufficient for a 5–10 min oral presentation (Figure 5). Students were encouraged to present any findings relevant to the topics covered during the lectures, lab and fieldwork, which may increase scientific knowledge of the site (Table 1). We also emphasised the potential use and application of their findings to local forest conservation.

This biological education programme not only taught scientific procedure and methods, but also trained the students to present to the public audience, sometimes for the first time. This last step was mandatory as it is a common method of communicating the results of any scientific project and is a valuable transferable skill for students to learn. Presentations generally involved demonstrating their results and conclusions to peers and the public audience through using scientific articles, conference presentations, posters and popular science articles. Each group prepared their presentation and selected designated speakers for a given number of slides in running order. At least one practice run was carried out per group. The students of each group presented their findings in the high school amphitheatre in front of about 80 participants consisting of students from other classes, parents, teachers, project partners and administrative employees. At the end of each presentation, five minutes were given for questions from the audience, generally with encouragement from the biology teachers (Figure 5). Finally, two months after the project, YJ and colleagues extended the programme by asking the students to review their knowledge through written scientific exercises on an online scientific blog. To date, 36 articles have been reviewed and published by the science department of the Ethiopian high school (with 19,248 views as of 17.08.2018).

Educational implications

Biology teachers wanting to conduct this field course, should first gain a basic understanding of insect ecology (with the help of entomologists) to aid in the implementation and learning outcomes of the course. When building the proposal for the project, we found it extremely important to integrate the programme "Des insectes et des Hommes" in advance into the school's formal annual plan of science

Figure 5. The French High School Guebre-Mariam, managed by the Mission laïque française and government-regulated with the AEFE (Agency for French education abroad). An example of results produced during the programme and, inside the high school, the amphitheatre that offers the opportunity for students to organise and follow their own symposium, a chance to develop maturity, motivation, leadership and self-confidence (Seq#5#6).

courses. This integration also provided teacher (YJ) an opportunity to be involved in participative science, a goal encouraged by the French Ministry of Education. This programme trains students effectively in scientific writing and communication in at least three different ways. First, students learnt a large array of scientific writing and communication skills, such as labelling, note taking, data entry, analysis, interpretation and oral presentation (e.g. cognitive dimension, Gaskins et al 1994). Second, the students strengthened their scientific reasoning and logic by generating research questions from examining and understanding the component of a problem (e.g. the conservation implications of regional forest fragmentation) and interpreting of the results (e.g. "we observed an increase in the abundance of insects following raining event"). In turn, this may provide an advantage for the students when applying for competitive jobs and university positions. The education project can introduce and illustrate potential vocations in biology such as biodiversity and conservation studies and forest management. Students also learned to use specific entomological equipment and followed rigorous protocols in the field and lab. Finally, we emphasise that being part of a young group of scientists has important social implications (Vérin 1995) and generates beneficial effects and positive group dynamics that enhances the whole experience.

Learning outcomes

Insects are often perceived as pests. Interestingly, this programme helped change students' attitudes towards insects through the experience of sampling and handling live insects. This is one of the main advantages of using light trapping, which yields a large number of live specimens. As we naturally fear what we do not understand, insects trigger strong emotions in us, both positive (diversity, shape and colours) and negative (fear of bites and diseases). Educating about insects through field experience is one way of convincing society at large that insects are more than just "creepy crawlies".

To investigate how the students perceived insects in general and understood their biodiversity and roles in ecosystem functioning, we sent an online questionnaire eighteen months after the programme concluded. We proposed a series of questions to 1) a test-group of students that followed the educational programme and 2) a control-group of students that did not attend the programme (i.e. the 3 absent students). The students who attended the programme gave a very good evaluation (100% of the students gave positive marks). Students were asked to choose a few words from a provided selection to describe the roles of insects in terrestrial ecosystems (Suppl. material 1: Figure S2). The students, who participated the programme, chose mainly "interesting" and "fascinating" (71% and 57%, respectively) and, to a lesser extent, "essential" (29%), "useful" (43%)," vital" (21%) and "curious" (21%). Students who did not participate to the programme described insects mostly as "disgusting", "small" and "ugly". However, with only three students who were absent and forming the control-group, we were not able to statistically confirm this trend. Only the test-group selected "useful" and "vital" and made the link with insect ecosystem services. Most of the students who cited insect groups as agents of ecosystem services included bees and butterflies (included in 60% of their answers). Finally, the students were asked to grade the programme "Des insectes et des Hommes" overall as an education tool for biology education. The answers confirmed that the aims of the programme were fulfilled, as illustrated by the use of specific vocabulary on the importance of biological education ("educational", "informative", "interesting", "rewarding", "patience"), ecological roles of insects ("pollination", "biodiversity", "useful", "fragile") and the emotional feeling associated with emerging curiosity about insects ("surprising", "fascinating", see Suppl. material 1: Figure S2).

Despite the often uncomfortable and unusual working environment, all students selected the fieldwork as their favourite activity during the project (Sequence #3). Our results confirmed that observing and collecting insects in the field can invoke interest, wonder and curiosity towards local natural ecosystems, increase understanding of their importance in terrestrial biodiversity and ecosystem functioning and finally leading the audience to increasing conservation awareness. Due to the limited number of students in the control-group, we were not able to adequately analyse the social benefits and cognitive education merits of our project. However, the overall positive feedback certainly assures the significance of our education programme. We recommended using similar online questionnaires after conducting the programme as this allows for comparisons in the perception of participants and non-participants towards biodiversity and nature conservation.

Conclusion

We conclude that students who participated in this programme gained a better understanding of the extent of terrestrial diversity and its relationship to the crucial ecosystem functions and services provided by insects. Many insect groups, which the students observed during the light trapping, were identified successfully using simple taxonomic identification keys, the illustrated plates and support from demonstrators. Furthermore, the students understood that entomologists are investigating insect functional traits (as observed under microscope) to help find solutions to the challenges our planet faces such as climate change and deforestation (Basset et al. 2017). They were able to relate the insect morphological traits to given functions in the ecosystem (e.g. those that might be disrupted), which increased their beneficial knowledge in ecology and conservation. As acknowledged in the introduction, entomology offers many options by using insects as an educational model in conservation, such as the study of specific functional groups responsible for crucial ecosystems services. Understanding trophic interactions, such as between plants and herbivorous insects and ecosystem functioning, represent an important cognitive knowledge base for increasing awareness of their local environment and conserving their natural habitats. We are encouraging scientists in biodiversity research to share their expertise and participate actively, collaborating with local biological education programmes, to increase essential knowledge in ecology and promote awareness about ecosystem conservation (see Ghimire et al. 2014). Both the French and Ethiopian institutions, local tourist committee and the French international education network AEFE confirmed the importance of environmental education and public outreach in places where natural ecosystems are most perturbed and at risk, such as in remote areas of Eastern Africa. We conclude the programme with a reminder that forest ecosystems in Ethiopia are of exceptional importance and deserve to be protected for future generations.

Acknowledgments

This work was supported by The Agency for French Education Abroad through the *Actions Pédagogiques Pilotes* (APP-AEFE) `2015–2016' under Grant 275H01. The European Research Council Grant (No. 669609) supported GPAL during the writing of the paper. We are thankful to education services and direction of the French High school Guebre-Mariam that helped during the week. Discover Abyssinia Tours provides the logistic support, safety and vehicles (http://www.dabyssinia.com.et/). We are thankful to the rangers and employees of the Menagesha-Suba Forest associate to the enthusiasm of all the students that participated in the project. We are grateful to Stéphane Brûlé and Vincent Vedel (SEAG) for sharing pictures. We thank Yves Basset, Simon Segar and Christopher Jeffs for valuable comments and discussions.

References

Basset B, Lamarre GPA, Ratz T, Segar S, Decaëns T, Rougerie R, Miller SE, Perez F, Bobadilla R, Lopez Y, Alejandro Ramirez J, Aiello A, Barrios H (2017) The Saturniidae of Barro Colorado Island, Panama: A model taxon for studying the long-term effects of climate change? Ecology and Evolution 7(23): 9991–10004. https://doi.org/10.1002/ece3.3515

Beynon SA, Wainwright WA, Christie M (2015) The application of an ecosystem services framework to estimate the economic value of dung beetles to the U.K. cattle industry. Ecological Entomology 40(S1): 124–135. https://doi.org/10.1111/een.12240

Borsos E, Patocskai M, Boric E (2018) Teaching in nature? Naturally! Journal of Biological Education 10: 1–11. https://doi.org/10.1080/00219266.2017.1420679

Campanaro A, Hardersen S, Redolfi De Zan L, Antonini G, Bardiani M, Maura M, Maurizi E, Mosconi F, Zauli A, Bologna MA, Roversi PF, Sabbatini Peverieri G, Mason F (2017) Analyses of occurrence data of protected insect species collected by citizens in Italy. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 265–297. https://doi.org/10.3897/natureconservation.20.12704

Clark JA, May RM (2002) Taxonomic bias in conservation research. Science 297(5579): 191–192. https://doi.org/10.1126/science.297.5579.191b

Demissew S (1988) The floristic composition of Menagesha State Forest and the need to conserve such forests in Ethiopia. Mountain Research and Development 8(2/3): 243–247. https://doi.org/10.2307/3673454

DeLucia EH, Casteel CL, Nabity PD, O'Neill BF (2008) Insects take a bigger bite out of plants in a warmer, higher carbon dioxide world. Proceedings of the National Academy of Sciences of the United States of America 105(6): 1781–1782. https://doi.org/10.1073/pnas.0712056105

Delvare G, Aberlenc HP (1989) Les insectes d'Afrique et d'Amérique tropicale. Clés pour la reconnaissance des familles. CIRAD-GERDAT, Prifas, Montpellier.

Evans G (1977) The Life of Beetles, 232 pp. Allen & Unwin, London.

FAO (2014) Agroecology for Food Security and Nutrition. Proceedings of the FAO International Symposium, 18–19 September 2014, Rome, Italy. Biodiversity & Ecosystem Services in Agricultural Production Systems. http://www.fao.org/3/a-i4729e.pdf

Fine PVA, Mesones I, Coley PD (2004) Herbivores promote habitat specialization by trees in Amazonian forests. Science 305(5684): 663–665. https://doi.org/10.1126/science.1098982

Gaskins IW, Guthrie JT, Satlow E, Ostertag J, Six L, Byrne J, Connor B (1994) Integrating instruction of science, reading, and writing: Goals, teacher development, and assessment. Journal of Research in Science Teaching 31(9): 1039–1056. https://doi.org/10.1002/tea.3660310914

Ghimire R, Green GT, Poudyal NC, Cordel HK (2014) Do outdoor recreation participants place their lands in conservation easements? Nature Conservation 9: 1–18. https://doi.org/10.3897/natureconservation.9.7981

Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, Stenmans W, Müller A, Sumser H, Hörren T, Goulson D, de Kroon H (2017) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS One 12(10): e0185809. https://doi.org/10.1371/journal.pone.0185809

Jeffs CT, Lewis OT (2013) Effects of climate warming on host-parasitoid interactions. Ecological Entomology 38(3): 209–218. https://doi.org/10.1111/een.12026

Jemal A, Getu E (2018) Diversity of butterfly communities at different altitudes of Menagesha-suba state forest, Ethiopia. Journal of Entomology and Zoology Studies 6(2): 2197–2202. http://www.entomoljournal.com/archives/2018/vol6issue2/PartX/6-1-213-212.pdf

Labandeira C, Dilcher D, Davis D, Wagner D (1994) Ninety-seven million years of angiosperm-insect association: Paleobiological insights into the meaning of coevolution. Proceedings of the National Academy of Sciences of the United States of America 91(25): 12278–12282. https://doi.org/10.1073/pnas.91.25.12278

Lamarre GPA, Baraloto C, Fortunel C, Davila Cardozo N, Mesones I, Grandez Rios J, Rios M, Valderrama E, Pilco MV, Fine PVA (2012a) Herbivory, growth strategies and habitat specialization in tropical tree lineages: Implications for Amazonian beta-diversity. Ecology 93(sp8): S195–S210. https://doi.org/10.1890/11-0397.1

Lamarre GPA, Molto Q, Fine PVA, Baraloto C (2012b) A comparison of common flight interception traps to survey tropical arthropods. ZooKeys 216: 43–55. https://doi.org/10.3897/zookeys.216.3332

Lamarre GPA, Hérault B, Fine PVA, Vedel V, Lupoli R, Mesones I, Baraloto C (2016) Taxonomic and functional composition of arthropod assemblages across contrasting Amazonian forests. Journal of Animal Ecology 85(1): 227–239. https://doi.org/10.1111/1365-2656.12445

Leather S (2009) Taxonomic chauvinism threatens the future of entomology. Biologist 56: 10–13. https://cdn.harper-adams.ac.uk/document/profile/Leather_Biologist_2009.pdf

Leather SR, Quicke DLJ (2009) Where would Darwin have been without taxonomy? Journal of Biological Education 43(2): 51–52. https://doi.org/10.1080/00219266.2009.9656151

Leather SR (2015) Influential entomology: A short review of the scientific, societal, economic and educational services provided by entomology. Ecological Entomology 40: 36–44. https://doi.org/10.1111/een.12207

Matthews RW, Flage LR (1997) Insects as teaching tools in primary and secondary education. Annual Review of Entomology 42: 269–289. https://doi.org/10.1146/annurev.ento.42.1.269

May RM (2010) Tropical Arthropod species, more or less? Science 329(5987): 41–42. https://doi.org/10.1126/science.1191058

Novotny N, Basset Y (2005) Host specificity of insect herbivores in tropical forests. Proceedings. Biological Sciences 272(1568): 1083–1090. https://doi.org/10.1098/rspb.2004.3023

Picanço A, Gil A, Rigal F, Borges PAV (2017) Pollination services mapping and economic valuation from insect communities: A case study in the Azores. Nature Conservation 18: 1–25. https://doi.org/10.3897/natureconservation.18.11523

Price PW (2002) Resource-driven terrestrial interaction webs. Ecological Research 17(2): 241–247. https://doi.org/10.1046/j.1440-1703.2002.00483.x

Rougeot PC (1977) Missions entomologiques en Ethiopie 1973–1975. Mémoires du Muséum National d'Histoire Naturelle. Série A, Zoologie. Muséum national d'histoire naturelle (Paris) – Direction des bibliothèques et de la documentation 105: 198.

Scheuch M, Panhuber T, Winter S, Kelemen-Finan J, Bardy Durchhalter M, Kapelari S (2018) Butterflies & wild bees: Biology teachers' PCK development through citizen science. Journal of Biological Education 52(1): 79–88. https://doi.org/10.1080/00219266.2017.1405530

Van Eldijk TJB, Wappler T, Strother PK, van der Weijst CMH, Rajaei H, Visscher H, van de Schootbrugge B (2018) A Triassic-Jurassic window into the evolution of Lepidoptera. Science Advances 4(1): e1701568. https://doi.org/10.1126/sciadv.1701568

Vérin A (1995) Mettre par écrit ses idées pour les faire évoluer en sciences. Repères 15: 21–36. https://www.persee.fr/docAsPDF/reper_1157-1330_1995_num_12_1_2155.pdf

Wilson EO (2017) Biodiversity research requires more boots on the ground. Nature Ecology & Evolution 1(11): 1590–1591. https://doi.org/10.1038/s41559-017-0360-y

Supplementary material 1

Supplementary figures

Authors: Greg P.A. Lamarre, Yohan Juin, Emmanuel Lapied, Philippe Le Gall, Akihiro Nakamura

Data type: multimedia

Link: https://doi.org/10.3897/natureconservation.29.26876.suppl1

Nature Conservation 29: 57–78 (2018)
doi: 10.3897/natureconservation.29.27273
http://natureconservation.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Nature Conservation
Launched to accelerate biodiversity conservation

Current status of habitat monitoring in the European Union according to Article 17 of the Habitats Directive, with an emphasis on habitat structure and functions and on Germany

Götz Ellwanger[1], Stephan Runge[2], Melanie Wagner[2], Werner Ackermann[3], Melanie Neukirchen[1], Wenke Frederking[1], Christina Müller[1], Axel Ssymank[1], Ulrich Sukopp[1]

1 *Federal Agency for Nature Conservation, Konstantinstr. 110, 53179 Bonn, Germany* **2** *LB Planer+Ingenieure GmbH, Luftbild Brandenburg, Eichenallee 1a, 15711 Königs Wusterhausen, Germany* **3** *PAN Planungsbüro für angewandten Naturschutz GmbH, Rosenkavalierplatz 8, 81925 München, Germany*

Corresponding author: *Götz Ellwanger* (goetz.ellwanger@bfn.de)

Academic editor: *S. Lengyel* | Received 21 June 2018 | Accepted 13 September 2018 | Published 4 October 2018

http://zoobank.org/CB795315-B99F-4D69-A3AE-9EBDBD74A596

Citation: Ellwanger G, Runge S, Wagner M, Ackermann W, Neukirchen M, Frederking W, Müller C, Ssymank A, Sukopp U (2018) Current status of habitat monitoring in the European Union according to Article 17 of the Habitats Directive, with an emphasis on habitat structure and functions and on Germany. Nature Conservation 29: 57–78. https://doi.org/10.3897/natureconservation.29.27273

Abstract

Since the beginning of the 1990s, monitoring of habitats has been a widespread tool to record and assess changes in habitat quality, for example due to land use change. Thus, Article 11 of the Habitats Directive (92/43/EEC) requires, inter alia, monitoring of the conservation status of habitat types listed in Annex I of the Habitats Directive, carried out by the Member States of the European Union (EU). This monitoring provides the foundation for the National Reports on the measures implemented and their effectiveness (Art. 17 Habitats Directive), which Member States have to submit to the European Commission every six years. Based on these requirements, Member States have developed different monitoring programmes or have adapted previously existing monitoring schemes to include relevant aspects of the Habitats Directive.

The parameter 'structure and functions' is a key parameter for the assessment of the conservation status of habitat types as it provides information on the quality of the habitats. A standardised questionnaire was developed and sent to the competent authorities of Member States to compare and analyse the assessment methods of the quality of habitat types. Responses were received from 13 of the 28 Member States, while it was possible to include another Member State in the analysis by evaluating appropriate literature.

The analysis revealed very different approaches and progress amongst the Member States in the development and implementation of monitoring programmes tailored to the reporting obligations of Article 17 of the Habitats Directive. Some Member States established a special standardised monitoring programme for Article 11 of the Habitats Directive, while others used data from already existing programmes (e.g. habitat mapping, large-scale forest inventories, landscape monitoring). Most Member States responding to the questionnaire use monitoring based on samples but the data collection, sample sizes and level of statistical certainty differ considerably. The same applies to the aggregation of data and the methods for the assessment of the parameter 'structure and functions'. In contrast to the assessment of conservation status as part of the reporting obligations according to Article 17 of the Habitats Directive, no standardised EU guidelines exist for monitoring. The present study discusses differences in the monitoring programmes and evaluates them with regard to the objectives of comparable assessments of conservation status of habitat types in the National Reports of Member States or at a biogeographical level.

Keywords

Habitats Directive, EU Member States, reporting, habitat type, structure and functions, assessment, biodiversity monitoring

Introduction

Monitoring of habitats has been a widespread tool for recording and assessing changes in habitat quality (e.g. due to land use change) since the beginning of the 1990s (Lengyel et al. 2008a). This development is driven by international conventions on the protection of biodiversity like the Convention on Biological Diversity (CBD) as well as different directives of the European Union (EU) referring to biodiversity which include monitoring of habitats and species (Henle et al. 2013). For example, monitoring conservation status of habitat types listed in Annex I of the Habitats Directive (92/43/EEC) is mandatory according to Article 11 of the Directive. This provides the foundation for the National Reports on the measures implemented and their effectiveness (Art. 17 Habitats Directive) which Member States have to submit to the European Commission (EC) every six years. Based on these National Reports, the EC compiles a composite report which represents an essential basis for the achievement of biodiversity targets (EC 2011a).

The monitoring of habitat types required for the assessment of conservation status concentrates on a large-scale spatial level, namely the biogeographical regions of Europe (Evans 2012, ETC-BD 2006) or their respective proportions of the EU Member States. However, of the 150 investigated monitoring schemes for European habitats examined in the EuMon project, only 17.6% had a national scope, while the main part focused on a local (e.g. conservation areas) or regional level (e.g. administrative regions). Furthermore, most of these schemes addressed only one (44%) or a few habitat types (Lengyel et al. 2008a, b). The EuMon database (http://eumon.ckff.si/monitoring/) currently (December 2017) contains 71 monitoring schemes from 11 EU Member States referring to the habitat types of the Habitats Directive. However, none of these monitoring schemes covers all habitat types of a Member State. It could be possible to combine several monitoring schemes, where appropriate, to a comprehensive monitoring system which includes all habitat types of the respective State.

The quality of the data for the National Reports according to the Habitats Directive still fluctuates considerably due to the ongoing need of Member States to develop targeted monitoring programmes or refine/complete existing programmes (de Bello et al. 2010, EEA 2015, EC 2015). The EU guidelines and reporting formats provide consistent cornerstones for appropriate monitoring programmes for all Member States (DG Environment 2017, EC 2016). The conservation status of habitat types is assessed by four parameters, which comprise quantitative ('range' and 'area' parameters) as well as qualitative ('structure and functions' parameter) criteria plus a forecast for the future ('future prospects' parameter) (EC 2016). An analysis of selective, nationwide mappings or recordings in conservation areas, some of which are also supported by remote sensing methods, provides data for the parameters range and area (e.g. Förster et al. 2008, Vanden Borre et al. 2011, 2017).

The assessment of habitat quality according to the requirements of the EC is based on the criteria 'habitat structures', 'habitat functions', 'typical species' and 'pressures and threats'. The criterion 'habitat structures' comprises physical components of a habitat type which are often formed by organisms (groups), including already dead organisms (e.g. standing or lying dead wood) as well as abiotic features (e.g. gravel banks for spawning). The criterion 'functions' means ecological processes which exist at a variety of temporal and spatial scales and differ considerably between habitat types (DG Environment 2017, p. 170). Typical species are those which mainly occur in a habitat type or at least in a subtype or a variant of a habitat type (DG Environment 2017, p. 172f.). Thus, individual attributes (or subcriteria) have to be selected for each habitat type to assess habitat quality. For the investigation of these criteria, sample-based monitoring is appropriate, as already implemented by some Member States and prepared by others (e.g. Bijlsma and Jansen 2014, McConville and Tucker 2015, Moser and Ellmauer 2009, Sachteleben and Behrens 2010, Stöhr et al. 2014).

While standardised EU requirements exist for the assessment of conservation status as an evaluation matrix for the biogeographical level (EC 2016), common minimum EU standards are missing for the development and design of specific monitoring schemes (e.g. for sample sizes or statistical certainty). However, the evaluation matrix requires the detection of differences of 6% in one reporting period (1% per year). The evaluation matrix also lacks a threshold value for the maximum proportion of the area assessed as 'unfavourable' that is permissible for a favourable status of the parameter 'structure and functions'. Therefore, a comparative analysis of the different assessment methods of the parameter 'structure and functions' in the EU Member States was compiled. The aim of the project was to obtain references for a possible improvement of habitat monitoring in Germany. Here we present the results of the analysis and a comparative discussion.

Methods

Research and analysis of reporting data from Member States

In a first step to find information on the approaches and methods of selected EU Member States concerning the assessment of the parameter 'structure and functions', an internet search was conducted for 15 countries which are part of the same biogeographical regions as Germany. The relevant data sources were considered regarding the accuracy of data aggregation and derivation of assessments, as well as validity of trends and amount of data (e.g. sample sizes for monitoring) and searched for further literature indications. Source documents were the Article 17 reports of Member States (EU obligations: Habitats Directive: Report on Implementation Measures) for the reporting period 2007–2012 (http://cdr.eionet.europa.eu), especially information on the 'most important achievements in the implementation of the Habitats Directive' (Annex A, field 1.1 or 1.2), as well as the collections of Internet links (especially field 2.3). German, English, French and Spanish homepages could be analysed directly, while information in other languages were examined for potentially relevant content by using translation aids like 'Google translate'. However, the internet search of publicly accessible documents did not yield sufficiently precise information on the assessment methodology used for the parameter 'structure and functions' for any of the selected Member States.

Questionnaire to Member States

In addition to the internet search, a standardised questionnaire was compiled for the selected EU Member States to answer essential questions. The questionnaire was in two parts (see Suppl. material 1: Table S1).

The first part focuses on sample selection and monitoring methods in general (e.g. monitoring scheme, number of habitat types monitored, number of sample plots, location of sample plots within or outside Special Areas of Conservation (SACs), utilisation of already existing monitoring systems).

The second part addresses assessment methods for the parameter 'structure and functions' at the level of biogeographical regions, based on the assessment of single habitat type occurrences (single plots). The objective of the analysis was to determine if Member States implement the assessment of conservation status based on individual habitat type occurrences (analogous to the standard data forms) similar to Germany or if the assessment is conducted based on other data.

The questionnaire contained 22 questions in total, as well as explanations on the approach in Germany. The questions related to monitoring in the reporting period 2007–2012, but some Member States have given outlooks for the current reporting period to show what will change.

In mid-August 2015, the German Federal Agency for Nature Conservation (Bundesamt für Naturschutz, BfN), which is responsible at a national level for monitoring under Article 11 of the Habitats Directive, sent the questionnaire to members of the Expert Group on Reporting (EGR) and the Habitat Committee, overall to 26 of 28 Member States. Croatia was not included because the country only joined the EU in 2013. Representatives of 13 Member States (Austria, Belgium [only Wallonia], Czech Republic, Denmark, Finland, Hungary, Ireland, Latvia, Lithuania, Portugal, Romania, Slovenia and Sweden) answered the questionnaire. The authors of this paper compiled the answers for Germany.

Additionally, Bijlsma and Jansen (2014) yielded relevant aspects of the approach in the Netherlands. Information from the region of Wallonia has been used for Belgium, although assessment requirements and algorithms for conservation status differ in the regions of Flanders and Brussels (Westra et al. 2018).

The answers of the Member State representatives have been compiled in a table (as of May 2016, after queries to individual States), with their consent for publication of the data.

Results

Answers from Member States

Analysis of the questionnaires revealed that only two of the selected Member States (Portugal and Slovenia) had not yet carried out monitoring according to the Article 11 requirements in the reporting period 2007–2012. Slovenia planned to establish monitoring for Natura 2000 areas in 2015 for the first time. Only a little information on Slovenia has been available from other sources so far. Thus, replies to the questions have been omitted for these two countries. Finland only answered some of the questions.

The Czech Republic assesses data from a nationwide biotope mapping programme. The complete update of all data takes place there over 12 years. This means that only part of the data is newly collected for each reporting period.

I. Was the assessment of the parameter 'structure and functions' for the biogeographical regions conducted on the basis of a monitoring programme?

Most Member States assess the parameter 'structure and functions' by monitoring similar to Germany, at least for some of the habitat types. Wallonia (southern part of Belgium; four ecologically grouped monitoring schemes – forests, waters, grassland and other semi-natural open landscapes) and Denmark have implemented comprehensive monitoring programmes.

Monitoring programmes for a part of the habitat types or just subparameters exist in Finland, Ireland (monitoring schemes for ecological groups [e.g. upland habitat types, dune habitat types and raised bog habitat types]), Latvia, Romania (part of the forest habitat types as part of the nationwide forest inventory), Sweden (nationwide forest inventory; inland waters as part of monitoring according to the Water Framework Directive; Article 11 requirements are integrated into a general landscape monitoring which is still under development) and Hungary (grassland habitat types since 2009, forest habitat types since 2011, wetland habitat types since 2014).

Lithuania and Austria are still developing monitoring programmes. In Austria, the monitoring programme has so far only been tested in the forest inventory for forest habitat types. The Czech Republic does not conduct separate monitoring but uses repeated biotope mapping of their entire land area instead.

I.a) When (year) was the monitoring programme installed?

The starting points in time differ between Member States. The first monitoring was established in Wallonia (Belgium), where monitoring forests as part of the forest inventory has existed since 1994. The second monitoring was implemented in Hungary in 1997. Most Member States established their monitoring in the first decade of the 21st century. Of course, older monitoring schemes for biotopes or landscapes existed in some Member States before the Habitats Directive came into force (e.g. the UK Countryside Survey).

The very different initialisation phases are a first indication of the varied development progresses and different methodical approaches of Member States. Like Germany, Denmark, Latvia and the Czech Republic used their monitoring results for the assessment of the parameter 'structure and functions' for the reporting period 2007–2012. In other countries, this has only been done for part of the habitat types (e.g. in Ireland) or has not yet been applied or just tested, as in Lithuania or Austria.

I.b) In which biogeographical region is the monitoring programme carried out?

Most of the Member States surveyed are conducting, or planning to carry out, a monitoring programme in all biogeographical regions. The marine parts of the biogeographical regions, which are recorded separately by the EC as marine regions, were not explicitly queried. So far, Ireland has conducted monitoring merely for the Atlantic region and the monitoring programme for marine-Atlantic habitat types only started in 2015.

I.c) How many habitat types occur in your country? How many habitat types have been processed in the monitoring?

Depending on country size and biogeographical setting, the Member States surveyed have between 41 (Wallonia) and 89 (Sweden) habitat types. The States with an already existing monitoring programme include all or a majority of the terrestrial/inland habitat types in their respective programmes (minimum 87% of the habitat types in Hungary).

I.d) Was the assessment conducted on selected sample plots or on the total habitat area (total census)?

Most of the monitoring approaches are based, at least partly, on sampling as undertaken in Germany. The assessments of habitat types in Wallonia, Denmark, Ireland, Lithuania, Sweden and Hungary are conducted fully with samples. In Austria and Romania, selection is carried out similar to Germany on the basis of sampling for widespread habitat types and total census for rare habitat types. Finland uses sampling for widespread habitat types (e.g. 9010 and 9050). In Lithuania, monitoring is carried out in transects.

In the Netherlands, the conservation degree of each habitat type of each Natura 2000 site is considered, weighted according to its area in the site (Bijlsma and Jansen 2014, page 12) and it is assumed that the area of the habitat type outside Natura 2000 sites is very small. If this assumption proves to be false for a habitat type, one or two virtual Natura 2000 sites with an estimated degree of conservation are added. In the Czech Republic, the entire area is considered.

I.e) What was the sample size per habitat type in each biogeographical region?

In most Member States, the number of sampling areas depends on the frequency of habitat type and other ecological and methodical factors, as well as effort and costs. The other States do not apply a standardised sample size with an upper limit like the 63-sample in Germany. For frequent of habitat types, the number of samples and sample size generally exceed those in Germany to achieve the necessary accuracy of results. Table 1 lists detailed information on the samples in Member States.

I.f) Which methods were used for the selection of sample plots (connected or unconnected, stratified or unstratified, weighted or unweighted samples)?

Each Member State selected sample plots differently. Overall, most Member States conducted at least a partly systematic selection based on distribution, size and characteristics of habitat types and/or other factors. Random samples without an expert assessment of their representativeness have not been used by any Member State.

I.g) Is the monitoring permanently repeated on the same sample plots once selected?

Apart from Ireland, all Member States use permanent observation plots at least for the majority of investigated habitat types or a total inventory of habitat types as in the Czech Republic.

Table 1. Number of sampling areas for habitat types in Member States. BGR: biogeographical region; WFD: Water Framework Directive.

Member State	Minimum and maximum number of sampling areas per habitat	Explanations
Austria	60–100 or total census	Depending on the variability and dynamic of the habitat type
Wallonia (Belgium)	No information	Forest habitat types as part of regional forest inventory – 1 sample plot/50 ha
		Meadows – all habitat types in 125 quadrants of 5 km × 5 km with occurrences of the habitat type
		Water bodies – 440 sample sites in total as part of WFD
Czech Republic	Total census	Consideration of total area
Denmark	200–3,000	Between 20 and 300 sample locations (stations) with 8–12 sample sites at each station for each habitat type
Hungary	1–530	Number of samples for each habitat type proportional to coverage of the habitat type
		4,800 samples in total, 55% for grassland habitat types, 35% for forest habitat types, 5% for wetland habitat types, 5% for other habitat types
Germany	63 per BGR or total census	5,128 sample plots for all habitat types in total
Ireland	Incomplete information	Representative sampling of national occurrences according to geographical spectrum, e.g. 60 sites of old oak forest, 40 sites of dune systems and 25 sites of meadows
		Depending on size of sample plots, minimum of 4 sample sites recommended
Latvia	1–224	2,393 samples for all habitat types in total
Lithuania	1–100	Spatial distribution based on quadrants with aim of covering at least 10% of national area
		Thus, coverage of 12–100% of respective habitat types occurring in the country
Netherlands	No information	All Natura 2000 sites of respective habitat type contribute to the assessment, plus additional virtual sites if considerable occurrences exist outside Natura 2000
Romania	20–1,000 or total census	Sample size per habitat type according to overall area of occurrences at national level
Sweden	No information	Depending on frequency of habitat type

I.h) How often is the monitoring carried out (survey intervals) during one reporting period?

Most Member States implemented at least one survey per reporting period. Several surveys per reporting period are mainly conducted in habitat types which depend on management, for example in Wallonia (aquatic monitoring) and Ireland (grassland). In the Czech Republic, one mapping cycle requires 12 years, thus only half of the land area is updated for each report.

I.i) Were only Natura 2000 sites included in the monitoring or also sites outside the Natura 2000 network? Can differences inside/outside Natura 2000 be detected from the monitoring?

Most Member States included areas within and outside Natura 2000 sites. So far, none of the Member States could find statistically reliable differences or the sample size was not large enough to allow a differentiation. Only a few Member States (Wallonia, Czech Republic, Denmark, Finland, Hungary and Sweden) assume that this will be possible for the next reporting periods.

I.j) Are there already existing monitoring systems and are they used for the Article 11 monitoring?

In a few States, the data for the National Reports are based on different monitoring systems and data. Monitoring according to Article 11 has been partly integrated into already existing procedures or only data has been extracted from already existing procedures. Independent monitoring programmes specially developed for Article 11 monitoring exist merely in a few countries (Germany, Denmark, Latvia, as well as the Czech Republic). Austria is currently developing Article 11 monitoring.

II.a) How is the evaluation of single habitat plots (degree of conservation) included in the assessment of the parameter 'structure and functions' as part of conservation status at biogeographical level?

The assessments of single monitoring plots are integrated into the assessment of the parameter 'structure and functions' in all examined monitoring approaches. However, the employed methods differ between States and are elaborated in the following questions. Table 2 shows a summary of all answers.

II.b) Is there a uniform assessment scheme for each habitat type on site or plot level in your State?

Wallonia, Germany, Denmark, Latvia, Lithuania, the Netherlands, Austria, Romania, Sweden, the Czech Republic and Hungary have a standardised assessment scheme. Denmark, Hungary and Germany revised their schemes for the reporting period 2013–2018. In Ireland, the assessment methods for each habitat type are similar but not always the same because different approaches work better for some habitat types depending on the heterogeneity of habitat type. Some countries, like Sweden or Hungary, have standardised assessment schemes for groups of habitat types, such as forest and grassland habitat types.

II.c) If no uniform assessment scheme exists: How is the evaluation of habitat types carried out?

Most Member States have standardised assessment schemes. In Hungary, assessment schemes are lacking only for a few habitat types. For these habitat types, experts conduct the assessment by using other data from nationwide biodiversity monitoring. In Sweden, habitat types without standardised assessment schemes are evaluated by experts based on existing data and expert opinion.

II.d) How is the review of the criteria/the degree of conservation on site or plot level carried out?

Except for Wallonia (Belgium), Member States calculated a degree of conservation (as in Germany) or a comparable assessment for each sample plot. In Wallonia, all features are aggregated at a biogeographical level and combined afterwards to a degree of conservation.

II.e) How was the assessment of the typical species referred to in Appendix V of the guidelines (Assessment and reporting under Article 17 of the Habitats Directive, Explanatory Notes & Guidelines) carried out for the period 2007–2012)? Which species groups were investigated?

The considered Member States, except for the Netherlands and Sweden, investigated plant species only; animal species are solely considered for assessment in special cases or as additional information. Member States either apply complete species lists or just use species typical for a habitat type or indicator species. In Germany, the assessment of habitat types dominated by vegetation is also conducted solely via number of typical plant species. Animal groups are included in the assessment of a few habitat types only (see question II.g in Suppl. material 1: Table S1).

II.f) What method for monitoring the vegetation is recognized: Compilation of a complete list of species, phytosociological relevés recording according to Braun-Blanquet, or other methods?

Many Member States compile, at least for some habitat types, lists of typical species. Only Denmark records complete species lists and three Member States perform phytosociological vegetation mapping (Wallonia, Ireland, Lithuania).

II.g) Is a faunistic list also recorded – a complete list or only for certain groups of species? From which faunistic groups are typical species recorded?

Most Member States rarely used animal species, especially for assessment of habitat types with sparse or no vegetation of higher plants. Only Sweden lists animal species for assessment of a few habitat types. The most frequent animal groups are birds, butterflies and beetles (see also question II.e).

II.h) Are the assessments of the criteria 'habitat structures', 'typical species', and 'pressures and threats' calculated separately for all sample plots? Or is the separately calculated degree of conservation of each sample plot used for aggregation?

Germany, Denmark, Ireland, Latvia, Austria, the Czech Republic and Hungary aggregate the degree of conservation which has been calculated for each sample plot separately. Denmark is revising the method for calculating conservation status for the next report according to Article 17. Ireland is testing another method with an indicator-based instead of plot-based assessment. For this method, individual features or criteria of many sample plots are directly aggregated at a biogeographical level without combining all relevant features or criteria of a single sample plot to a degree of conservation. Similar assessments are already underway in Sweden. In Wallonia, assessments of some features are aggregated at the biogeographical level, while others are aggregated at the level of single sample plots.

In the Netherlands, the conservation degree of a habitat type (based on the requirements of the standard data forms) of each SAC is assessed separately and conservation degrees of respective habitat type of several SACs are subsequently aggregated according to their area percentage (see question II.d).

II.i) How is the parameter 'structure and functions' calculated at biogeographical level?

From the information supplied, it is concluded that four different approaches can be distinguished regarding the methods of Member States (see Table 2):

- Holistic assessment of single plots or single Natura 2000 sites and aggregation of the parameter 'structure and functions' according to a threshold for percentage of single assessments (Denmark, Wallonia – grassland habitat types, Ireland, Latvia, Austria, Romania, Czech Republic). This corresponds to monitoring of sample plots in Germany and assessment of the parameter 'structure and functions' according to the proportion of C assessments of conservation degree.
- Calculation of an assessment at a biogeographical level of each individual feature and subsequent derivation of the parameter 'structure and functions' (Wallonia – forest habitat types). In Germany, a consistent value at a biogeographical level is only determined for the features 'forest development phase', 'habitat trees' and 'dead wood' of frequent forest habitats which are assessed based on data from the National Forest Inventory.
- Expert evaluation of the parameter 'structure and functions' without fixed rules for aggregation but based on individual features of all sample plots (Sweden). This method was occasionally used in Germany if monitoring would have resulted in 'unknown' for some reasons (missing data for a part or all of sample plots).
- Assessment of conservation degrees in single Natura 2000 sites and aggregation to a status of the subparameter 'structure and functions (without typical species)' according to a threshold value for the area percentages of conservation degrees at a

Table 2. Assessment of parameter 'structure and functions' in Member States – row colours: violet – assessment at single plot level, blue – assessment at Natura 2000 site level, orange – assessment at biogeographical level.

Member State	Habitat types	Threshold for favourable status FV ('green')	Threshold for unfavourable-inadequate status U1 ('yellow')	Threshold for unfavourable-bad status U2 ('red')	Explanations
Austria	All	A-proportion ≥ 50% and C-proportion < 33.3%	All other combinations	C-proportion > 33.3% and A-proportion < 50%	Assessment of conservation degrees of single plots
Wallonia (Belgium)	Forest	All three criteria (structure, functions, regeneration) at biogeographical level FV	At least one criterion at biogeographical level U1 and no criterion U2	At least one criterion at biogeographical level U2	Assessment of each feature of single plots → averaging all assessments of a feature at biogeographical level (C weighted more strongly: A = 1, B = 2 and C = 4) → FV < 1.5 < U1 < 2.5 < U2
Wallonia (Belgium)	Grassland	No information	No information	No information	Assessment of conservation degrees of single plots
Czech Republic	All	< 10% of partial areas assessed as 'less favourable' and 'unfavourable'	10-25% of partial areas assessed as 'less favourable' and 'unfavourable'	> 25% of partial areas assessed as 'less favourable' and 'unfavourable'	Assessment of conservation degrees of single plots
Denmark	All	> 50% A-proportion and > 75% A+B-proportion (= C-proportion < 25% and A-proportion > 50%)	All other combinations	< 25% A-proportion and < 50% A+B-proportion (= C-proportion > 50% and A-proportion < 25%)	Assessment of conservation degrees of single plots
Germany	All	C-proportion ≤ 20%	C-proportion > 20 and ≤ 25%	C-proportion > 25%	Assessment of conservation degrees of single plots
Hungary	All	No information	No information	No information	Assessment of conservation degrees of single plots. Naturalness-based habitat quality index (Németh and Seregélyes 1989)
Ireland	All	C-proportion < 1%	C-proportion 1–25%	C-proportion > 25%	Assessment of conservation degrees of single plots
Latvia	All	No information	No information	No information	Assessment of conservation degrees of single plots (no assessment if total area of habitat type decreases > 1% per year during one reporting period)
Lithuania	All	No information	No information	No information	Assessment of conservation degree at Natura 2000 site level
Netherlands	All	If A-proportion ≥ 75% and C-proportion ≤ 15%	All other combinations	C-proportion > 25% and A < B + C	Assessment of subparameter 'structure and functions (without typical species)' at Natura 2000 site level
Netherlands	All	Proportion of FV ≥ 75% and proportion of U2 ≤ 15%	All other combinations	Proportion of U2 > 25% and proportion of FV < proportion of (U1 + U2)	Assessment of subparameter 'typical species' at biogeographical level in relation to proportion of species belonging to different species groups according to Red List

Member State	Habitat types	Threshold for favourable status FV ('green')	Threshold for unfavourable-inadequate status U1 ('yellow')	Threshold for unfavourable-bad status U2 ('red')	Explanations
Netherlands	All	2 × FV	At least 1 × U1	At least 1 × U2	Assessment of conservation status by aggregating assessments of subparameter 'structure and functions (without typical species)' and 'typical species' at biogeographical level according to EU evaluation matrix
Romania	All	C-proportion < 20% and A-proportion ≥ 50%	All other combinations	C-proportion ≥ 50% and A-proportion < 20%	Assessment of conservation degrees of single plots
Sweden	All	–	–	–	Expert evaluation

biogeographical level. Furthermore, determination of the status of the subparameter 'typical species' at a biogeographical level based on the categories of the Red List of all typical species and subsequent aggregation of both subparameters at a biogeographical level to the parameter 'structure and functions (including typical species)' according to the EU evaluation matrix. Only the Netherlands uses this method.

Although most Member States apply the first method, considerable differences exist between threshold values (see Table 2 and section 3.2). In Wallonia, the first two methods are used.

II.j) What is the significance and the statistical power of the monitoring/the assessment? How big is the minimum detectable difference between two reporting cycles (six years)?

None of the Member States surveyed made a statement on theoretical statistical strength of the samples or the monitoring. Either they did not understand the question or no calculations exist. The question did not aim at complete determined differences, but at the size of possibly detectable differences between two reporting periods.

Germany specified a significance level (α error) and a β error of 0.2 each. The power is 0.8. The applied Chi^2 test yielded a minimum detectable difference of ≥ 30% for a sample size of 63 (Sachteleben and Behrens 2010).

II.k) Are reference documents or websites available for methods used? Is it possible to provide digital documents?

The information and further literature of Member States differ considerably. Additional information on Article 17 methodology beyond the questionnaire replies is not included. Lithuania, Denmark and Hungary only have information in their respective national language. The Czech Republic added all relevant information which has been integrated into the answers above. For the Netherlands, the documents from Bijlsma and Jansen (2014) and Jansen et al. (2014) were analysed.

Assessment at biogeographical level

Besides the design of data collection (questions to point I), the assessment methods of the parameter 'structure and functions' at a biogeographical level are also particularly important. The approaches of Member States differ considerably (see Table 2). However, most Member States who replied use the three grades of the EU assessment system for the conservation degree. The assessment of the degree of conservation comprises three grades: A – excellent; B – good; and C – average or bad. Some States assess degrees of conservation at the level of single plots; others assess degrees of conservation of habitat type areas in the respective Natura 2000 sites (Netherlands) and some States evaluate subparameters for assessment of the structure and functions directly at site or biogeographical level (Ireland, Wallonia – e.g. structural diversity in forests). Sometimes, experts are consulted in borderline cases or for subparameters which are difficult to obtain.

Only four Member States (Germany, Ireland, Netherlands, Czech Republic) apply the threshold value defined in the reporting format for an inadequate-bad status if more than 25% of the area is inadequate (Table 2), but in the Netherlands, it is linked to additional conditions. Another three Member States (Denmark, Austria, Romania) also assess the proportion of C assessments but use different threshold values and consider the proportion of A assessments as well.

The 25% specification of the reporting format is unclear if individual features are assessed at a biogeographical level and then aggregated to the criteria or directly to the parameter 'structure and functions'. Although it is possible to aggregate individual features according to their proportion (based on area or quantity) of the respective assessments (A, B or C), Member States can decide if one or more features of a criterion have to exceed 25% for a U2 assessment of the criterion.

If assessment of the individual features of conservation degrees is conducted by averaging for assessment of the three features of the criterion 'species composition of the vegetation' (as in Wallonia), the proportion of A assessments partly balances the C assessments. Thus, the 'unfavourable' status of a feature shows only with a much higher proportion of C assessments. The described example of Wallonia tries to compensate for this effect by weighting the proportion of C assessments higher than the other assessments.

An assessment of single features of the parameter 'structure and functions' may not be appropriate at the level of single plots (e.g. proportion of dead wood or habitat trees on very small sample plots of a few 100 m^2). In this case, averaging of the value of the feature is conducted for all sample plots (e.g. forest habitat types in Wallonia, frequent forest habitat types in Germany). The assessment is ultimately carried out via a threshold for the calculated average values. In these cases, it is not possible to apply the 25% rule.

Discussion

Our analysis of relevant monitoring programmes of EU Member States reveals considerable differences in the interpretation and application of monitoring according to Article 11 of the EU Habitats Directive and, thus, differences in the quality and quantity of monitoring data used for assessment of conservation status of habitat types. Only a few of the States who replied (Wallonia, Denmark, Germany, Hungary, Ireland) have established and already applied a special, standardised monitoring programme according to Article 11. Some States have used data from existing monitoring programmes (e.g. large-scale forest inventories: Wallonia, Austria, Romania, Sweden, Germany [for 5 frequent forest habitat types]; landscape monitoring in Sweden) for monitoring according to Article 11. Many States are still developing or implementing their monitoring schemes (e.g. Austria, Lithuania) or revising it (e.g. Sweden – landscape monitoring).

Monitoring of structure and functions of a habitat type targets the determination of significant changes in conservation status. For its sample plots, each Member State defines which type of changes it specifically investigates, the criteria or indicators used for the analysis, the number of repetitions (in a reporting period) and the extent of tolerable changes in criteria/indicators. Thus, it is impossible to combine assessments of sample plots from different Member States at a biogeographical level or compare them directly.

An overall monitoring programme in all Member States would have many potential advantages but it would result in an enormous effort of coordination and development, not least because of very different manifestations of many broadly defined habitat types (e.g. regarding their floristic composition) within their European distribution area (compare Bunce et al. 2013 on the field identification of habitat types). Nevertheless, we recommend regional cross-border cooperation, especially for implementation of a monitoring programme in small Member States or if a Member State has only a small proportion of a biogeographical region. Thus, disproportionate effort in areas with a small occurrence of a habitat type can be avoided without completely foregoing monitoring in these parts. An analogous cooperation has been suggested for the Alpine region of Austria and Germany (Bavaria) (National Report 2013).

Lengyel et al. (2008b) describe the essential requirements for integration of data and monitoring schemes. The most important foundation is a consistent typology of habitat types, as mostly given in the Habitats Directive (EC 2013, Evans 2006; see Evans 2010 on the differences between Member States). In Germany, this typology of habitat types enabled joint monitoring of habitats conducted by the Federal States in the first place (Sachteleben and Behrens 2010). The Federal States had been developing their own classifications for decades before the Habitats Directive was implemented (Riecken et al. 2003). Various vegetation classifications have also been developed at the regional/national level of the European States, which form the basis for the definition of habitats and may only be partially compatible with the recently published European checklist (Mucina et al. 2016).

Most Member States apply sample-based monitoring schemes which are based on field mapping of single occurrences of specified target habitats (‘field mapping-based, targeted monitoring schemes’ *sensu* Lengyel et al. 2008a). The survey method in the field and the design of sample plots are very important for comparability and integration of results of monitoring schemes.

The number of sample plots per habitat type and methods of selecting samples differ considerably between Member States. The number of sample plots depends mainly on the frequency of a habitat type in most Member States. Sample plots are partly selected systematically based on criteria such as spatial distribution, size or manifestation of habitat types, while the representativeness of plots is usually evaluated by an expert.

This analysis did not include the application of remote sensing methods for monitoring the structure and functions of habitat types. Nevertheless, it can be assumed that remote sensing methods are used only sporadically in inaccessible regions (e.g. high mountains in the Alpine region), apart from the application of aerial images for mapping of habitat types. On the one hand, it is almost impossible to recognise habitat types even with satellite data; on the other hand, the responsible project managers so far lack access to the necessary data, computing capacity, standardised analysing tools and specific knowledge. The rapid development in this field could possibly lead to many innovations, as well as in monitoring of habitats (e.g. Buck et al. 2015, Corbane et al. 2015, Schmidt et al. 2017, Vanden Borre et al. 2017).

The individual Member States (or even sub-regions like Wallonia in Belgium) also define the criteria and thresholds for assessment of the quality of single occurrences of habitat types. Although the EC was able to provide a standardised reporting format for the reporting period 2001–2006, as required by the Habitats Directive, further development of this format, including some methodical harmonisations, required long and comprehensive preparations by EU working groups for both subsequent reports. Thus, it seems unlikely that the detection of changes in habitat types at the level of single sample plots could be successfully standardised for many Member States or complete biogeographical regions. Even though methodical approaches of European monitoring of habitats have been developed (Bunce et al. 2005, Brus et al. 2011, Metzger et al. 2013), they focused on common habitats or general ecosystem monitoring. Therefore, integration of previously existing Member State monitoring schemes is the preferable option (see Henry et al. 2008).

Harmonisation/integration could be achieved by standardisation of derivation methods of the overall assessment of the parameter 'structure and functions' at a biogeographical level by Member States. Most Member States who replied have standardised schemes for assessment of the conservation degree of single sample plots. The degree of conservation is defined by the EU decision on the standard data forms of the SACs and is composed of three criteria: 'conservation degree of the structure (including typical species)'; 'conservation degree of the functions'; and 'restoration possibility' (EC 2011b). The criteria of Member States partially comply with the EU definition, but mostly pick up the three grades of the EU assessment system (see section 3.2). Thus, overall assessment of all sample plots at a biogeographical level offers the best chance to harmonise assessment methods.

The evaluation matrix according to Annex E of the reporting format (EC 2016) is essential for this final assessment step, in this context for the parameter 'structure and functions'. It defines the status of the structure and functions of a habitat type as favourable if all the criteria mentioned above are assessed as good and no significant deterioration or pressures exist. An unfavourable status is subdivided into 'unfavourable-inadequate' and 'unfavourable-bad'. The latter is attained if more than 25% of the area of a habitat type is evaluated as 'unfavourable' regarding structure and functions (including typical species). Some Member States deviate from these specifications (see 3.2). The 25% threshold is sometimes ignored, sometimes replaced or supplemented by minimum proportions of sample plots with 'excellent status (A)'. The inclusion of proportion of sample plots with an A status into the assessment seems appropriate considering the protection of habitat type. The changes in A-plots to B-plots, which do not affect the assessment if only the C proportion is considered, still constitute a deterioration in status of a habitat type. Furthermore, A-plots can substitute (regarding their function) C-plots only if these are in close spatial proximity. Thus, it is critical to comply with the prescribed maximum C proportions solely mathematically regarding comparability of the results of Member States. The matrix does not contain a threshold for the definition of a favourable status which has led to values between 1% and 20% for the proportion of C assessments in Member States. The EC suggests an area proportion of 90% with a 'good' status of structure and functions (DG Environment 2017). As this was controversial within Member States, the threshold value for the next reporting period is expected to be discussed again.

Our analysis also revealed that consideration of animal species is a weak point in monitoring schemes of habitat types in almost all Member States. This contradicts the definition of 'conservation status of a natural habitat' according to Article 1e) of the Habitats Directive, which states that the conservation status of a habitat type is favourable if, inter alia, the conservation status of the typical species of this habitat type are favourable. This definition includes animal species, but also fungi, which are presumably also not, or hardly taken into account in monitoring programmes.

Another difficulty of monitoring schemes is that sample plots may be selected regarding the number and distribution of occurrences, but regardless of widely varying area sizes of different occurrences of a habitat type. At least in Germany, this is the case and leads to a lack of representativeness in terms of total distribution area of a habitat type. The different quantities of sample plots per habitat type both absolute and concerning the relative size of a Member State have been mentioned already. Furthermore, in most Member States, no information is available on statistical robustness regarding the detectability of changes in status of a habitat type (in two or more reporting periods) based on the analysed samples. For assessment of conservation status of a habitat type, information on occurrences inside conservation areas as well as outside these areas is necessary. Thus, most Member States consider sample plots within and outside their protected areas in their monitoring schemes. No Member State has been able to detect statistically robust differences so far, but some expect to do so in the next reporting periods.

A European-wide analysis (meta-analysis), considering data from different national monitoring programmes based on one or more indicators (e.g. completeness of the species inventory, habitat quality) or trends, could possibly result in improved assessment of habitat types at a biogeographical level in the EU. Thus, it could be possible to enhance the sample size, the accuracy of estimation (e.g. of trends), temporal coverage and probably statistical power without increasing the number of sample plots within Member States (Henry et al. 2008). An appropriate evaluation method remains to be developed and will need to take account of different sample designs (number and selection of sample plots), for example by weighting (post stratification). Henry et al. (2008) discuss methodical approaches to that. It has to be determined if the usage of classes (A-B-C assessment of the sample plots), which have been calculated based on different indicators, enables a sufficiently precise detectability of changes in status of habitat types.

Nevertheless, Member States are obliged to fill the gaps in geographical coverage of single habitat types, i.e. to develop monitoring programmes at a national level where they are still missing. To improve comparability of results of assessments of a habitat type between Member States, simple minimum requirements regarding sample size and assessment methods for biogeographical regions (within the Member States) should be agreed upon at the EU level.

From the authors' point of view, the following points describe best practices implemented so far and emerge as potential recommendations for sample-based habitat monitoring for the parameter 'structure and functions':

- assessment of all habitat types of a Member State according to individual assessment schemes, separated by biogeographical regions,
- sufficiently large sample size to be able to estimate changes in condition of a habitat type with sufficient certainty (Denmark appears exemplary here),
- stratification of samples according to the areal proportion of habitat types and whether they are located within or outside the SACs,
- survey of habitat types on fixed permanent sampling plots,
- examination of each sampling plot at least in one year of each reporting period, several times in the case of anthropozoogenic habitat types which respond quickly to changing land use or pressures,
- consideration of typical plant species at least by means of roughly quantified species lists or vegetation surveys,
- consideration of typical animal species of well-known groups of species with a known indicator function in the assessment of habitat types,
- normally, status of a habitat type is evaluated first at the level of the sample areas and then aggregated at the level of the biogeographical region.

Currently there is no monitoring programme for any of the Member States examined that already covers all these points. However, some recommendations are already being implemented in some Member States´ monitoring programmes. Further points to consider in the evaluation of habitat monitoring programmes, including benchmarks to compare current practices, can be found in Lengyel et al. (2018).

Acknowledgements

This research was funded by the German Federal Agency for Nature Conservation (BfN) with financial resources of the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (Research and Development project 'Adaptation and development of the methods for the compilation of the National Report according to Article 17 of the Habitats Directive', FKZ 3514 82 0600).

We would like to especially thank the representatives of the Member States who took the time to answer the questionnaire, sometimes in great detail. Austria: Thomas Ellmauer and Alexander Schuster; Czech Republic: Karel Chobot; Denmark: Jesper Fredshavn; Ireland: Deirdre Lynn; Finland: Olli Ojala; Hungary: Kinga Bata and Ildikó Varga; Latvia: Gita Strode; Lithuania: Algirdas Klimavičius; Portugal: Helena Ceia; Romania: Iovu Biris; Slovenia: Matej Petkovšek; Sweden: Anders Jacobson; Wallonia (Belgium): Jean Marc Couvreur and Lionel Wibail. We would also like to thank the reviewers Doug Evans and Szabolcs Lengyel for their valuable comments on the manuscript. We thank Mark Sixsmith for his linguistic correction of the manuscript.

References

Bijlsma RJ, Jansen JAM (2014) Structuur en functie van habitattypen; Onderdeel van de documentatie van Habitatrichtlijn artikel 17-rapportage 2013. Wettelijke Onderzoekstaken Natuur & Milieu, WOt-technical report 33: 1–42. http://edepot.wur.nl/341898

Brus DJ, Knotters M, Metzger MJ, Walvoort DJJ (2011) Towards a European-wide sampling design for statistical monitoring of common habitats. Alterra report 2213: 1–66. https://www.wur.nl/upload_mm/4/0/9/3c60c015-861e-46cc-85fc-b54d969f3f6a_EBONED-33TowardsaEuropeanwidesamplingdesign.pdf

Buck O, García Millán VE, Klink A, Pakzad K (2015) Using information layers for mapping grassland habitat distribution at local to regional scales. International Journal of Applied Earth Observation and Geoinformation 37: 83–89. https://doi.org/10.1016/j.jag.2014.10.012

Bunce RGH, Bogers MMB, Evans D, Jongman RHG (2013) Field identification of habitats directive Annex I habitats as major European biodiversity indicator. Ecological Indicators 33: 105–110. https://doi.org/10.1016/j.ecolind.2012.10.004

Bunce RGH, Groom GB, Jongman RHG, Padoa-Schioppa E (Eds) (2005) Handbook for Surveillance and Monitoring of European Habitats. Alterra report 1219: 1–107. http://library.wur.nl/WebQuery/wurpubs/fulltext/30274

Corbane C, Lang S, Pipkins K, Alleaume S, Deshayes M, García Millán VE, Strasser T, Vanden Borre J, Spanhove T, Förster M (2015) Remote sensing for mapping natural habitats and their conservation status – New opportunities and challenges. International Journal of Applied Earth Observation and Geoinformation 37: 7–16. https://doi.org/10.1016/j.jag.2014.11.005

de Bello F, Lavorel S, Gerhold P, Reier U, Pärtel M (2010) A biodiversity monitoring framework for practical conservation of grassland and shrublands. Biological Conservation 143(1): 9–17. https://doi.org/10.1016/j.biocon.2009.04.022

DG Environment (2017) Reporting under Article 17 of the Habitats Directive: Explanatory notes and guidelines for the period 2013–2018. Final version – May 2017. Brussels: 1–188. http://cdr.eionet.europa.eu/help/habitats_art17/index_html

EC/European Commission (2011a) The EU Biodiversity Strategy to 2020. Publications Office of the European Union, Luxembourg, 1–27. https://doi.org/10.2779/39229

EC/European Commission (2011b) Commission Implementing Decision of 11 July 2011 concerning a site information format for Natura 2000 sites (notified under document C(2011) 4892) (2011/484/EU). Official Journal of the European Union L 198: 39–70. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011D0484&from=EN

EC/European Commission (2013) Interpretation Manual of European Union habitats. EUR 28: 1–144. http://ec.europa.eu/environment/nature/legislation/habitatsdirective/docs/Int_Manual_EU28.pdf

EC/European Commission (2015) The state of Nature in the European Union. Report on the status of and trends for habitat types and species covered by the Birds and Habitats Directives for the 2007–2012 period as required under Article 17 of the Habitats Directive and Article 12 of the Birds Directive. Brussels (COM(2015) 219 final): 1–19. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52015DC0219&from=EN

EC/European Commission (2016) Reporting under Article 17 of the Habitats Directive. Report format for the period 2013–2018, Final Version – November 2016, Brussels, 1–29. http://cdr.eionet.europa.eu/help/habitats_art17/index_html

EEA/European Environment Agency (2015) State of nature in the EU. Results from reporting under the nature directives 2007–2012. EEA Technical report No 2/2015: 1–173. https://www.eea.europa.eu/publications/state-of-nature-in-the-eu

ETC-BD/European Topic Centre on Biological Diversity (2006) The Indicative Map of European Biogeographical Regions: Methodology and Development. http://www.eea.europa.eu/data-andmaps/data/biogeographical-regions-europe-2005/methodology-description-pdf-format/methodology-description-pdf-format/at_download/file

Evans D (2006) The habitats of the European Union Habitats Directive. Biology and Environment 106B(3): 167–173. https://doi.org/10.3318/BIOE.2006.106.3.167

Evans D (2010) Interpreting the habitats of Annex I – Past, present and future. Acta Botanica Gallica 157(4): 677–686. https://doi.org/10.1080/12538078.2010.10516241

Evans D (2012) Building the European Union's Natura 2000 network. Nature Conservation 1: 11–26. https://doi.org/10.3897/natureconservation.1.1808

Förster M, Frick A, Walentowski H, Kleinschmit B (2008) (2008) Approaches to utilising QuickBird data for the monitoring of Natura 2000 habitats. Community Ecology 9(2): 155–168. https://doi.org/10.1556/ComEc.9.2008.2.4

Henle K, Bauch B, Auliya M, Külvik M, Pe'er G, Schmeller DS, Framstad E (2013) Priorities for biodiversity monitoring in Europe: A review of supranational policies and a novel scheme for integrative priorization. Ecological Indicators 33: 5–18. https://doi.org/10.1016/j.ecolind.2013.03.028

Henry P-Y, Lengyel S, Nowicki P, Julliard R, Clobert J, Čelik T, Gruber B, Schmeller DS, Babij V, Henle K (2008) Integrating ongoing biodiversity monitoring: Potential benefits and methods. Biodiversity and Conservation 17(14): 3357–3382. https://doi.org/10.1007/s10531-008-9417-1

Jansen JAM, Weeda EJ, Schippers P, Bijlsma RJ, Schaminée JHJ, Arts GHP, Deerenberg CM, Bos OG, Jak RG (2014) Habitattypen in Natura 2000-gebieden. Beoordeling van Oppervlakte, representativiteit en behoudsstatus in de Standard Data Forms (SDFs). Wageningen, Wettelijke Onderzoekstaken Natuur & Milieu, WOt-technical report 8: 1–196. http://edepot.wur.nl/314180

Lengyel S, Déri E, Varga Z, Horváth R, Tóthmérész B, Henry P-Y, Kobler A, Kutnar L, Babij V, Selskar A, Christia C, Papastergiadou E, Gruber B, Henle K (2008a) Habitat monitoring in Europe: A description of current practices. Biodiversity and Conservation 17(14): 3327–3339. https://doi.org/10.1007/s10531-008-9395-3

Lengyel S, Kobler A, Kutnar L, Framstad E, Henry P-Y, Babij V, Gruber B, Schmeller D, Henle K (2008b) A review and a framework for the integration of biodiversity monitoring at the habitat level. Biodiversity and Conservation 17(14): 3341–3356. https://doi.org/10.1007/s10531-008-9359-7

Lengyel S, Kosztyi B, Schmeller DS, Henry P-Y, Kotarac M, Lin Y-P, Henle K (2018) Evaluating and benchmarking biodiversity monitoring: Metadata-based indicators for sampling design, sampling effort and data analysis. Ecological Indicators 85: 624–633. https://doi.org/10.1016/j.ecolind.2017.11.012

Mucina L, Bültmann H, Dierßen K, Theurillat J-P, Raus T, Čarni A, Šumberová K, Willner W, Dengler J, Gavilán García R, Chytrý M, Hájek M, Di Pietro R, Iakushenko D, Pallas J, Daniëls FJA, Bergmeier E, Santos Guerra A, Ermakov N, Valachovič M, Schaminée JHJ, Lysenko T, Didukh YP, Pignatti S, Rodwell JS, Capelo J, Weber HE, Solomeshch A, Dimopoulos P, Aguiar C, Hennekens SM, Tichý L (2016) Vegetation of Europe: Hierarchical floristic classification system of vascular plant, bryophyte, lichen, and algal communities. Applied Vegetation Science 19: 3–264. https://doi.org/10.1111/avsc.12257

Metzger MJ, Brus DJ, Bunce RGH, Carey PD, Gonçalves J, Honrado JP, Jongman RHG, Trabucco A, Zomer R (2013) The Environmental stratifications as the basis for national, European and global ecological monitoring. Ecological Indicators 33: 26–35. https://doi.org/10.1016/j.ecolind.2012.11.009

McConville AJ, Tucker GM (2015) Review of Favourable Conservation Status and Birds Directive article 2 interpretation within the European Union. Natural England Commissioned Reports 176: 1–106.

Moser D, Ellmauer T (2009) Konzept zu einem Monitoring nach Artikel 11 der Fauna-Flora-Habitat-Richtlinie in Österreich. Im Auftrag der neun Bundesländer Österreichs. Umweltbundesamt, Wien, 1–131.

Riecken U, Finck P, Raths U, Schröder E, Ssymank S (2003) Standard-Biotoptypenliste für Deutschland, 2. Fassung: Februar 2003. Schriftenreihe für Landschaftspflege und Naturschutz 75: 1–65.

Sachteleben J, Behrens M (2010) Konzept zum Monitoring des Erhaltungszustandes von Lebensraumtypen und Arten der FFH-Richtlinie in Deutschland. BfN-Skripten (Bonn-

Bad Godesberg) 278: 1–180. https://www.bfn.de/fileadmin/MDB/documents/service/skript_278.pdf

Schmidt J, Fassnacht FE, Neff C, Lausch A, Kleinschmit B, Förster M, Schmidtlein S (2017) Adapting a Natura 2000 field guideline for a remote sensing-based assessment of heathland conservation status. International Journal of Applied Earth Observation and Geoinformation 60: 61–71. https://doi.org/10.1016/j.jag.2017.04.005

Stöhr O, Ragger C, Gewolf S, Drapela-Dhiflaoui J, Weinke E, Egger G, Lang S (2014) Basiserhebung von Lebensraumtypen und Arten von gemeinschaftlichen Bedeutung in Österreich. Natur und Landschaft 89(11): 471–477.

Vanden Borre J, Paelinckx D, Mücher CA, Kooistra L, Haest B, De Blust G, Schmidt AM (2011) Integrating remote sensing in Natura 2000 habitat monitoring: Prospects on the way forward. Journal for Nature Conservation 19(2): 116–125. https://doi.org/10.1016/j.jnc.2010.07.003

Vanden Borre J, Spanhove T, Haest B (2017) Towards a mature age of remote sensing for Natura 2000 habitat conservation: Poor method transferability as a prime obstacle. In: Díaz-Delgado F, et al. (Eds) The roles of remote sensing in nature conservation. Springer, 11–37. https://doi.org/10.1007/978-3-319-64332-8_2

Westra T, Onkelinx T, Oosterlynck P (2018) Design and implementation of a monitoring scheme to assess habitat quality of European protected habitats in Flanders (Belgium). Nature and Forest Research Institute (INBO). https://pureportal.inbo.be/portal/files/15005144/thu_c_2_westra_2525.pdf

Supplementary material 1

Table S1. Content of the questionnaire with explanations of the German approach

Authors: Götz Ellwanger, Stephan Runge, Melanie Wagner, Werner Ackermann, Melanie Neukirchen, Wenke Frederking, Axel Ssymank, Ulrich Sukopp

Data type: table

Link: https://doi.org/10.3897/natureconservation.29.27273.suppl1

Nature Conservation 29: 79–92 (2018)
doi: 10.3897/natureconservation.29.28943
http://natureconservation.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Nature Conservation
Launched to accelerate biodiversity conservation

Vulnerability of mammals to land-use changes in Colombia's post-conflict era

Bayron R. Calle-Rendón[1,2], Flavio Moreno[3], Renato R. Hilário[1,2]

1 *Programa de Pós-Graduação em Biodiversidade Tropical (PPGBio), Universidade Federal do Amapá, Macapá, Brazil* **2** *Laboratório de Ecologia (LabEco), Universidade Federal do Amapá, Macapá, Brazil* **3** *Departamento de Ciencias Forestales, Universidad Nacional de Colombia Sede Medellín, Medellín, Colombia*

Corresponding author: *Bayron R. Calle-Rendón* (brcalle@unal.edu.co)

Academic editor: *C. Knogge* | Received 8 August 2018 | Accepted 12 September 2018 | Published 9 October 2018

http://zoobank.org/D76BEBBE-4115-48CA-8EE7-83025E77D4FC

Citation: Calle-Rendón BR, Moreno F, Hilário RR (2018) Vulnerability of mammals to land-use changes in Colombia's post-conflict era. Nature Conservation 29: 79–92. https://doi.org/10.3897/natureconservation.29.28943

Abstract

Colombia, one of the most biodiverse countries in the world, is entering a peaceful period after more than fifty years of armed conflict. Due to land use changes resulting from this new situation, negative effects on biodiversity, including mammals are expected. We think that mammal populations will be more sensitive in municipalities where activities related to post-conflict will be carried out. In that order, we aim to: 1) identify which mammal species would be more sensitive and 2) identify the critical regions where there is higher richness of sensitive mammals. We used the distributions of 95 mammal taxa and calculated a sensitivity index by combining four factors: 1) the proportion of each species distribution within protected areas in relation to their proposed extinction thresholds, 2) the proportion within post-conflict municipalities, 3) the proportion of five types of potential land use in post-conflict municipalities and 4) the threat status of each species. Using this index, we drew a map of species richness for mammals classified at high-risk and very high-risk categories. Primates were the most sensitive group to post-conflict changes. Urabá and the region near to the Serranía de San Lucas were the areas with the highest richness of sensitive species. We suggest using primates as flagship species to carry out conservation schemes in the post-conflict era in programmes led by local farmers and former fighters who have been reintegrated into civilian life.

Keywords

Armed conflict, biodiversity loss, flagship species, primates, protected areas

Introduction

After more than 50 years of armed conflict in Colombia, the Government and FARC (Revolutionary Armed Forces of Colombia), the oldest guerrilla army from Latin America, signed a peace agreement in 2016. One of the central points in such agreement is rural reform (Gobierno Nacional and FARC-EP 2016). As a consequence, it is expected that many people who suffered forced displacement will return to rural areas. This immigration process could represent a risk for biodiversity due to expansion of the agricultural frontier in formerly abandoned lands (Negret et al. 2017).

Colombia is a megadiverse country (Andrade 2011) that encompasses two biodiversity hotspots (Myers et al. 2000) and holds 518 mammal species (Ramírez-Chaves et al. 2016). Mammals are important due to their economic importance and ecological value (Ceballos and Brown 1994), but their populations around the world are threatened by several anthropogenic factors, including those related to armed conflicts (Ceballos and Ehrlich 2002, Dudley et al. 2002, Daskin and Pringle 2018). Notwithstanding, there are no specific studies analysing the relationship between warfare and mammals population in Colombia.

War in Colombia has had both negative and positive effects on biodiversity. Forests from many areas were cleared for illicit crop plantations, mining and land grabbing for cattle ranching. In addition, terrorist attacks spilled oil causing pollution over vast areas (Dávalos et al. 2011, Sánchez-Cuervo and Mitchell 2013, Asociación Colombiana del Petróleo 2014). On the other hand, due to the violence, vegetation regrowth and absence of human disturbance in many abandoned areas may have preserved biodiversity (Sánchez-Cuervo and Mitchell 2013).

In the current post-conflict era, many social and environmental changes are expected in Colombia (Baptiste et al. 2017, Negret et al. 2017). Experiences from other countries have shown that some activities related to post-conflict, such as resettlement and rural reforms, can have negative effects on biodiversity (Suarez et al. 2018). These effects may be exacerbated in Colombia by the low representation of threatened areas and endemic species within protected areas (Forero-Medina and Joppa 2010). In the Colombian case, we expect biodiversity –and specifically mammal species– will be affected mainly in those areas where post-conflict activities will be carried out. As many mammal species are sensitive to human activities, it is important to identify which areas and species would need more attention in that new scenario. With the premise that environmental changes will follow in municipalities where peace agreements are to be implemented, we aim to: 1) identify which mammal species are most sensitive and, 2) identify critical regions where negative effects on mammals are most likely.

Methods

Study Polygons selection

We selected mammals from six orders (Artiodactyla, Carnivora, Cingulata, Perissodactyla, Pilosa and Primates) with known distributions in Colombia. Species distributions were downloaded from the IUCN Red List of Threatened Species (IUCN 2017) and the digital tool BioModelos (Instituto Humboldt 2017).

We considered as post-conflict areas, the polygons retrieved from the Departamento Administrativo Nacional de Estadístca (DANE 2017) of the 170 municipalities, where activities related to rural reform will be carried out to develop the rural economy, according to Decree 893 of 2017 (Ministerio de Agricultura y Desarrollo Rural 2017). Additionally, we used polygons of national protected areas retrieved from the Colombian National Parks (Parques Nacionales Naturales de Colombia 2017) and polygons of the types of potential land use retrieved from the Instituto Geográfico Agustín Codazi (IGAC 2018).

Risk evaluation

To identify the sensitivity of mammal species to post-conflict land use change, we calculated the proportion of its distribution that overlaps with post-conflict areas and protected areas. Additionally, we calculated the proportion of species distribution that overlaps with five main types of potential land use for Colombia, only within municipalities of post-conflict in QGIS software (2.14.8-Essen). Then, we developed a sensitivity index (S) for each species considering four factors:

$$S = TS + PAET + Post + PLU$$

where TS is the threat status according to the classification of the IUCN Red List of Threatened Species (IUCN 2017). $PAET$ is an index that relates the proportion of the distribution of each species within national protected areas and its proposed extinction threshold. $Post$ is the proportion of the distribution of each species within post-conflict areas and PLU is the proportion of the distribution of each species within a type of potential land use in post-conflict municipalities. TS, $PAET$, $Post$ and PLU ranged between 0 and 5 each. Thus S varied from 0 to 20 and was classified into five categories: non-risk ($S = 0$), low-risk ($0 < S < 5$), middle-risk ($5 \leq S < 10$), high-risk ($10 \leq S < 15$) and very high-risk ($S \geq 15$).

As detailed information about population structure of each species across all the country is unknown, we therefore used the threat status (TS) of the species as a proxy of extinction proneness since it is related to a quantitative measure of reduction in population size in a temporal scale and geographic range (IUCN 2012). We assigned TS values for each species according to the following: Least Concern (LC) = 0; Data Deficient (DD) or Not Evaluated (NE) = 1; Near Threatened (NT) = 2; Vulnerable (VU) = 3; Endangered (EN) = 4; Critically Endangered (CR) = 5.

Considering that any place outside protected areas will be more vulnerable to changes in land use in the post-conflict era, it is expected that species with a higher proportion of their distribution within protected areas will be less vulnerable. Then, we considered each protected area as a 'patch of habitat' and that each species would need an amount of habitat (i.e. proportion of its distribution overlapped with protected areas) equivalent to its extinction threshold to maintain a population in equilibrium (Fahrig 2001). Previous simulations highlight that the four main factors determining extinction threshold are (from least to most important): habitat pattern, matrix quality, dispersal rate and reproductive rate (Fahrig 2001). As body weight of mammals is negatively related to reproductive rate (Western 1979), we expect that the greater the body size, the higher the extinction threshold. Thus, we used body mass of each species (or the average mass of the genus) (Jones et al. 2009) to classify them into four categories: small (< 1 kg), medium (1–5 kg), large (5–15 kg) and very large (> 15 kg). We defined the extinction threshold for each species knowing *a priori* that a species like *Panthera onca* (a very large species) need at least 50% of its distribution within protected areas to maintain its subpopulations in lower risk (de la Torre et al. 2018). Then, we used species body weight to classify the extinction threshold for our species as 50% of remaining habitat for very-large, 40% for large, 30% for medium and 20% for small species. We used each threshold as the amount of habitat needed within protected areas to guarantee adequate protection for each species, and calculated the relationship between the proportion of the distribution of each species within national protected areas and its proposed extinction threshold (*PAET*) as:

$$PAET = \frac{5}{\left(\frac{pa * 4}{et}\right) + 1}$$

where *pa* is the proportion of the distribution of each species within national protected areas and *et* is the extinction threshold applied for the species considering its body weight.

We assumed that the greater the post-conflict area overlapped with species distribution, the greater the negative effect on the species. However, the effects will be more negative in municipalities where more people will return in the post-conflict era since this immigration process is a driver of environmental impacts in the post-conflict era (Suarez et al. 2018). Consequently, we used the number of displaced people in each municipality between 1993 and 2013 (Consejería Presidencial para los Derechos Humanos 2015, Unidad para las Víctimas 2018) as the surrogate number of people that might return to each municipality. Then we calculated the proportion of the distribution of each species within post-conflict areas (*Post*) as:

$$Post = 5 * \left(\sum_{i=1}^{n} A_i * disp\right)$$

where A_j is the proportion of a species distribution within the municipality *i* and *disp* is a factor associated with each municipality according to the number of displaced people (dp): *disp* = 0.7 (dp < 10,000), *disp* = 0.8 (10,000 ≤ dp < 25,000), *disd* = 0.9 (25,000 ≤ dp < 50,000) and *disp* = 1 (dp ≥ 50,000).

Peace agreements between the Colombian Government and FARC have a special focus on rural reform to carry out agricultural activities according to the potential land use (Gobierno Nacional FARC-EP 2016). Negative effects on biodiversity depend of the land use, being most negative on croplands and least negative on uses that maintain natural vegetation to some extent (i.e. extractivism) (see Newbold et al. 2015). We selected five main types of potential land use proposed for Colombia: conservation, forestry, agroforestry, cattle ranch and agriculture. These potential uses are based on the natural capacity of the land to support a given activity under sustainable conditions (IGAC 2012). We calculated the proportion of the distribution of each species within a type of potential land use in post-conflict municipalities (PLU) as:

$$PLU = 5 * \left(\sum_{j=1}^{n} A_j * lu\right)$$

where A_j is the proportion of a species within a type of potential land use j and lu is a factor associated with each land use according to the intensity of use: lu = 0.2 (conservation), lu = 0.4 (forestry), lu = 0.6 (agroforestry), lu = 0.8 (cattle ranch) and lu = 1 (agriculture).

Finally, we created a grid on a map of Colombia using squared cells of 0.1° (approximately 10.6 km). Then the species richness was calculated in each cell by overlapping all species distributions and considering that a given species was present if the cell occupancy was greater than 50%. We created two maps: one considering all present species and another considering only species classified at high-risk and very high-risk according to our sensitivity index to identify critical regions where negative effects on mammals are most likely. Both maps were designed using the SAM Software Version 4.0 (Rangel et al. 2010).

Results

We obtained spatial distributions of 95 taxa: 44 primates, 26 Carnivora, nine Artiodactyla, seven Pilosa, six Cingulata, and three Perissodactyla (see Suppl. material 1: Table S1: mammal species used in the analysis). A total of 36.84% of all species were classified as low-risk, 48.42% as middle-risk, 13.68% as high-risk and 1.05% as very high-risk (represented only by the primate species *Plecturocebus caquetensis*). No species was classified as non-risk (Figure 1). With 12 out of 44 species classified as high-risk and very high-risk, primates are the mammal group that is most sensitive to post-conflict changes in Colombia. *Tapirus bairdii* (Perissodactyla) and *Mazama temama* (Artiodactyla) were also classified as high-risk (Figure 2).

Amazon and some points from the Orinoco region (including the eastern side of the Cordillera Oriental) were the areas with greatest overall species richness, followed by the Serranía de San Lucas and the transition zone between the Caribbean and the Pacific region near to the Urabá Gulf (Figure 3a). The areas with highest richness of sensitive species were those near to Urabá Gulf (municipalities of Turbo, Chigorodó

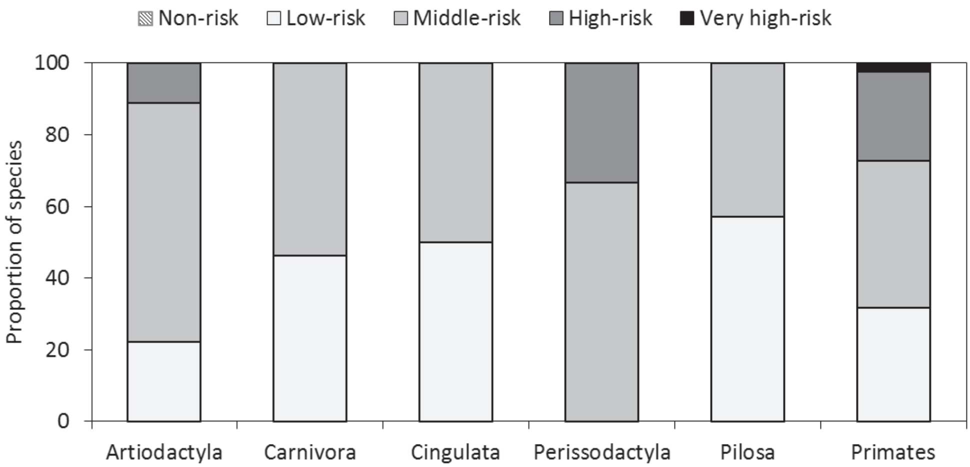

Figure 1. Proportion of mammal species amongst six orders within each category of sensitivity in the Colombian post-conflict era. Non-risk (S = 0), low-risk (0 < S < 5), middle-risk (5 ≤ S < 10), high-risk (10 ≤ S < 15) and very high-risk (S ≥ 15).

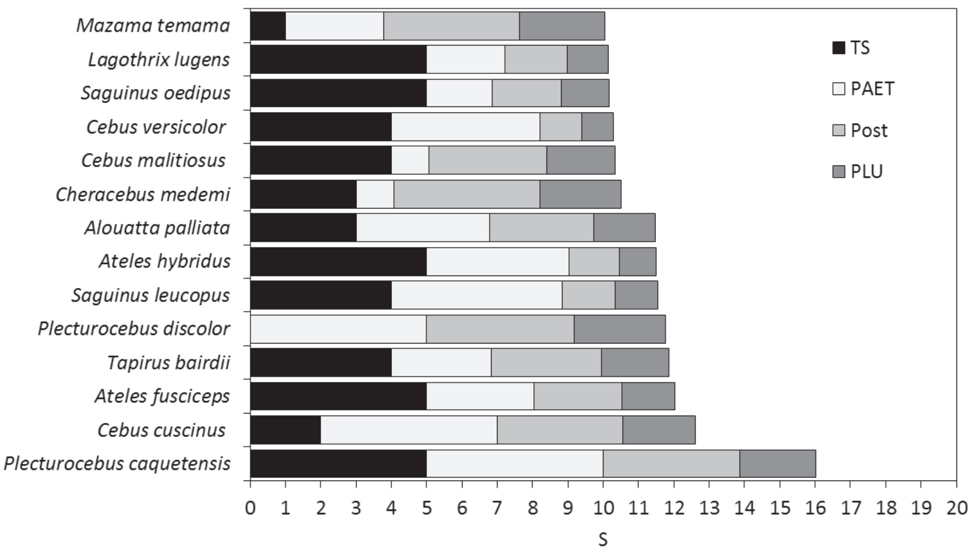

Figure 2. Most sensitive (*S* ≥ 10) mammal species in Colombian postconflict areas and the contribution of each of the four factors to the sensitivity index (S). ***TS***: threat status; ***PAET***: relationship between the proportion of the distribution of each species within national protected areas and its proposed extinction threshold; ***Post***: proportion of the distribution of each species within post-conflict areas; ***PLU***: proportion of the distribution of each species within a type of potential land use in post-conflict municipalities.

and Mutatá in Antioquia, Tierralta in Cordoba and Riosucio in Chocó) and the region near to the Serranía de San Lucas (municipalities of Yondó, Segovia, El Bagre and Remedios in Antioquia and Arenal, Cantagallo, Morales, San Pablo, Santa Rosa del Sur and Simití in Bolivar) (Figure 3b).

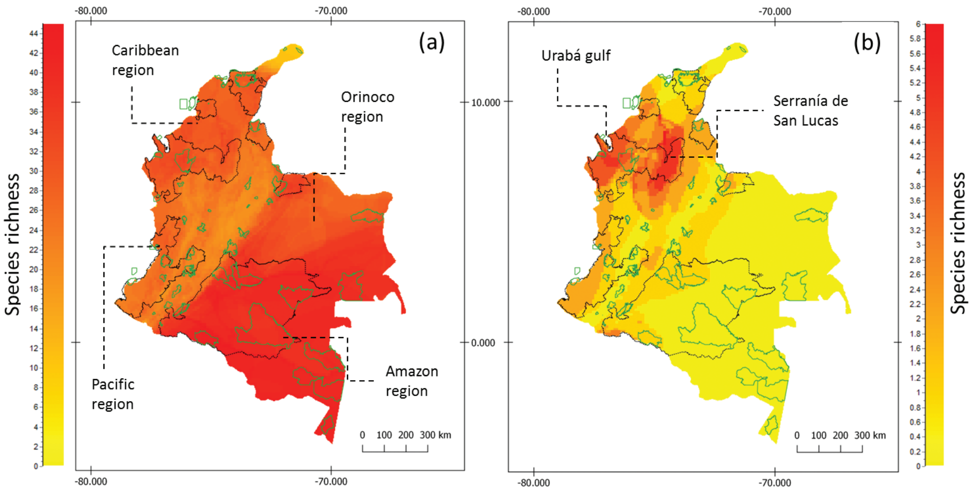

Figure 3. a Mammal species richness in Colombia considering the distribution of 95 taxa of six orders. **b** Critical regions (Urabá and Serranía de San Lucas) where negative effects on mammals are most likely considering only those species classified as high-risk and very high-risk in post-conflict era ($S \geq 10$). Black polygons correspond to the 170 municipalities used as post-conflict areas and green polygons to protected areas.

Discussion

Increased attention has been given to the effects of warfare on biodiversity in the last two decades and Colombia is one of the areas of special attention (Dudley et al. 2002, Hanson et al. 2009, Lawrence et al. 2015, Daskin and Pringle 2018, Hanson 2018, Suarez et al. 2018). Colombia not only holds the Tropical Andes and the Tumbes-Chocó-Magdalena as biodiversity hotspots, but is also part of the Amazon region, the greatest extension of tropical forest on the earth (Myers et al. 2000, FAO ITTO 2011). Preserving these areas is not only of national concern, but also of global interest due to their contribution of these regions to global biodiversity, high endemism of plants and fauna and regulation of global climate (Myers et al. 2000, Werth and Avissar 2002, Malhi et al. 2008).

We found that post-conflict alterations in Colombia represent a threat to many mammal species and that primates are the most vulnerable group to such alterations. As forest dwelling animals, primates are highly sensitive to deforestation, as well as other animals such as the Baird's Tapir, that also depends on closed canopy forests (Matola et al. 1997). Other international examples support the fact that many species might be highly impacted by forest loss during the post-conflict era in Colombia. When civil war ended in Nicaragua, deforestation increased because many people returned to rural areas (Stevens et al. 2011). In addition, rapid forest loss was also documented in post-conflict periods in Bosnia, Liberia, Rwanda and Sierra Leone (Suarez et al. 2018).

Other human activities, such as hunting, may increase the threats to some primate species. For example, four Atelids and the Central American Red Brocket, which are classified as high-risk species in our study, face known hunting pressure (Weber and Gonzalez 2003, de Thoisy et al. 2005, Aquino et al. 2009) concomitant with post-conflict land-use change. Previous studies demonstrated that in the Rwanda Republic and the Democratic Republic of Congo for example, bushmeat hunting increased after peace negotiation, affecting ungulates and the emblematic flagship species *Gorilla* spp. (Plumptre et al. 1997, Glew and Hudson 2007). Increased hunting also caused wildlife decline in Cambodia during its post-conflict era (Loucks et al. 2009).

We found that areas near to the Serranía de San Lucas and Urabá gulf are the most critical regions since they host the largest numbers of mammals considered at risk according to our sensitivity index. Historically, some guerrillas in San Lucas imposed environmental restrictions for locals to preserve the area, such as prohibition of hunting and logging and they planted landmines to avoid mining and logging by foreign people (Dávalos 2001). Nevertheless, after the peace agreement, FARC abandoned this area, ending this protective measure. The National Liberation Army (ELN) is another armed group that still occupies some areas in Colombia and has similar environmental policies (Dávalos 2001). However, ELN and the Colombian Government have been under negotiations since 2017 and a similar effect on biodiversity could arise should such negotiations succeed, not only due to the absence of environmental control imposed by guerrillas, but also due to the changes related to land use inherent in the new peace agreement. On the other hand, armed conflict displaced more than 10,000 people in some municipalities from Urabá (see database in Consejería Presidencial para los Derechos Humanos 2015 and Unidad para las Víctimas 2018). As a consequence, we expect the return of a large number of people to this region, leading to deforestation, increased hunting and raising the risk of local extinctions.

The relationship between biodiversity and warfare can be separated in three stages: 1) preparations, 2) war and, 3) post-war activities (Machlis and Hanson 2008). As the Colombian conflict is old (more than 50 years), it is difficult to gather information related to biodiversity in the former stage (i.e. pre-war). However, current geopolitical and social scenarios provide an opportunity to guide the Colombian government in thinking about the last two stages. Concerning the second stage, there are other armed groups in Colombia disputing territories in a few regions of the country and they have dominion or take advantage of the gap left by the former armed groups. In this case, the government can carry out actions to reduce the negative effects of this regionalised war on biodiversity and environment. Such actions may include (1) increasing research, (2) applying the resolutions of the United Nations against pollution in war time and the environmental protection in conflicts areas and (3) taking into account the "International Day for Preventing the Exploitation of the Environment in War and Armed Conflict" (Hanson 2018). Concerning the third stage, primates, similar to birds (Ocampo-Peñuela and Scott 2017), could be targeted by touristic activities in the post-conflict era or became flagship species in a "green economy based on low-emissions land/resource use systems" (Baptiste et al. 2017).

Increasing the protected areas should be a government strategy associated with the rural reform to prevent biodiversity losses during the post-conflict period. The critical regions for the implementation of such protected areas would be areas near to Urabá Gulf and Serranía de San Lucas, since they harbour most of the high-risk species from this study. Most of the species classified as high-risk or very high-risk in our analysis have less than 10% of their distribution under legal protection. The most critical case was *Plecturocebus caquetensis*, the single very high-risk species in our analysis that occurs outside of the most critical areas in post-conflict: its distribution does not overlap any protected area, its entire distribution is within post-conflict area and it was recently classified amongst the world's 25 most endangered primates (Defler et al. 2017). This species, similar to others, demands urgent conservation schemes, such as economic incentives for the establishment of private protected areas and agro-silvoforestry plots (Baptiste et al. 2017) in programmes led by local farmers and former fighters who have been reintegrated into civilian life. This option is highly feasible taking into account the forestry vocation of Colombia (IGAC 2012). For this reason, the government needs to consider the current types of potential land use of Colombia in the post-conflict era to avoid biodiversity loss, as evidence around the world has shown that some environmental drivers of change in post-conflict countries are "ineffective land use planning" and "unsustainable agricultural practices" (Suarez et al. 2018).

Conclusions

Around the world, in the last half of the past century, more than 80% of armed conflicts took place within biodiversity hotspots (Hanson et al. 2009). Therefore, mitigating warfare impacts is imperative for biodiversity conservation, as many conflicts of state-based violence and non-state violence have been increasing in the last century throughout the globe, including countries such as Mexico, Somalia, Syria and Myanmar (UCDP 2018), which host biodiversity hotspots (Myers et al. 2000, Mittermeier et al. 2004). As other conflicts are certain to occur in the future, approaches such as ours may aid other conflict areas to promote biodiversity conservation when these conflicts are over.

When the environmental context is not cohesive with peace agreements, several drivers of environmental change can emerge (Suarez et al. 2018). In that order, conservation planning is vital for peace building in regions of high biodiversity (Lujala and Rustad 2012). Some countries reached a peace agreements in the last 30 years, for example: El Salvador (1992), Rwanda (1993), Bosnia and Herzegovina (1995), Sierra Leone (2000), Liberia (2003) and Burundi (2008) (Suarez et al. 2018). These six countries and Colombia have a common denominator and can prospectively induce positive changes within their territories if peace is ongoing, because high biodiversity make people more resilient when war has devastated their society (Hanson 2018). Since biodiversity can be seen as an opportunity for peace building, it is important to work together with locals, researchers and former fighters with financial support to bring welfare to the people without threatening the biodiversity (Hanson 2018).

This first evaluation of the possible consequences of the peace agreement between Colombian government and FARC on mammals can help to improve the current scenario of peace in Colombia. Fourteen species classified at high-risk or very high-risk categories need to be included in schemes for conservation based in local initiatives led by former fighters and victims of armed conflict. Experiences from Asia, Africa and Central America have shown how the lack of planning can have negative effects on biodiversity, since impacts on the environment increase after cessation of conflict or peace treaties (Loucks et al. 2005, Glew and Hudson 2007, Suarez et al. 2018). In the current scenario of political division in Colombia after the negative result of a plebiscite for peace in October 2016, there is a challenge for the new government that started on August 2018, independent of its political ideology, to include in its environmental strategies the creation of protected areas in the biodiversity hotspot Tumbes-Chocó-Magdalena, specifically in Urabá and Serranía de San Lucas regions. As well as the sustainable use of the biodiversity (e.g. ecotourism approaches with locals and former fighters), using mammals as flagships species, especially primates, to prevent the biodiversity loss and its consequences. Additionally, we think the same analysis with other biological groups would be extremely useful for the design of conservation schemes, land use policies and rural reform programmes in order to prevent extinctions and to decrease threats to the species in Colombia, one of the most biodiverse countries in the world.

Acknowledgments

We are grateful to C. Murcia who made valuable comments on a first version of this paper, with X. Carretero-Pinzón who revised the final version in English and with the two reviewers who contributed to improve the manuscript. B.R.C.R. is supported by a PhD scholarship from the CAPES (Federal Agency for Support and Evaluation of Graduate Education).

References

Andrade CMG (2011) Estado del conocimiento de la biodiversidad en Colombia y sus amenazas: Consideraciones para fortalecer la interacción ciencia-política. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 35: 491–507.

Aquino R, Torrones W, Navarro R, Torrones C, Cornejo FM (2009) Caza y estado de conservación de primates en la cuenca del río Itaya, Loreto, Perú. Revista Peruana de Biología 15: 33–39. https://doi.org/10.15381/rpb.v15i2.1719

Colombiana del Petróleo A (2014) Un terrorismo que contamina. ACP Hidrocarburos 7: 40–43.

Baptiste B, Pinedo-Vasquez M, Gutierrez-Velez VH, Andrade GI, Vieira P, Estupiñán-Suárez LM, Londoño MC, Laurance W, Lee TM (2017) Greening peace in Colombia. Nature Ecology & Evolution 1(4): 1–3. https://doi.org/10.1038/s41559-017-0102

Ceballos G, Brown JH (1994) Global pattern of mammal diversity, endemism and endangerment. Conservation Biology 9(3): 559–568. https://doi.org/10.1046/j.1523-1739.1995.09030559.x

Ceballos G, Ehrlich PR (2002) Mammal population losses and the extinction crisis. Science 296(5569): 904–907. https://doi.org/10.1126/science.1069349

Consejería Presidencial para los Derechos Humanos (2015) Atlas del impacto regional del conflicto armado en Colombia: dinámicas locales y regionales en el período 1990–2013. Presidencia de Colombia, Bogotá, Colombia. http://www.derechoshumanos.gov.co/observatorio/publicaciones/Documents/150422-atlas-impacto.pdf

DANE (2017) Geoportal. Descarga del Marco Geoestadistico Nacional (MGN). Departamento Estadístico Nacional de Estadística, Bogotá, Colombia. https://geoportal.dane.gov.co/v2/?page=elementoDescargaMGN

Daskin JH, Pringle RM (2018) Warfare and wildlife declines in Africa's protected areas. Nature 553(7688): 328–332. https://doi.org/10.1038/nature25194

Dávalos LM (2001) The San Lucas mountain range in Colombia: How much conservation is owed to the violence? Biodiversity and Conservation 10(1): 69–78. https://doi.org/10.1023/A:1016651011294

Dávalos LM, Bejarano AC, Hall MA, Correa HL, Corthals A, Espejo O (2011) Forests and drugs: Coca-driven deforestation in tropical biodiversity Hotspots. Environmental Science & Technology 45(4): 1219–1227. https://doi.org/10.1021/es102373d

Defler TR, García J, Guzmán-Caro DC (2017) Caquetá Titi Monkey. In: Schwitzer C, Mittermeier RA, Rylands AB, Chiozza F, Williamson EA, Macfie EJ, Wallis J, Cotton A (Eds) Primates in Peril: the world's 25 most endangered primates 2016–2018. IUCN SSC Primate Specialist Group, International Primatological Society, Conservation International, and Bristol Zoological Society, Arlington, USA, 80–82. https://portals.iucn.org/library/sites/library/files/documents/2017-059.pdf

de la Torre JA, González-Maya JF, Zarza H, Ceballos G, Medellín RA (2018) The jaguar's spots are darker than they appear: Assessing the global conservation status of the jaguar *Panthera onca*. Oryx 52(02): 300–315. https://doi.org/10.1017/S0030605316001046

de Thoisy B, Renoux F, Julliot C (2005) Hunting in northern French Guiana and its impact on primate communities. Oryx 39(02): 149–157. https://doi.org/10.1017/S0030605305000384

Dudley J, Ginsberg JR, Plumptre AJ, Hart JA, Campos LC (2002) Effects of war and civil strife on wildlife and wildlife habitats. Conservation Biology 16(2): 319–329. https://doi.org/10.1046/j.1523-1739.2002.00306.x

Fahrig L (2001) How much habitat is enough? Biological Conservation 100(1): 65–74. https://doi.org/10.1016/S0006-3207(00)00208-1

FAO ITTO (2011) The state of forests in the Amazon basin, Congo basin and Southeast Asia. Food and Agriculture Organization of the United Nations, International Tropical Timber Organization, Rome, Italy. http://www.fao.org/docrep/014/i2247e/i2247e00.pdf

Forero-Medina G, Joppa L (2010) Representation of global and national conservation priorities by Colombia's protected area network. PLoS One 5(10): e13210. https://doi.org/10.1371/journal.pone.0013210

Glew L, Hudson MD (2007) Gorillas in the midst: The impact of armed conflict on the conservation of protected areas in sub-Saharan Africa. Oryx 41(02): 140–150. https://doi.org/10.1017/S0030605307001755

Gobierno Nacional FARC-EP (2016) Acuerdo final para la terminación del conflicto y la construcción de una paz estable y duradera. Oficina del Alto Comisionado para la Paz, Bogotá, Colombia. http://www.altocomisionadoparalapaz.gov.co/procesos-y-conversaciones/Documentos%20compartidos/24-11-2016NuevoAcuerdoFinal.pdf

Hanson T (2018) Biodiversity conservation and armed conflict: A warfare ecology perspective. Annals of the New York Academy of Sciences. https://doi.org/10.1111/nyas.13689

Hanson T, Brooks TM, da Fonseca GAB, Hoffmann M, Lamoreux JF, Machlis G, Mittermeier CG, Mittermeier RA, Pilgrim JD (2009) Warfare in biodiversity hotspots. Conservation Biology 23(3): 578–587. https://doi.org/10.1111/j.1523-1739.2009.01166.x

IGAC (2012) Estudio de los conflictos de uso del territorio colombiano, escala 1:100.000. Instituto Geográfico Agustín Codazzi, Bogotá, Colombia.

IGAC (2018) Mapas de clasificación de las tierras por su vocación de uso a escala 1:100.000. Datos abiertos, subdidrección de agrología. Instituto Geográfico Agustín Codazzi, Bogotá, Colombia. http://datos.igac.gov.co/pages/agrologia

Instituto Humboldt (2017) BioModelos. Programa de Evaluación y Monitoreo de la Biodiversidad. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá, Colombia. http://biomodelos.humboldt.org.co/

IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. International Union for Conservation of Nature, Gland, Switzerland. http://s3.amazonaws.com/iucnredlist-newcms/staging/public/attachments/3097/redlist_cats_crit_en.pdf

IUCN (2017) The IUCN Red List of Threatened Species. Version 2017-3. http://www.iucnredlist.org

Jones KE, Bielby J, Cardillo M, Fritz SA, O'Dell J, Orme CDL, Safi K, Sechrest W, Boakes EH, Carbone C, Connolly C, Cutts MJ, Foster JK, Grenyer R, Habib M, Plaster CA, Price SA, Rigby EA, Rist J, Teacher A, Bininda-Emonds ORP, Gittleman JL, Mace GM, Purvis A (2009) PanTHERIA: A species-level database of life history, ecology, and geography of extant and recently extinct mammals. Ecology 90(9): 2648. https://doi.org/10.1890/08-1494.1

Lawrence MJ, Stemberger HLJ, Zolderdo AJ, Struthers DP, Cooke SJ (2015) The effects of modern war and military activities on biodiversity and the environment. Environmental Reviews 23(4): 443–460. https://doi.org/10.1139/er-2015-0039

Loucks C, Mascia MB, Maxwell A, Huy K, Duong K, Chea N, Long B, Cox N, Seng T (2009) Wildlife decline in Cambodia, 1953–2005: Exploring the legacy of armed conflict. Conservation Letters 2(2): 82–92. https://doi.org/10.1111/j.1755-263X.2008.00044.x

Lujala P, Rustad SA (2012) High-value natural resources: a blessing or a curse for peace? In: Lujala P, Rustad SA (Eds) High-value natural resources and post-conflict peacebuilding. Environmental Law Institute and United Nations Environment Programme, London, UK, 3–18.

Machlis GE, Hanson T (2008) Warfare ecology. Bioscience 58(8): 729–736. https://doi.org/10.1641/B580809

Malhi Y, Roberts JT, Betts RA, Killeen TJ, Li W, Nobre CA (2008) Climate change, deforestation, and the fate of the Amazon. Science 319(5860): 169–172. https://doi.org/10.1126/science.1146961

Matola S, Cuaron AD, Rubio-Torgler H (1997) Status and action plan of Baird's Tapir (*Tapirus bairdii*). In: Brooks DM, Bodmer RE, Matola S (Eds) Status survey and conservation action plan: Tapirs. IUCN/SSC Tapir Specialist Group, Gland, Switzerland, 29–45 https://portals.iucn.org/library/sites/library/files/documents/1997-053.pdf

Ministerio de Agricultura y Desarrollo Rural (2017) Decreto 893 de 2017: Programas de Desarrollo con Enfoque Territorial – PDET. Ministerio de Agricultura y Desarrollo Rural, Bogotá, Colombia. http://es.presidencia.gov.co/normativa/normativa/DECRETO%20893%20DEL%2028%20DE%20MAYO%20DE%202017.pdf

Mittermeier RA, Gil PR, Hoffmann M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, da Fonseca GAB (2004) Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions. CEMEX/Agrupación Sierra Madre, Mexico City, Mexico.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403(6772): 853–858. https://doi.org/10.1038/35002501

Negret PJ, Allan J, Braczkowski A, Maron M, Watson JEM (2017) Need for conservation planning in postconflict Colombia. Conservation Biology 31(3): 499–500. https://doi.org/10.1111/cobi.12935

Newbold T, Hudson LN, Hill SLL, Contu S, Lysenko I, Senior RA, Börger L, Bennett DJ, Choimes A, Collen B, Day J, Palma A, Díaz S, Echeverria-Londoño S, Edgar MJ, Feldman A, Garon M, Harrison MLK, Alhusseini T, Ingram DJ, Itescu Y, Kattge J, Kemp V, Kirkpatrick L, Kleyer M, Correia DLP, Martin CD, Meiri S, Novosolov M, Pan Y, Phillips HRP, Purves DW, Robinson A, Simpson J, Tuck SL, Weiher E, White HJ, Ewers RM, Mace GM, Scharlemann JPW, Purvis A (2015) Global effects of land use on local terrestrial biodiversity. Nature 520(7545): 45–50. https://doi.org/10.1038/nature14324

Ocampo-Peñuela N, Scott R (2017) Economic and conservation potential of bird-watching tourism in postconflict Colombia. Tropical Conservation Science 10: 1–6. https://doi.org/10.1177/1940082917733862

Parques Nacionales Naturales de Colombia (2017) Información cartográfica (Shape): límite Parques Nacionales. Parques Nacionales Naturales de Colombia, Bogotá, Colombia. http://www.parquesnacionales.gov.co/portal/es/servicio-al-ciudadano/datos/abiertos/

Plumptre AJ, Bizumuremyi J-B, Uwimana F, Ndaruhebeye J-D (1997) The effects of the Rwandan civil war on poaching of ungulates in the Parc National des Volcans. Oryx 31(04): 265–273. https://doi.org/10.1017/S0030605300022225

Ramírez-Chaves HE, Suárez-Castro AF, González-Maya JF (2016) Cambios recientes a la lista de los mamíferos de Colombia. Mammalogy Notes 3: 1–9.

Rangel TF, Diniz-Filho JAF, Bini LM (2010) SAM: A comprehensive application for spatial analysis in macroecology. Ecography 33(1): 1–5. https://doi.org/10.1111/j.1600-0587.2009.06299.x

Sánchez-Cuervo AM, Mitchell T (2013) Consequences of the armed conflict, forced human displacement, and land abandonment on forest cover change in Colombia: A multi-scaled analysis. Ecosystems (New York, N.Y.) 16(6): 1052–1070. https://doi.org/10.1007/s10021-013-9667-y

Stevens K, Campbell L, Urquhart G, Kramer D, Qi J (2011) Examining complexities of forest cover change during armed conflict on Nicaragua's Atlantic Coast. Biodiversity and Conservation 20(12): 2597–2613. https://doi.org/10.1007/s10531-011-0093-1

Suarez A, Árias-Arévalo PA, Martínez-Mera E (2018) Environmental sustainability in post-conflict countries: Insights for rural Colombia. Environment, Development and Sustainability 20(3): 997–1015. https://doi.org/10.1007/s10668-017-9925-9

UCDP (2018) Uppsala Conflict Data Program. Department of Peace and Conflict Research, Uppsala, Sweden. http://ucdp.uu.se/

Unidad para las Víctimas (2018) Desplazamiento-Personas. Red Nacional de Información, Bogotá, Colombia. https://cifras.unidadvictimas.gov.co/Home/Desplazamiento

Weber M, Gonzalez S (2003) Latin American deer diversity and conservation: A review of status and distribution. Ecoscience 10(4): 443–454. https://doi.org/10.1080/11956860.2003.11682792

Werth D, Avissar R (2002) The local and global effects of Amazon deforestation. Journal of Geophysical Research 107: 55–1–55–8. https://doi.org/10.1029/2001JD000717

Western D (1979) Size, life history and ecology in mammals. African Journal of Ecology 17(4): 185–204. https://doi.org/10.1111/j.1365-2028.1979.tb00256.x

Supplementary material 1

Table S1

Authors: Bayron R. Calle-Rendón, Flavio Moreno, Renato R. Hilário

Data type: species data

Explanation note: Mammal species used in the analysis and value of each factor to calculate the sensitivity index of each species (*S*) is available for this article online.

Link: https://doi.org/10.3897/natureconservation.29.28943.suppl1