

Antarctic macrobenthic communities: A compilation of circumpolar information

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Abstract

Comprehensive information on Antarctic macrobenthic community structure has been publicly available since the 1960s. It stems from trawl, dredge, grab, and corer samples as well as from direct and camera observations (Table 1–2). The quality of this information varies considerably; it consists of pure descriptions, figures for presence (absence) and abundance of some key taxa or proxies for such parameters, e.g. sea-floor cover. Some data sets even cover a defined and complete proportion of the macrobenthos with further analyses on diversity and zoogeography. As a consequence the acquisition of data from approximately 90 different campaigns assembled here was not standardised. Nevertheless, it was possible to classify this broad variety of known macrobenthic assemblages to the best of expert knowledge (Gutt 2007; Fig. 1). This overview does not replace statistically sound community and diversity analyses. However, it shows from where which kind of information is available and it acts as an example of the feasibility and power of such data collections. The data set provides unique georeferenced biological basic information for the planning of future coordinated research activities, e.g. under the umbrella of the biology program “Antarctic Thresholds - Ecosystem Resilience and Adaptation” (AnT-ERA) of the Scientific Committee on Antarctic Research (SCAR) and especially for actual conservation issues, e.g. the planning of Marine Protected Areas (MPAs) by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR).

Keywords

Macrobenthic communities, trawls, dredges, grabs, corers, direct observations (scuba-diving, sea-bed video, sea-bed photography)

Data resources

Data published through GBIF: <http://ipt.biodiversity.aq/resource.do?r=macrobenthos>

Seabed images through Pangaea: <http://www.pangaea.de/> (sample: <http://doi.pangaea.de/10.1594/PANGAEA.702075>)

General description

Additional information: Additional files uploaded: list of references (Table 1–2) and classification of macrobenthic communities (Fig. 1).

Project details

Project title: Antarctic macrobenthic communities: A compilation of circumpolar information.

Personnel: Julian Gutt.

Taxonomic coverage

General taxonomic coverage description (for detailed information see references in Table 1): Macrobenthic communities have been uploaded in the category “vernacularNames”, abbreviations in “taxonRemarks”.

“Sessile suspension feeders and associated fauna” can be dominated by both demosponges, e.g., *Cinachyra* or *Mycale* and hexactinellid (glass) sponges. The most common genus is *Rossella*. The sponges include fast growing genera, such as *Homaxinella* or those that grow slowly, at least during the adult life stage, such as the also common hexactinellid genus *Anoxycalix*. The associated fauna comprises specialised predators, such as nudibranches, asteroids (especially *Acodontaster conspicuus* and *Perknaster fuscus*, which control fast growing *Mycale acerata* populations) and gastropods. Other fauna groups are symbionts, amphipods and other macroorganisms that prefer an epibiotic life-mode (mainly from the echinoderms, such as sedentary holothurians, ophiuroids and crinoids). If space is not monopolised by sponges, then, cnidarians (such as gorgonians, pennatularians, alconarians or hydrocorals), solitary and compound ascidians, and a variety of bryozoans can be most abundant. A recently described population of lithodid crabs is speculated to grow fast due to oceanic warming and was associated with the “mobile deposit feeders, infauna and grazers”. Other mobile epifauna assemblages can be dominated in shallow areas by the asteroid *Acodontaster validus*, by two species of the grazing echinoid *Sterechinus*, a variety of deposit feeding and scavenging ophiuroids and mobile holothurians. The infauna is comparably rare; however, polychaetes and the clams *Yoldia* as well as *Laternula* can reach high densities, especially in shallow muddy sediments. A

general depth gradient exists for biomass and abundances. In addition, very low biomass and abundances are found in shallow habitats that are physically and permanently disturbed by sea-scour, in intermediately deep shelf areas that are scoured by icebergs and in extremely oligotrophic situations under or close to the ice-shelves. Intensively disturbed assemblages can be dominated by very few species, appearing to be almost "monospecific", during recolonisation by pioneers such as the ascidian *Mogula pedunculata*, bryozones like *Cellarinella* and *Cellaria* or the gorgonian *Primnoisis antarctica* or in physically disturbed areas, where only opportunistic mobile species survive. Locally clams of the species *Adamussium colbecki* can live in several layers on top of each other simply due to suitable environmental conditions and low competition. Species can also become very abundant when they are better local competitors for space, such as the demosponge *Cinachyra barbata*, s.l. Recently, fauna-rich vent sites and far poorer seeps have been discovered.

Common names: sessile suspension feeders and associated fauna (SSFA), sessile suspension feeders and associated fauna - predator driven (SSFA-PRED), sessile suspension feeders and associated fauna - dominated by sponges (SSFA-SPO), sessile suspension feeders and associated fauna - dominated by taxa other than sponges (SSFA-OTH), mixed assemblage (MIX), very low biomass or absence of trophic guilds (VLB), "monospecific" (MONO), physically controlled (PHYCO), mobile deposit feeders, infauna and grazers (MOIN), mobile deposit feeders, infauna and grazers - infauna dominated (MOIN-INF), mobile deposit feeders, infauna and grazers - epi-fauna dominated (MOIN-EPI), vent (VENT), and seep (SEEP).

Spatial coverage

General spatial coverage: The study area generally covers almost the entire Southern Ocean, including single ice-shelf covered sites (Fig. 2). The vast majority of information is from shelf areas around the continent at water depth shallower than 800m. Non-ice shelf covered shelf areas can be up to 300km wide or the shelf-edge at 600 to 800m depth can "disappear" beneath the floating ice-shelf. Shallow areas (<50m) are rare because 86% of the coast-line is glaciated or consists of an ice-shelf edge. A non-glaciated coast mainly exists along the Antarctic Peninsula. The coastline is either extremely complex with bays, channels, peninsulas, islands etc. or less structured, especially where it is formed by the ice-shelf. Overdeepened basins (inner-shelf depressions) can reach >1200m water depth. Most islands exist west of the Antarctic Peninsula and along the Scotia Arc linking the Peninsula with the southern tip of South America. The coastal waters are mainly affected by the Antarctic Coastal Current (East Wind Drift), whilst the largest off-shore part of the Southern Ocean is dominated by the Antarctic Circumpolar Current (West Wind Drift) with gyres of different size. Sediments are predominantly poorly sorted but also cobble "fields", bedrock, and pure soft sediments exist. The Antarctic marine ecosystem is shaped by a distinct seasonality of the sea-ice cover affecting a short and intensive primary production in austral summer, by predominantly stable low temperature to which most organisms are thought

to be specifically adapted to, and very little terrestrial run-off. Most of the shelf-inhabiting macrobenthic species are endemic; some taxa reach above-average species richness (Clarke and Johnston 2003). Only few marine habitats are protected, most of which are small. Plans and proposals for large Marine Protected Areas (MPA's), e.g. in East Antarctica, in the Ross and Weddell Seas, exist but require good scientific knowledge and data to be meaningful.

Coordinates: 83°0'0"S and 52°0'0"S Latitude; 180°0'0"W and 180°0'0"E Longitude.

Temporal coverage: March 1, 1956–February 21, 2010.

Methods

Method step description: Attribution of the information from the different sources (for references see Table 1, for hyperlinks see Table 2) to the classified macrobenthic assemblages (Fig. 1) was done to the best of expert knowledge. This was done for the entire data set simultaneously and the results were made publically available for the first time via the database “Antarctic Biodiversity Facility” (ANTABIF). The principal parameter on which these assumptions have been made was biomass or a proxy for biomass such as sea-floor coverage. Some information on benthic functioning is also included directly or indirectly, e.g. predation, competition, succession after ice-berg scouring, epi-biotic life-mode and oligotrophic conditions under ice shelves. The source publications listed (Table 1) comprise descriptions of catches, other observations, and data on fauna and were mainly from historical and modern peer-reviewed articles. Other information sources were sea-bed videos and still images together with associated meta-data (Table 2). All the latter source material has an associated DOI and is available at the database PANGAEA (www.pangaea.de).

Study extent description: Southern Ocean with emphasize on coastal shelf areas and some islands without specific temporal patterns of sampling.

Sampling description: This project aggregates data from various expeditions with a full range of benthic sampling methods, such as grabs, corers, dredges, and trawls as well as non-invasive observations by scuba divers, stationary, towed, or ROV-based still and video-cameras. For detail descriptions see original publications in journals (Table 1) or data repositories (Table 2).

Quality control description: A first version of the classification of the macrobenthic communities had been published in a peer-reviewed journal (Gutt 2007). A modified version had been published in the Antarctic Climate Change and the Environment report (ACCE, Turner et al. 2009). The actual version is depicted in Fig. 1. Data presented here is available at ANTABIF/SCAR-MarBIN and will contribute to the biogeographic atlas project of SCAR and the Census of Marine Life (De Broyer et al. in prep.), <http://atlas.biodiversity.aq/>.

Table 1. References of results and data used for the compilation of information on Antarctic macrobenthic communities presented in this article.

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Table 2. Hyperlinks (DataCite DOIs), which provide access to seabed images and metadata from single stations where the images have been taken. The macrobenthos depicted in these images was classified and used for the compilation of information on Antarctic macro-benthic communities presented in this article.

doi: 10.1594/PANGAEA.702075	doi: 10.1594/PANGAEA.770359
doi: 10.1594/PANGAEA.702059	doi: 10.1594/PANGAEA.198690
doi: 10.1594/PANGAEA.702076	doi: 10.1594/PANGAEA.198691
doi: 10.1594/PANGAEA.702077	doi: 10.1594/PANGAEA.198692
doi: 10.1594/PANGAEA.702062	doi: 10.1594/PANGAEA.198693
doi: 10.1594/PANGAEA.702078	doi: 10.1594/PANGAEA.198694
doi: 10.1594/PANGAEA.702064	doi: 10.1594/PANGAEA.198695
doi: 10.1594/PANGAEA.702065	doi: 10.1594/PANGAEA.198696
doi: 10.1594/PANGAEA.702066	doi: 10.1594/PANGAEA.198697
doi: 10.1594/PANGAEA.702067	doi: 10.1594/PANGAEA.198698
doi: 10.1594/PANGAEA.702079	doi: 10.1594/PANGAEA.198699
doi: 10.1594/PANGAEA.702069	doi: 10.1594/PANGAEA.198667
doi: 10.1594/PANGAEA.702070	doi: 10.1594/PANGAEA.198668
doi: 10.1594/PANGAEA.702080	doi: 10.1594/PANGAEA.198669
doi: 10.1594/PANGAEA.702072	doi: 10.1594/PANGAEA.198670
doi: 10.1594/PANGAEA.702073	doi: 10.1594/PANGAEA.198671
doi: 10.1594/PANGAEA.702074	doi: 10.1594/PANGAEA.198672

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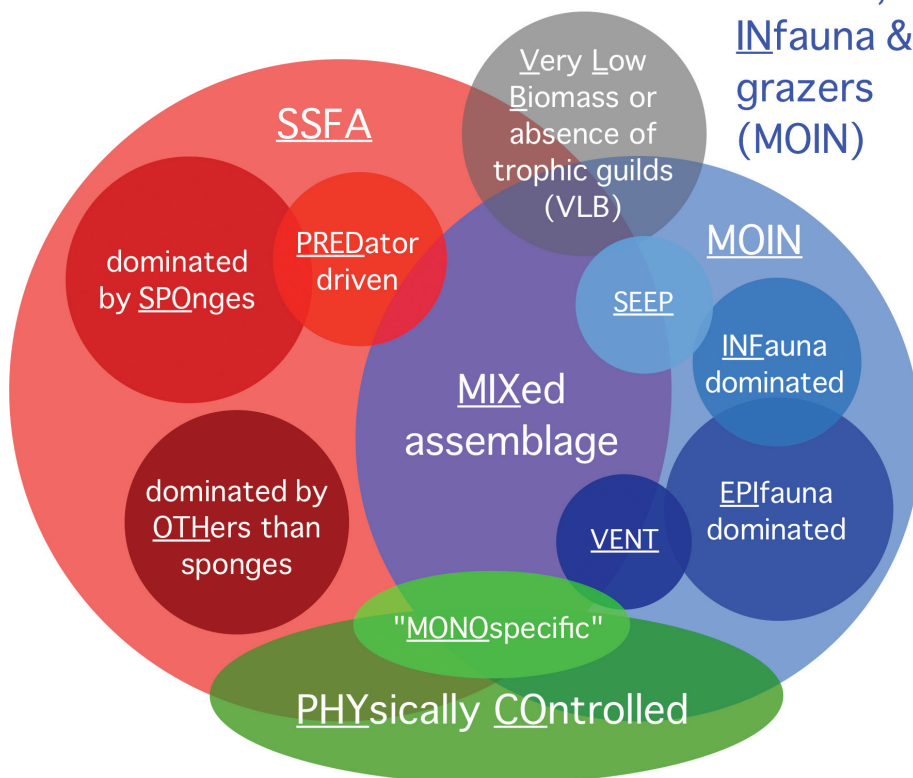


Figure 1. Classification of Antarctic macro-benthic communities (after Gutt 2007 and Turner et al. 2009).

Datasets

Dataset description

Object name: Darwin Core Archive Antarctic macrobenthic communities: A compilation of circumpolar information

Character encoding: UTF-8

Format name: Darwin Core Archive format

Format version: 1.0

Distribution: <http://ipt.biodiversity.aq/archive.do?r=macrobenthos>

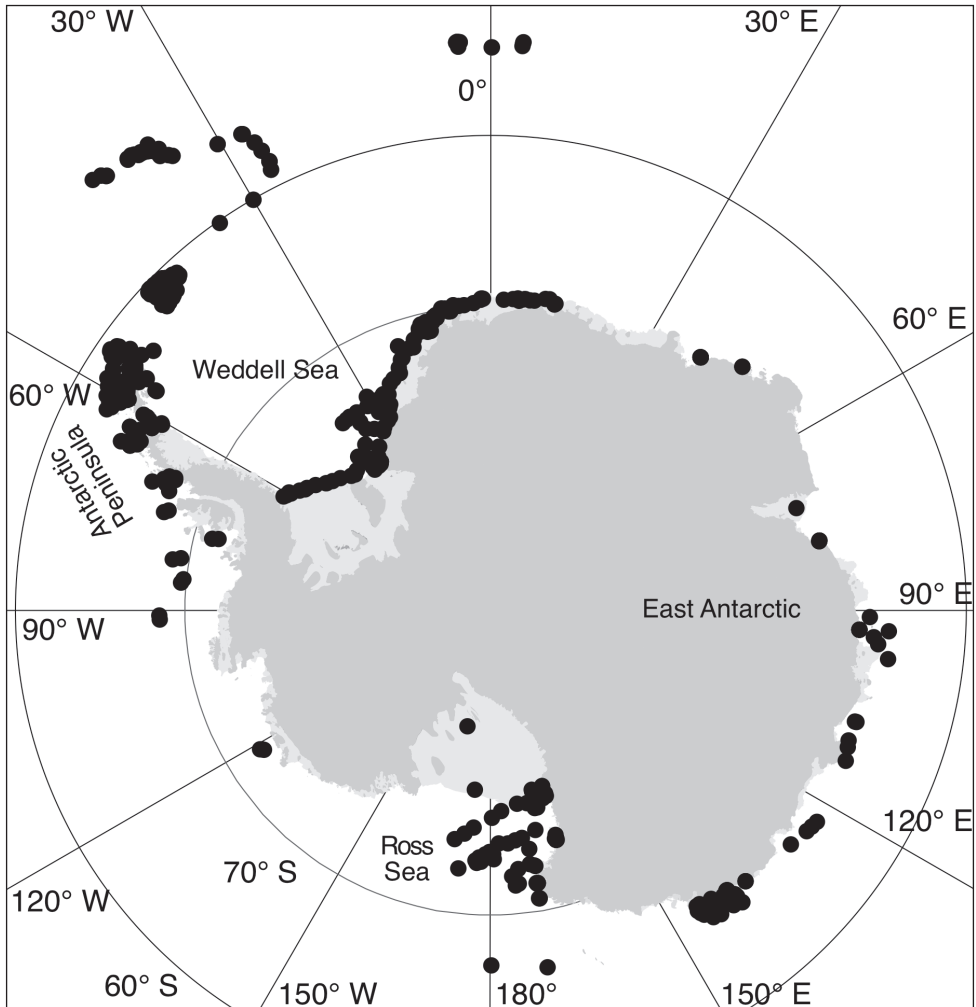


Figure 2. Geographic coverage of the circumpolar distribution of information on Antarctic macrobenthic communities provided by ANTABIF.

Publication date of data: 2012-07-19

Language: English

Licenses of use: This data-set is entitled “Antarctic macrobenthic communities: A compilation of circumpolar information” and has been uploaded to (ANTABIF). The data set has been made available under the Open Data Commons Attribution License: <http://www.opendatacommons.org/licenses/by/1.0/>

Metadata language: English

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Protected Areas legislation and the conservation of the Colombian Orinoco basin natural ecosystems

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Abstract

Colombia has shown a strong commitment to the achievement of the CBD's biodiversity target, by promoting the conservation of at least 10% of its natural ecosystems. Protected Area categories in Colombia are undergoing a standardization process that should enhance the country's capacity to protect its natural ecosystems. In this study we use a spatial analysis to examine how the legislation and the civil society's initiatives help in the conservation of natural ecosystems in the Colombian Orinoco Basin. We found that differentiation in use restriction legislation limits the conservation potential of some Protected Area categories. The only fully Protected Areas in Colombia are the Natural National Parks System Areas, which protect only 10% of the area of natural ecosystems and less than 50% of the natural ecosystems in the Colombian Orinoco Basin. Indigenous Reserves help significantly in the conservation of the natural ecosystems in the Colombian Orinoco Basin, but are not a Protected Area category, making it difficult for indigenous groups to aid in the accomplishment of conservation goals in Colombia.

A small percentage of ecosystems of the Colombian Orinoco Basin fall outside of any Protected Area or Indigenous Reserve and urgent actions may be needed to protect them. Future similar studies should use current and updated information on Protected Areas and take into account changes in land cover, for a better understanding of the role of different categories of Protected Areas in the achievement of conservation objectives in Colombia.

Keywords

National System of Protected Areas, Orinoco Basin, Ecosystem Coverage, Indigenous Reserves, Private Protected Areas

Introduction

Colombia has shown a strong commitment to the achievement of the CBD's 2010 biodiversity target. Just recently the Ministry of Environment passed a decree to standardize the Protected Area categories and to organize the National System of Protected Areas (SINAP in Spanish). The SINAP will need guidelines for the management and establishment of Protected Areas in Colombia. Thus, evaluations of the ecosystem coverage and effectiveness of Protected Areas is necessary. Here we present an overview of the Protected Areas management in Colombia and the implications for the conservation of the natural ecosystems in the Colombian Orinoco Basin.

Protected Areas in Colombia

The first Protected Areas in Colombia were established by the 2nd law of 1959 under the category of Forest Reserves, with the purpose of preserving Colombia's water supply and wildlife. But the concept of Protected Area was not clearly defined by Colombian legislation until 1994, when the government ratified the CBD with the law 165 of 1994, using the same wording used by the Convention to give a definition to the concept of a Protected Area: "a geographically defined area, which is designated or regulated and managed to achieve specific conservation objectives" (Ponce de Leon 2005).

Since then, a great number of Protected Areas have been established without specific standards or criteria, mainly due to flaws within the legislation. For example, some areas have been created by institutions authorized to do so but without proper use of the categories recognized by Colombian legislation (Ponce de Leon 2005; Vásquez-V. and Serrano-G. 2009). This situation lead to a great number of Protected Areas to be left out of any protection scheme, as they were not strictly legally established Protected Areas.

In a recent inventory of Protected Areas in Colombia, the National Natural Parks Office reported a total of 419 Protected Areas in Colombia, established under 181 designations of which only 11 were legally supported (Tamayo 2009).

National system of Protected Areas

Aware of the problem identified above, the Ministry of Environment has been working together with all the stakeholders on consolidating and coordinating the National System of Protected Areas (Sistema Nacional de Areas Protegidas – SINAP) which has been defined by the decree 2372 of 2010 as: "the set of protected areas, social and institutional stakeholders and the strategies and management tools that bring them together, contributing as a whole to the fulfillment of the general conservation objec-

tives of the country”. This decree classifies and describes the categories of the SINAP, setting a target to redefine all categories and designations used before, so that they fit the legally accepted categories, by July 2011, going from more than 43 to only 8 subcategories and designations. Legally, only three Protected Area management categories will be accepted: areas managed by the national government, areas managed by the regional government and areas managed by private landowners. The national government Protected Areas are administered by the Ministry of Environment, and are divided into three subcategories. The regional government Protected Areas are divided into five subcategories and are administered by the Regional Environmental Agencies (Corporaciones Autonomas Regionales – CARs). In 1993 the law 99 allowed the existence of Protected Areas managed by private landowners, following an initiative of the private landowners themselves, to aid in the *in situ* conservation of biological diversity in Colombia. Most Private Protected Areas in Colombia are grouped into different non-governmental associations that help coordinate conservation actions between landowners.

Protected Areas with full protection

The World Conservation Union (IUCN) defines a Protected Area as: “A clearly defined geographical space, recognized, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values” (Dudley 2008). Strict compliance to this definition would result in some of the Protected Area categories and subcategories in Colombia not to be considered as Protected Areas. For example, the constitution states that all of Colombia’s subsoil is property of the State, under this premise, any land portion that has been proven to contain oil or any other mineral is subject to exploitation by the government or a private organization commissioned by the State, and although a number of laws have been emitted in order to prevent this from happening in Protected Areas, only some categories have been effectively protected.

Regarding oil exploitation, the decree 622 from 1997 prohibits any exploration and extraction inside one of the subcategories of the national government Protected Areas, the National Natural Parks System Areas (NNPSA). Areas that are forbidden for extraction of other minerals are the NNPSA’s and some subcategories within the national and regional governmental Protected Areas. A further examination of the legislation shows that the only PA’s established in perpetuity are the NNPSA’s, as has been determined by the constitution. Unfortunately this is almost impossible to implement for the other Protected Area categories in Colombia. As a result of these gaps in Colombian legislation for Protected Areas, only a very small proportion is truly protected: the 55 NNPSA’s, as the other ca. 400 are not protected from mining, oil exploration and exploitation, and possible disappearance.

Other areas of special interest for biodiversity conservation in Colombia

The constitution allows ethnic groups to own and administer the territories they occupy, in order to protect their cultural heritage and, traditional knowledge and ways of life. A total of 738 ethnic collective territories have been assigned to afro-descendant communities and indigenous groups in Colombia, adding up to 435,000 km² (Tamayo 2009). These territories have not been created to achieve conservation objectives and thus are not included in the SINAP. But they pose a great opportunity for biodiversity conservation, as the traditional ways of life of indigenous people are usually sustainable and environmentally friendly.

Colombian Orinoco basin

The Orinoco River flows 2,140 km from the Andes in Colombia to the Atlantic in Venezuela. Its tributary rivers form a basin considered to be the 3rd most important river system on the planet, and one of the most biologically and hydrologically diverse areas of the world (WWF 2010). The area of the basin has been estimated to be about 991,587 km²; including landscapes of the Andes, plains of the Llanos and the Guiana shield. Nearly 65% of the Orinoco Basin is located in Venezuela and the remaining 35% in Colombia (Romero et al. 2004). This area contains high levels of species diversity. To date there are records of 17,420 species of plants, 1,300 species of birds, nearly 1,000 species of fish, 318 species of mammals, 266 species of amphibians and 290 species of reptiles (Lasso et al. 2010, WWF 2010). This area also has great ethnic diversity as it is home to indigenous groups such as the *Achagua*, *Amorua*, *Baniba*, *Bare*, *Betoye*, *Chiricoa*, *Cuiba*, *Guahibos*, *Hoti*, *Kapo*, *Karina*, *Kuripako*, *Makaguaje*, *Masiguare*, *Ninam*, *Panare*, *Pemon*, *Piapoko*, *Piaroa*, *Puinave*, *Saliba*, *Sanema*, *Uwa*, *Warao*, *Yanomami*, *Yekuana* and *Yeral* (WWF 2010).

A previous study, aiming to identify the gaps of the NNPSA's on a national scale, based on an ecosystem classification published in 1998 by Etter, reported for a subarea inside of the Colombian Orinoco Basin, eight totally unprotected ecosystems (Arango et al. 2003). More recently, a set of studies identifying priority areas for conservation and the representativeness of the Protected Areas on a global scale has been published. These were based on more recent ecosystem classifications and species diversity information. Some of these studies have been done on a national scale (Corzo 2009, Forero-Medina & Joppa 2010), and some on a more regional scale (Rodríguez et al. 2009, Romero et al. 2009), but none of them has looked at the whole of the Colombian Orinoco Basin and the implications of differences in legislation applicable to the various Protected Area categories.

The aim of this paper is to examine how the different Protected Area categories at present are protecting the natural ecosystems of the Colombian Orinoco Basin, and to observe how the protection could improve with new regulations, providing information for the design of future conservation strategies in this area. This was achieved by carrying out a spatial analysis to determine the level of protection of each ecosystem present in the study area.

Methods

Study area

The Colombian Orinoco Basin covers 3,447,713 km² that correspond to 30.4% of Colombia's terrestrial area (Romero et al. 2004). Ecosystems present in this area serve as regulators of a highly fluctuating water cycle that provides 33.8% of Colombia's fresh water supply (Andrade-Pérez et al. 2009). Romero et al. (2004) produced the Map of the Ecosystems of the Colombian Orinoco Basin. The map was created in a 1:100,000 scale using Landsat and ETM images from the 1999-2001 period, and cartography from the Instituto Geográfico Agustín Codazzi (IGAC) at the same scale, based on the methodology proposed by Rodríguez et al. (2004). This classification identified 4 great biomes (*Orobioma Del Zonobioma Humedo Tropical*, *Pedobioma Del Zonobioma Humedo Tropical*, *Zonobioma Humedo Tropical*, *Zonoecotono Del Zonobioma Humedo Tropical Y Pedobioma*), 22 types of biomes and 207 ecosystems, of which 51 are classified as transformed ecosystems and the other 156 as natural (Romero et al. 2004).

Data collection

Information regarding the name, management category, date of establishment, exact location and digital geographical information of all the Protected Areas and Indigenous Reserves in the Colombian Orinoco Basin was gathered from various sources. The main source of information was the inventory of Colombia's Natural Protected Areas published by Vásquez-V. and Serrano-G. (2009), the authors made available the digital maps and shapes of all the Natural Protected Areas managed by governmental organizations, at the national, regional and local scales, established up to December 2008.

Information from Private Protected Areas was provided by the Colombian Orinoco Basin information node, of the Asociación Red Colombiana de Reservas Naturales de la Sociedad Civil – Resnatur - (Nodo Orinoquia 2010); Miguel Andrés Suarez; and the National Natural Parks Office. The Instituto Geográfico Agustín Codazzi - IGAC, provided information from Indigenous Reserves, established up to December 2008.

The user license of the digital version of ecosystem classification map was provided by Instituto de Investigación de Recursos Biológicos Alexander von Humboldt – IAvH.

Data analysis

A digital map of all the Protected Areas and Indigenous Reserves established in the Colombian Orinoco Basin, up to December 2008, was created using ArcGIS 9.3.

A major difficulty in the construction of the map was that some areas overlapped. The majority of the overlaps were cases in which a small Protected Area was embedded inside a bigger Protected Area; for those cases we completely eliminated the small

Protected Area from the map, taking into account that the bigger area for all cases had a better protection scheme than the smaller one. In cases where the limits of the areas overlapped, we used a hierarchical system, erasing parts of the local government Protected Areas first, the regional governmental Protected Areas secondly and finally from the Indigenous Reserves giving priority to the National Parks over all the Protected Areas.

The map of Protected Areas and Indigenous Reserves of the Colombian Orinoco Basin was superimposed over the ecosystem classification map, using the intersect function of the analysis tools in ArcGIS 9.3. The resulting shape file in the Magna Colombia Bogotá coordinate system was projected to calculate the areas in km² using ArcView GIS 3.2. The resulting attribute tables were exported to Microsoft Excel and area totals were calculated using pivot tables. The ecosystems were organized by order of protection, from no protection at all to total protection, in terms of proportion of the total area included in Protected Areas and Indigenous Reserves, and classified into 4 levels of protection: without protection, less than 10% of total area protected, 10-50% of total area protected, and more than 50% of total area protected, based on the criterion of the CBD that the minimum of protection for an ecosystem should be 10% of its area.

Three different analyses were carried out: an analysis of ecosystem coverage of the Protected Areas included in the National Natural Parks System, considered by us to be the only truly Protected Areas in Colombia; an analysis of ecosystem coverage of all the areas of the National System of Protected Areas (SINAP) which includes National Parks, Protected Areas of the Regional Governments and Private Protected Areas; and an analysis of ecosystem coverage of all the Protected Areas plus the Indigenous Reserves to explore the benefits of declaring Indigenous Reserves as Protected Areas.

Results

A total of 132 Protected Areas and 123 Indigenous Reserves have been established in the Colombian Orinoco Basin during the period of 1945 – 2008. Due to overlapping problems, we had to completely eliminate from the analysis some PA's and Indigenous Reserves resulting with a final map of 106 Protected Areas and 115 Indigenous Reserves. All the Protected Areas included in the final map sum to 52,819.69 km²; half of this area belongs to the National Natural Parks System Areas. About 80% of the total area of the Colombian Orinoco Basin (ca. 280,000 km²) is covered by natural ecosystems.

Analysis of ecosystem protection by existing fully Protected Areas

According to the definition of Protected Area given by the IUCN (Dudley 2008), the National Natural Parks System Areas (NNPSA) are the only Protected Areas in Colombia that should be considered as truly protected. Thus, an analysis of ecosystem coverage with only this Protected Area subcategory was carried out.

Table 1. Protection levels of natural ecosystems in National Natural Parks System Areas of the Colombian Orinoco Basin.

Level of protection	Number of natural ecosystems	Proportion of total natural ecosystems
Without protection	67	43%
Less than 10% of total area protected	32	21%
10–50% of total area protected	35	22%
More than 50% of total area protected	22	14%
Total	156	100%

The National Natural Parks System in the Colombian Orinoco basin has 2 National Nature Reserves (Puinawai and Nukak), 2 National Natural Parks (El Tuparro and Sierra de La Macarena), and small fragments of other 7 National Natural Parks. All of these areas add up to 28,508.78 km², of which ca. 27,000 km² are covered with natural ecosystems (94%). The NNPSA's protect almost 10% of the 280,000 km² of natural ecosystems found in the Colombian Orinoco Basin.

The ecosystem coverage analysis shows that with the NNPSA's 43% of natural ecosystems are not protected at all and 21% are inadequately protected (Table 1). On the other hand, 1% (2) of ecosystems is completely protected inside the NNPSA's.

Analysis of potential ecosystem protection

Taking in to account that by July 2011, all previously established Protected Areas should be included in the National System of Protected Areas (SINAP), we carried out an analysis to determine ecosystem protection by all the Protected Area categories in the Colombian Orinoco Basin. It is important to highlight that legalization of the previously established Protected Areas, by including them in the SINAP, is not enough to ensure their protection. We believe ecosystem protection will not be satisfactorily achieved, unless all the categories in the SINAP are legally protected in the same way that the NNPSA are, preventing the possible loss of these areas to mining and drilling projects and other unfortunate circumstances. This is why we refer to this analysis as potential ecosystem protection.

The 106 Protected Areas included in this analysis sum to 52,819.69 km², with ca. 42,000 km² covered with natural ecosystems (79%), which equates to 15% of the 280,000 km² of natural ecosystems found in the Colombian Orinoco Basin. This analysis shows that Protected Areas in the Colombian Orinoco Basin leave 24% of natural ecosystems totally unprotected and 27% inadequately protected (Table 2). However, 8% (13) of natural ecosystems would be completely protected by all the PA's of the SINAP.

Table 2. Protection levels of natural ecosystems in Protected Areas of the SINAP in the Colombian Orinoco Basin.

Level of protection	Number of natural ecosystems	Proportion of total natural ecosystems
Without protection	38	24%
Less than 10% of total area protected	42	27%
10–50% of total area protected	36	23%
More than 50% of total area protected	40	26%
Total	156	100%

The Indigenous Reserve as a Protected area category

The 115 Indigenous Reserves (IR) of the Colombian Orinoco Basin used in this study sum to 87,729.95 km², covering 25% of the study area. We estimate that nearly 97% of the area of Indigenous Reserves is covered with natural ecosystems. Incorporating the IR's as a fully Protected Area category in the SINAP would increase the area protected in the Colombian Orinoco Basin up to 140,549.64 km² (50% of the Colombian Orinoco Basin) of which approx. 125,000 km² are covered with natural ecosystems. This would represent protection for 45% of the 280,000 km² covered with natural ecosystems in the Colombian Orinoco Basin. Integrating IR's into the SINAP as a fully Protected Area category leaves only 6% of natural ecosystems totally unprotected (Tables 3 and 4). On the other hand, 12% (20) of natural ecosystems would be completely protected under this scenario.

Discussion

Data collection for this analysis was very difficult because the information on Protected Areas is scattered around many institutions. Although the objective was to observe the establishment of Protected Areas up to the present time, this was not possible due to limitations of the information provided by some of the sources, which had information only up to December 2008.

In regards to the proportion of land identified as covered with natural ecosystems in the Colombian Orinoco Basin, a study on deforestation (Sanchez-Cuervo et al. 2012) estimated that some areas in the Llanos ecoregion have lost up to 27% of their woody vegetation cover in the last decade. Another study on land cover change of the natural savannas by Etter et al. (2010), has shown that, for the 2000–2007 period, the natural savannas have been destroyed at an annual rate of more than 1,000 km² and converted to pastures and oil palm plantations. This affects the results reported here as the images used for the ecosystem classification are dated in 1999–2001, and it is uncertain how land cover has changed for other natural ecosystems. For future studies we recommend to evaluate change in land cover and conversion of natural ecosystems inside the Protected Areas.

Table 3. Protection levels of natural ecosystems in Protected Areas and Indigenous Reserves of the Colombian Orinoco Basin.

Level of protection	Number of natural ecosystems	Proportion of total natural ecosystems
Without protection	10	6%
Less than 10% of total area protected	21	13%
10–50% of total area protected	45	29%
More than 50% of total area protected	80	51%
Total	156	100%

Table 4. Names and total area of ecosystems of the Orinoco River Basin that appear to be unprotected. Descriptions and distribution of these ecosystems can be found in Romero et al. 2004.

Natural Ecosystem	Area (Has)
<i>Anfibroma de Arauca - Casanare_Sabana en piedemonte antiguo y tectonizado</i>	10,408.8
<i>Anfibroma de Arauca - Casanare_Sabana inundable en terraza alta aluvial de rio andinense</i>	94,584.0
<i>Helobioma de la Orinoquia y Amazonia_Sabana de desborde en llanura aluvial con influencia eolica</i>	20,550.1
<i>Orobioma de paramo de la cordillera Oriental_paramo humedo en montaña estructural erosional</i>	19,807.5
<i>Orobioma de paramo de la cordillera Oriental_Subparamo muy humedo en montaña estructural erosional</i>	999.8
<i>Orobioma subandino cordillera Oriental_BMD seco en montaña estructural erosional</i>	5,704.5
<i>ZHT del piedemonte Meta_BMD muy humedo a húmedo en piedemonte aluvio diluvial</i>	38,361.9
<i>ZHT del piedemonte Meta_BMD muy humedo a húmedo en terraza alta aluvial de rio andinense</i>	5,784.9
<i>Zonocotono del zoniobioma humedo tropical y pedobioma_BMD en piedemonte antiguo y tectonizado</i>	16,814.1
<i>Orobioma subandino cordillera Oriental_BMD humedo en montaña estructural erosional</i>	1,757.2

Ecosystem protection by existing fully Protected Areas

Our findings show a similar pattern to the results of previous studies regarding the proportion of inadequately protected ecosystems. In 2003 Arango et al. found that for a smaller area inside the Colombian Orinoco Basin, 57% of natural ecosystems were not protected at all and 21% did not have enough area protected by the NNPSA's. They based their study on an earlier published national classification of ecosystems. Taking into account that the area studied by Arango et al. (2003) is smaller and the ecosystem classification they used broader, it is reasonable to state that ecosystem protection by NNPSA's has not improved in the last 7 years, mainly because no new areas have been established.

A recent study of fire regimes by Romero-Ruiz et al. (2009) reported that NNPSA's burn very often due to the presence of Indigenous people inside the NNPSA's. Also, it has been reported that some of these areas have management problems such as the lack of control on the population growth inside the parks and the unsustainable use of the resources (Vásquez-V. and Serrano-G. 2009), these matters should be looked at in more detail as an evaluation on the effectiveness of management plans inside the NNPSA's.

Potential ecosystem protection

We estimate that all PA's of the SINAP in the Colombian Orinoco Basin protect an area of 52,819.69 km². This number could be higher as this analysis only included areas registered up to December 2008. But it could also decrease in the future as some PA's could be left out of the SINAP on the standardization process, depending on the criteria used to include them and the budget limitations for their management. So it is important to carry out a new census in the future, to re-evaluate ecosystem protection after the new legislation is implemented, and the SINAP organized. We recommend repeating this kind of study regularly to provide a periodical evaluation of the SINAP at regional and national scales. An important finding of this analysis, compared to the analysis of fully protected areas, is that although the protected area is almost doubled, ecosystem protection does not increase proportionally, this is a good indicative of the lack of planning and management capacities of the regional and local governments that established most of these areas.

The work that the Private Protected Areas are doing on complementing the governmental Protected Areas must be highlighted; although these areas are small, one natural ecosystem: *Helobioma de la Orinoquia y Amazonia_Sabana de desborde en llanura aluvial de rio andinense*, is being adequately protected only by private landowners, and other ecosystems have reached an acceptable level of protection (more than 10% of the area) mainly because of the Private Protected Areas.

Recent studies on fire regimes in the Orinoco Basin savannas have found that private ranches dedicated to cattle farming on native vegetation, tend to burn less often than other areas in this region (Romero-Ruiz et al. 2009). This might be a good indicative of the high-quality management capacity of private landowners.

The national and regional governments could rely on the civil society to help them achieve the nation's conservation goals but this is only possible if the Private Protected Areas become fully protected, and new incentives and mechanisms are given to private landowners to establish and maintain Private Protected Areas.

There is some agreement between the distribution of unprotected and inadequately protected ecosystems reported here with published maps from other studies of identification of priority areas for conservation, (Rodríguez et al. 2009, Romero et al. 2009, Corzo 2009), although none of the previous studies looked at the whole of the Colombian Orinoco Basin.

The Indigenous Reserve as a Protected Area category

Our results show that most of the lands owned and managed by indigenous people are still in very good ecological condition, based on the percentage of natural ecosystems they hold, specially compared to the Protected Areas of the SINAP. Making the Indigenous Reserves a Protected Area category would help greatly on the conservation of the Colombian Orinoco Basin, but this is a significant national debate, with a lot of controversy around it.

At the moment, if any indigenous group wants to protect biodiversity in its territories in the long term, it should follow the extraordinary example Ayawa people in the Amazon region, who got the Ministry of Environment to declare their Indigenous Reserve a National Natural Park, the Yaigoje Apaporis, committing to promote their traditional ways of life and implement management plans (Macuna 2009).

Another example of the willingness of Indigenous people to aid in the conservation of biodiversity in their territories is the Selva Matavén Indigenous Reserve; this reserve was created after 16 Indigenous Reserves in its surroundings, from different ethnic groups, got together to protect this forest that is sacred to all of them (Mora et al. 2002). Now the ca. 20,000 km² of Selva Matavén need the double protection scheme, with the help of the National Natural Parks System, just like the Yaigoje Apaporis, or another legally viable solution.

Previously published literature on fire regimes in this area has shown that burning occurs more often in the natural savannas inside Indigenous Reserves than in private ranches. This should be looked at in more detail as it could help understand the effect of traditional practices on natural ecosystems (Romero-Ruiz et al. 2009).

General considerations

Special attention should be paid to the 10 ecosystems that appear to be unprotected in the three levels of analysis (Table 4). Urgent action may be needed to protect those ecosystems from disappearing, as they are not present in any of the PA's or Indigenous Reserves of the Colombian Orinoco Basin. Land tenure is a truly problematic situation in Colombia, and awareness of unprotected ecosystems should be raised to the Ministry of Agriculture when allocating State property land to the people. These ecosystems should be excluded from any land reform, or the property titles of these areas should be given to NGO's and private landowners committed to biodiversity conservation.

Etter et al. (2010) made projections on the change in land cover of the Colombian Orinoco Basin, showing that it is possible to lose more than 22,350 km² of natural savannas in the next 10 years, their finding should be taken into account in future efforts to protect the ecosystems that are currently unprotected to avoid losing them forever.

Conclusions

There are 132 Protected Areas and 123 Indigenous Reserves in the Colombian Orinoco Basin. This area has 52,819.69 km² of Protected Areas with 79% of their area covered with natural ecosystems; and 87,729.95 km² of Indigenous Reserves with 97% of their area covered with natural ecosystems. An estimated 36% of ecosystems are adequately protected by fully protected areas; these areas leave 43% of natural ecosystems totally unprotected, and 21% inadequately protected, defined as less than 10% of total area inside a Protected Area. Ecosystem protection would improve if all categories in the SINAP

would be legally protected from sudden disappearance or losing areas for mining and drilling. A much better protection of ecosystems would be achieved if the Indigenous Reserves were a Protected Area category or if Indigenous Reserves could have a mechanism to declare some of their territories fully Protected Areas. Ultimately, 10 natural ecosystems were found to be completely unprotected in the three levels of analysis of this study.

Recommendations

Legislation should be modified so that all Protected Area categories in Colombia are fully protected, to ensure real *in situ*, long-term conservation of biodiversity and natural ecosystems.

Incentives should be used to promote the creation and maintenance of Private Protected Areas

Legal mechanisms should be implemented so that Indigenous Reserves can officially aid in the *in situ* conservation of natural ecosystems in Colombia more easily.

Urgent actions should be taken to ensure protection of the natural ecosystems that fall outside Protected Areas and Indigenous Reserves in the Colombian Orinoco Basin, as these may be significantly endangered.

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Retention forestry and biodiversity conservation: a parallel with agroforestry

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In forested landscapes two general management systems – retention forestry and agroforestry – have been proposed as potentially efficient components of landscape approaches to ease the conflict between biodiversity objectives and human needs. In two recent reviews, Gustafsson et al. (2012) and Lindenmayer et al. (2012) provide a global overview of current knowledge about the practice and ecological roles of retention forestry. A few years ago, Bhagwat et al. (2008) produced a similar review addressing the role of agroforestry in biodiversity conservation. Here we draw a parallel between research on the ecological effects of retention forestry and agroforestry. We argue that conservation science and practice would benefit from bridging these two separate fields and the experiences achieved.

Gustafsson et al. (2012) defined retention forestry as “an approach to forest management based on the long-term retention of structures and organisms, such as live and dead trees and small areas of intact forests, at the time of harvest”. The retention approach is broadly applicable to tropical, temperate and boreal forests (Lindenmayer et al. 2012). Agroforestry is defined as “intentional management of shade trees with agricultural crops” (Bhagwat et al. 2008). The agricultural component of agroforestry systems may also consist of pasture (Mosquera-Losada et al. 2008). Agroforestry systems are widespread in the tropics but also relevant to temperate regions (e.g. Gordon

and Newman 1997; Mosquera-Losada et al. 2008). In even-aged forest management, there are typically three possible management regimes for a given stand: clearcutting (or shelterwood without long-term tree retention), harvesting with retention, and no harvesting. Analogously, in the context of agriculture, three broad types of local land use are likely in regions where the natural vegetation is forest: intensive agriculture or plantation, agroforestry, and no management (i.e. forest).

There are important similarities between retention forestry and agroforestry, the most salient being that they both result in a tree cover which is intermediate between treeless vegetation and continuous forest. The original reasons for leaving some tree cover may differ between the two approaches, but from the biodiversity conservation perspective they still share many important features: both approaches (1) maintain or restore compositional, structural and functional diversity within ecosystems, (2) facilitate dispersal in fragmented landscapes through increased connectivity for forest-dwelling species, (3) provide habitat for tree-dependent species outside forest, and (4) minimize off-site impacts of management on, for example, aquatic systems (Bhagwat et al. 2008, Jose 2009, Gustafsson et al. 2012). There are also differences between the two approaches as regards biodiversity conservation. For example, in even-aged forestry retention trees may play a temporary life boating role over the first stages of forest succession (Gustafsson et al. 2012) as opposed to a more static function in most agroforestry systems. In retention forestry, the level and spatial patterning of retention is usually based on conservation objectives (and influenced by operational limitations), whereas the tree cover characteristics in agroforestry systems has traditionally been influenced mostly by agricultural production objectives. Nevertheless, the large overlap in the features important for biodiversity conservation implies a clear potential for bridging the two fields. For example, although the two approaches are relevant to both temperate and tropical regions, the biodiversity benefits of retention forestry have mostly been studied in temperate and boreal ecosystems. Hence, tropical retention forestry could benefit not only from the knowledge about retention forestry outside the tropics, but also from some of the experiences acquired in tropical agroforestry.

How have retention forestry and agroforestry succeeded at conserving biodiversity in practice? Bhagwat et al. (2008) compared species richness and composition between tropical forest reserves and agroforestry systems, and concluded that the latter may help conserve a large proportion of tropical biodiversity in the face of an increasing land-use pressure. Research on retention forestry has shown that species richness may be relatively high on retention sites, but that several specialized species requiring interior-forest conditions cannot persist there (Rosenvald and Löhms 2008). Bhagwat et al. (2008) also raised the issue that agroforestry systems may be impoverished in specialist and endemic species, an area which clearly requires more research. Hence, the use of coarse-resolution biological response variables such as total species richness or abundance within higher taxa may not be sufficient for evaluating the conservation value of the two management systems (Waltert et al. 2011). To guide conservation, we need better knowledge about which particular groups of species tend to be systematically absent or underrepresented in various types of retention sites and agroforestry

systems relative to naturally dynamic forest and traditional woodlands (e.g. ancient tree-bearing cultural systems; Kirby and Watkins 1998). Other important areas for future research include the role of tree species, density and spatial configuration, effects on the reproductive success of threatened species, and modeling of the long-term effects of the two management systems on biodiversity in complex landscapes (see e.g. Ranius and Roberge 2011 for tree retention).

Given that retention forestry and agroforestry imply different costs and benefits compared to their respective alternatives, integrated cost-effectiveness analysis (Hughey et al. 2003) is necessary to assess their feasibility for conservation practice. For example, Mönkkönen et al. (2011) compared the cost-effectiveness of a number of alternative conservation approaches – including tree retention – for long-term conservation of boreal forest biodiversity. Some attempts have recently been made to assess the cost-effectiveness of agroforestry systems in the context of climate change mitigation (e.g. Makundi and Sathaye 2004), but surprisingly little has been done in relation to biodiversity conservation outcomes. An important question is whether it is possible to develop high-biodiversity approaches which simultaneously provide high economic returns (Clough et al. 2011, Tikkanen et al. 2012). Ideally, cost-effectiveness analyses should consider not only the local scale but also the management systems' roles as part of wider landscape-scale strategies (e.g. Côté et al. 2010).

Notwithstanding the importance of protected forests, we concur with Lindenmayer et al.'s (2012, see also Franklin and Lindenmayer 2009) and Bhagwat et al.'s (2008) conclusions that the matrix deserves increased attention, and that retention forestry and agroforestry are likely to constitute crucial tools for matrix management and restoration. We call for further research about the cost-effectiveness of retention forestry and agroforestry as complements to other existing approaches in various socio-ecological systems for the conservation of biodiversity. To this aim, we encourage increased collaboration between researchers and practitioners across the two fields.

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Effectiveness of the Natura 2000 network to cover threatened species

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Abstract

The world's biodiversity is currently in rapid decline - Europe being no exception - with as principal cause a human-mediated global change. The Natura 2000 network is an important conservation tool for European biodiversity; it is a network of natural and semi-natural sites within Europe with high heritage values due to the exceptional flora and fauna they contain. Here, we evaluated the coverage of 300 threatened species by the Natura 2000 network, and determined potential factors influencing the designation of sites and the structure of the network within a country (social, ecological and demographic national factors). Our analysis was based on a coverage ratio between the Natura 2000 sites and distribution maps of threatened European species. We showed that the distributions of a large proportion of threatened species of mammals, birds and reptiles considered in our study were highly covered (above 90%) by the current Natura 2000 network, demonstrating that the Natura 2000 network also covers species not listed in the annexes of the Nature Directives. However, our results confirm that a large proportion of threatened species (some of them listed on the European annexes), especially fishes, are currently poorly covered by the Natura 2000 network. The coverage of species likely seemed to be highly related to national demographic factors, i.e. the proportion of the national urban population. Our analysis also suggested that the designation of sites depends too strongly on governmental politics, economic and cultural criteria, and interactions between society and the environment. A more effective process might be necessary to ensure the Natura 2000 network reaches its potential as the most important and comprehensive network of protected areas intended to halt the loss of biodiversity in Europe in the near future.

Keywords

Reserve sites, threatened species, biodiversity loss, conservation tools

Introduction

The world's biodiversity is currently in rapid decline. In recent decades, this trend has accelerated globally, Europe being no exception. The international community reacted by adopting the Rio de Janeiro Convention on Biological Diversity (CBD) in 1992 (Balmford et al. 2005; Vié et al. 2008), which the European Community ratified in 1993. The CBD recommends that strategies that aim to ensure the conservation and sustainable use of biodiversity by anticipating and preventing significant reduction in or loss of biodiversity (for Europe see DG Environment 2002) need to be adopted. In response to the CBD, Europe established a network of protected sites called Natura 2000. The Natura 2000 network is governed by the Directive 79/409/EEC, adopted in April 1979 for the conservation of wild birds (also called "Birds Directive") and the Directive 92/43/EEC, adopted in May 1992 for the conservation of natural habitats, wild fauna and flora (also called "Habitats Directive"). Even if the Natura 2000 network is a European network of natural and semi-natural sites with high heritage values due to the exceptional flora and fauna they contain, the effectiveness of this network still remains unclear (Gruber et al. 2012).

The goal of the Natura 2000 network is to maintain the biological diversity of environments, while taking into account economic, social, cultural and regional logic of sustainable development. Compared to other nature conservation programs (Ramsar (www.ramsar.org) and MedWet (www.medwet.org), the Natura 2000 network can be considered as the main contribution by the European Union (EU) to fulfil the recommendations of the CBD, aiming to establish regional and national systems of protected areas on land (by 2010) and sea (by 2012). Currently, the Natura 2000 network covers almost 18% of the area of the 27 member states (more than 26,000 sites; European Commission 2010), covering all biogeographical regions of Europe, each site with its own characteristic blend of vegetation, climate and geology.

The Natura 2000 network comprises two major site categories, Special Protection Areas (SPAs) and Special Areas for Conservation (SACs). SPAs are sites of conservation value for rare and threatened European bird species designated internationally under the Birds Directive (DG Environment 1979). Special Areas for Conservation (SACs) are sites to protect plants, animals and wildlife habitats of EU importance as designated by the Habitats Directive (DG Environment 1992). For SPAs and SACs, the percentage of national territory designated to the Natura 2000 network ranges from 3% in Ireland to 25.1% in Slovakia and from 6.8% in the United Kingdom to 31.4% in Slovenia respectively (DG Environment 2010; but see also Evans 2005). Both SPAs and SACs can overlap, but differ in their designation processes.

While the designation of SPAs is based on the presence of bird species listed in the annexes of the Birds Directive, including a validation stage of the EU, SACs designation (Habitats Directive) is more complex and involves several stages (Evans 2012). Nationally, Natura 2000 sites are selected on the basis of national lists proposed by the member states. For each biogeographical region, the European Commission adopts a list of Sites of Community Importance (SCI) which then become part of the network.

Finally, the SCI are designated at the national level as Special Areas of Conservation (SAC) which subsequently undergo implementation measures. Faced to this complex method of establishment, the European Directives did not specify the method of consultation to be followed for reserve site selection. Therefore, management of the Natura 2000 network and the responsibilities of member states remain unclear, and so far have not followed a standardized framework (DG Environment 2002). Procedures have varied considerably between member states according to their administrative system. The detailed work involved is often delegated to various national agencies or, in the case of federal states, to regions. Several studies (Alphandéry and Fortier 2001, Pinton 2001, Mischi 2009) focused on problems in the identification of sites (SACs and SPAs) at the national level (in France), corresponding to the first phase of implementation guidelines. Similar problems, e.g. administrative, scientific (lack of data and tools) and social, were also encountered in other countries (in UK: Ledoux et al. 2000; in Greece: Apostolopoulou and Pantis 2009; in Finland: Björkell 2008, Hiedanpää 2002; in Germany: Stoll-Kleeman 2001a, b; in Ireland: Bryan 2012) and at the European scale (Keulartz 2009; Julien et al. 2000; Jackson 2011). Many environmental diagnoses were questioned, notably for potentially unreliable methods due to insufficient financial and human resources and a lack of data control, which slowed the implementation of new Natura 2000 sites at local level.

Species listed in the annexes of the European Directives depend on the criteria from the European and Member state's scales. Consequently, several species listed in these annexes are not mentioned on the IUCN Red List and vice versa. However, despite not being the primary aim, the Natura 2000 network might help to protect all threatened species. Here, we were interested in the effectiveness of the Natura 2000 network to cover also non-target, but threatened species [IUCN Red List categories: vulnerable (VU), endangered (EN) or critically endangered (CR)]. We were especially interested in the following questions: Are there differences in the coverage related to countries, taxonomic groups or biogeographical regions? Can the differences between countries be explained by national indicators such as population density, gross domestic product, etc.? Because an arbitrary threshold, such as 10 % of the area, is often assumed to assure an efficient protection to a species (Rosati et al. 2008), we also focused our analysis on species with a coverage of less than 10% by the Natura 2000 network.

Methods

The distribution areas of threatened species as listed on the IUCN Red List were studied within the Natura 2000 network at the national scale, at the scale of biogeographical regions and at the European scale. For abbreviations of each member state from the European Union we followed the two-letter nomenclature established for internet resources (i.e. FR = France, DE = Germany, etc.). Biogeographical regions were abbreviated as follows: Alpine (ALP), Atlantic (ATL), Black Sea (BLA), Boreal (BOR), Continental (CON), Macaronesian (MAC), Mediterranean (MED), Pannonian (PAN), Steppic (STE).

Data collection

As marine sites have been implemented very recently, we decided to focus on terrestrial and freshwater Natura 2000 sites. The database from the IUCN Red List (IUCN 2007) was used to obtain a list of all threatened [vulnerable (VU), endangered (EN) or critically endangered (CR)] terrestrial and freshwater plant and animal species in the European Union (see Appendix 1). In total, 707 terrestrial and freshwater species fall into these categories. For our analysis on the representation of threatened species in the Natura 2000 network, we used distribution maps in Image Bitmap format (sources: <http://www.iucnredlist.org/>; EIONET 2009 available on <http://biodiversity.eionet.europa.eu/>). We were able to obtain distribution maps in Image Bitmap files for 300 threatened species (amphibians: $n = 17$; birds: $n = 20$; fishes: $n = 124$; insects: $n = 26$; mammals: $n = 20$; molluscs: $n = 13$; plants: $n = 61$; reptiles: $n = 19$). The distribution maps from the IUCN website used numerous information sources and high data quality (IUCN 2007) suggesting that map precision was relatively high. Because distribution maps from spatial data of member state reports (EIONET 2009; ETC/BD 2008) were built using different approaches and data were captured at a variety of resolutions, they were re-projected by the European Topic Centre on Biological Diversity (ETC/BD) to a standard projection and were harmonised to give range and distribution on a $10 \text{ km} \times 10 \text{ km}$ or equivalent grid (ETC/BD 2008). Of these 300 species, 43.6% were VU, 26.7% were EN and 29.7% were CR. More than half (54.8%) were included in the annexes II, III or IV of the Habitats Directive or the Birds Directive. The Natura 2000 network map, the biogeographical regions map and the member states map were available in Image Bitmap format (EEA 2010) through the European Commission.

Among all species, distribution maps are prone to errors. The maps used from the IUCN website ($n = 145$) are depending on how a species present in a given site when underlying distributional maps was considered. Indeed, because information of species abundance was not available yet from the distribution maps of the IUCN website, the species present in a given site could be constantly present, or promptly present (for example present during the migration, for reproduction access, or accidentally present). These map limitations could be a potential source of bias (Alagador et al. 2011; Araújo 2004). Moreover, the distribution maps ($n = 155$) from EIONET (2009) have a relatively low resolution ($10 \text{ km} \times 10 \text{ km}$) and are harmonised depending on the resolution of the method used in each member state (ETC/BD 2008). For instance, French Article 17 report maps were built at a very coarse resolution compared to the neighbouring countries. Overall, the currently available data has certain limits, likely introducing a not quantified bias in our analysis.

Data processing

To estimate the coverage of the Natura 2000 network in regard to the distribution of threatened species in Europe, we used an image processing protocol employing

ADOBE PHOTOSHOP CS v8.0 (Adobe Systems Incorporated 2003). By overlaying distribution maps of species and Natura 2000 maps, we were able to calculate the ratio at which a species distribution falls within sites of the Natura 2000 network. The first step consisted of overlaying the Natura 2000 network map with a distribution map of a species. When the maps were overlaid, the distribution map of the species was modified in transparency, in order to highlight the Natura 2000 sites covered by the distribution map. Through transparency, several colours were obtained on the screen. For example, we had red pixels for Natura 2000 sites included in the distribution of the given species and grey pixels for the rest of the distribution map (not covered by the Natura 2000 network). Consequently for each coloured area, all pixels were selected and the number of pixels was obtained. Coverage was then obtained as follows: the proportion of the distribution of a given species in the Natura 2000 network within a member state/biogeographical region (number of pixels corresponding to the overlay between the distribution map and the Natura 2000 network map; i.e. the number of red pixels) divided by the distribution of the given species within a member state/biogeographical region (sum of grey and red pixels corresponding to the global distribution map). The cover ratios per species were then obtained 1) per country, 2) per biogeographical region, and 3) at the European scale (by adding the total number of pixels included in the Natura 2000 network divided by the total distribution map pixels). To validate the method using Image Bitmap files, we also obtained cover ratios from GIS data (polygon vector files) for species groups for which such data was available (mammals, birds, reptiles and amphibians). The comparison of the two approaches revealed a non-significant difference (Mann-Whitney test: $W = 1995.5$, $n = 64$, $P = 0.959$).

In order to determine if country and Natura 2000 parameters could explain the coverage of threatened species by a national Natura 2000 network, we calculated the average coverage by country and compared it to seven socio-economic parameters of countries and three Natura 2000 indicators (Table 1; Appendix 1).

Statistical analysis

For each species, coverage could range between 0 and 1, following a Poisson distribution. Therefore, we used a non-parametric Kruskal-Wallis ANOVA to test for differences between different species groups, member states and biogeographical regions. For refinement of the ANOVAs we employed the Tukey's honestly significant difference (HSD) posthoc test to compare member states/biogeographical region where significant differences were found with the ANOVA. We also used a non-parametric ANOVA to test if the surface of biogeographical regions is correlated to the mean coverage, to the number of threatened species present within and to the proportion of Natura 2000 network per region.

We then determined an arbitrary threshold of 10% of coverage to detect threatened species for which the Natura 2000 network has a poor coverage. This threshold

Table 1. Details of all national indicators used with definition, abbreviations and units. Abbr. = abbreviation.

Class	Indicator	Abbr.	Definition	Unit	Ref.
Economic indicators	Gross domestic product	GDP	market value of all final goods and services made within the borders of a country/year	Million €	1
Demographic indicators	Total population	TP	all persons residing in the country	Inhab.	1
	Population density	PD	number of individuals per surface units	Inhab./km ²	1
	National surface	NS	total surface of a country	km ²	1
	Urban population	UP	number of individuals residing in cities compared to the total population	% of total population	1
Ecological indicators	Ecological footprint	FP	amount of biologically productive land and sea area needed to regenerate the resources a human population consumes and to absorb and render harmless the corresponding waste	ha/person	2
	CO ₂ consumption	CO ₂	weighted emissions of greenhouse gas emissions	Million tonnes of CO ₂	1
Natura2000 indicators	Number of sites	NS	Number of Natura2000 sites	Number of sites	3
	Total area of sites	TA	Total area of all Natura2000 sites	km ²	3
	Natura2000 surface	%size	National network surface compared to total national surface	% of total surface	3

References: 1: UNDP (2006); 2: EEA (2008); 3: DG ENVIRONMENT (2010).

of 10% is assumed to be the minimum of coverage to assure an efficient protection to a given species (Rosati et al. 2008). Under this threshold, the representation of the species may be defined as an under-protection (“total gap”, see Rosati et al. 2008). But we can suggest that an effective coverage ratio (sufficient for a good protection) for a small insect may be low in areas with high densities, whereas we could imagine that a similar ratio should be not sufficient for mammal or bird species. Hence, the arbitrary threshold of 10% determined in this study was not used to highlight threatened species not correctly protected by the network, but only used to see how the Natura 2000 network overlaid the distribution of threatened species at the European scale. In parallel to a low coverage of the network (10% or less), we also detected high coverage using a threshold of 90%.

We used linear models to analyse the extent country and Natura 2000 indicators (Table 1) explain the variation in coverage of threatened species by Natura 2000 (dependent variable ‘coverage’) per country (average of coverage ratios from all species living within the country) and the number of species with a coverage of less than 10% (dependent variable ‘Nspecies<10%’) using a Gaussian distribution and an identity link function. Because fishes were numerous in our database and poorly covered by the Natura 2000 network, we also conducted the same analysis only with these species to

see if the national indicators could explain their specific coverage. Data were not available for Cyprus and Luxembourg, which were therefore excluded from this analysis. The best model among all possible sub-models was then selected using the corrected Akaike's information criterion ($AICc = 2 * [\text{model performance log-likelihood} + \text{number of parameters estimated}]$): models explaining the most variation with the fewest predictors have the lowest $AICc$ and were considered the 'best models'. With a selection by AIC , one best model can be selected (if the difference of their respective $AICc$ is < 2 ; Anderson et al. 1994). All statistical analyses were performed with the software R (R Development Core Team 2008).

Results

European scale

The global mean ratio of threatened species coverage was 0.359 ± 0.255 (mean \pm SD; median = 0.304). Depending on the taxonomic group, the global mean ratio varied from 0.292 ± 0.159 in insects (median = 0.261) to 0.452 ± 0.239 in reptiles (median = 0.412; see Fig. 1) but differences between taxonomic groups were statistically not significant ($F_{8,294} = 0.936$, $P = 0.487$). For only 6.6% ($n = 20$) of the analysed species, 90% of their distribution was covered by the Natura 2000 network. While 12% ($n = 36$) of the analysed species had only 10% of their distribution covered. The taxonomic group the least covered by Natura 2000 were fishes [22 (17.8%) threatened fish species]. Seven of these fish species are currently listed in the annexes of the Habitats Directive. In birds, only one species had a coverage of less than 10% in the Natura 2000 network. Overall, 42% (15 out of 36 species) of threatened species with a low coverage and 30% (6 out of 20 species) of threatened species with a high coverage by Natura 2000 were listed on the annexes I, II or V of the European Directives (see Appendix 1). However, comparing the coverage of threatened species listed by the European Natura Directives (0.339 ± 0.210) with the coverage of threatened species from the IUCN Red List (0.359 ± 0.255) did not reveal a significant difference ($F_{1,299} = 2.512$, $P = 0.114$).

Biogeographical regions scale

The mean coverage across biogeographical regions was 0.352 ± 0.244 . We did not detect any difference in the coverage at the taxonomic group level ($F_{8,483} = 1.601$, $P = 0.122$; Fig. 2a) in regard to the biogeographical regions, but found a significant difference at the species level ($F_{8,483} = 7.01$, $P < 0.001$). Threatened species were best covered in the Black Sea (0.587 ± 0.300 ; median = 0.602), compared to the mean coverage of the Continental (0.271 ± 0.203 ; median = 0.244), Atlantic (0.282 ± 0.269 ; median = 0.194), Boreal (0.191 ± 0.156 ; median = 0.146), Mediterranean (0.354 ± 0.254 ;

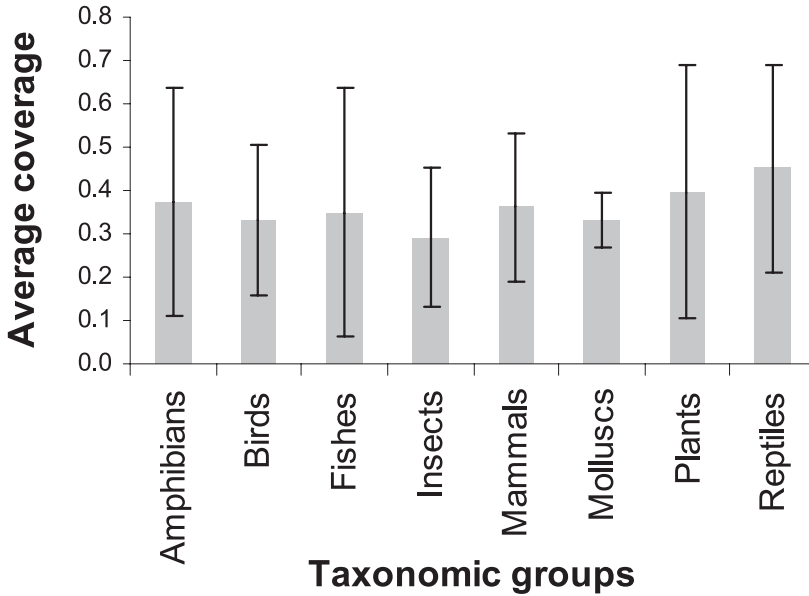


Figure 1. The average Natura2000 coverage of threatened species by taxonomic group.

median = 0.279) and Pannonian (0.287 ± 0.106 ; median = 0.270) regions (all Tukey's HSD tests: $P < 0.001$). Threatened species were also well covered in the Alpine region (0.451 ± 0.210 ; median = 0.409), compared to the mean coverage of the Atlantic (Tukey's HSD test: $P = 0.004$), Boreal (Tukey's HSD test: $P = 0.004$) and Continental (Tukey's HSD test: $P < 0.001$) regions. We found a poor coverage of threatened species in the Atlantic (17 out of 54 species = 31.5%), Boreal (26.7%) and Continental regions (14.7%; Fig. 2b and 2c).

We did not find a size effect between the mean coverage and the surface of biogeographical regions ($F_{1,7} = 2.036$, $P = 0.197$). We also did not find a relationship between the surface of biogeographical regions and the number of threatened species present within ($F_{1,7} = 2.41$, $P = 0.164$), whereas we found a correlation between the surface of biogeographical regions and the proportion of the Natura 2000 network per region ($F_{1,7} = 6.06$, $P = 0.043$).

Member states scale

The mean coverage across countries was 0.323 ± 0.225 (median = 0.282). Our comparison of the coverage of threatened species by a country's Natura 2000 sites revealed significant differences at the taxonomic group level ($F_{8,583} = 2.929$, $P = 0.003$). Insects appeared to be much less covered by the national Natura 2000 networks (0.242 ± 0.169) compared to reptiles (0.435 ± 0.229 ; Tukey's HSD test: $P = 0.034$) and plants (0.388 ± 0.29 ; Tukey's HSD test: $P = 0.057$).

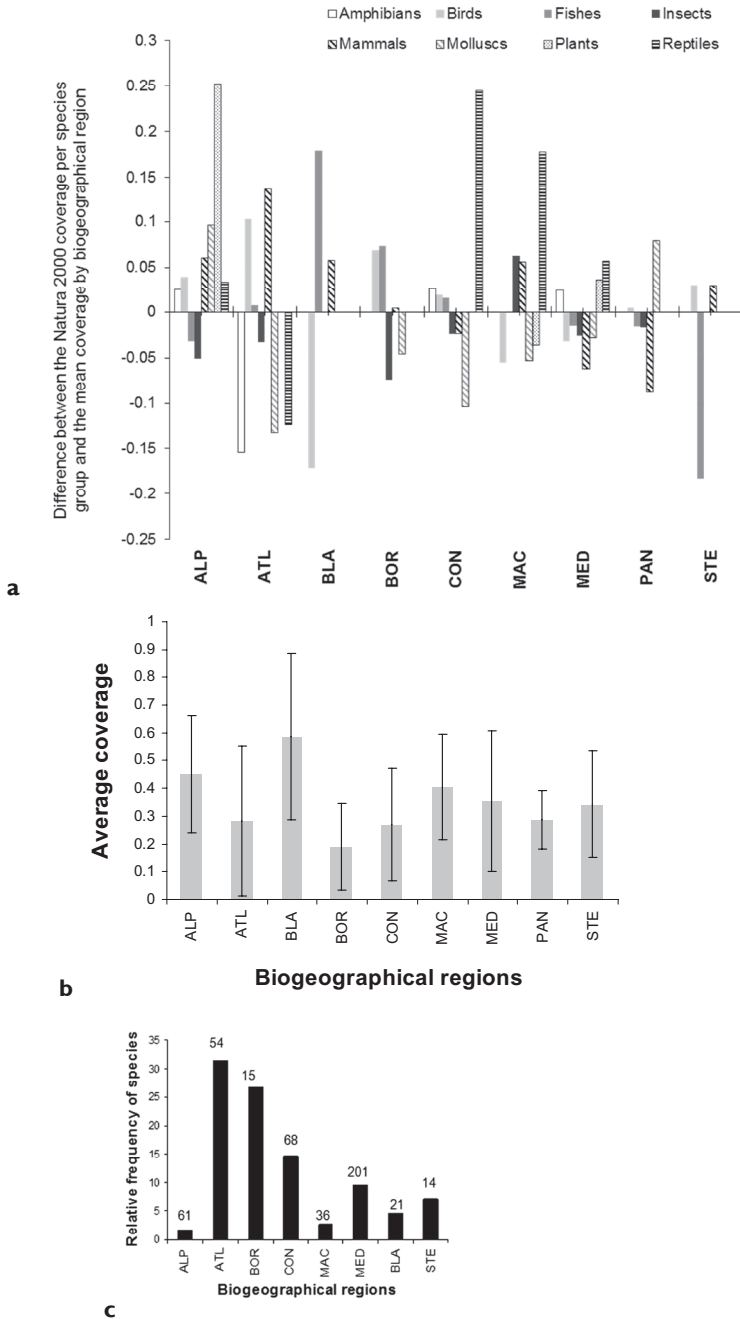


Figure 2. **a** Difference between the Natura2000 coverage per species group and the mean coverage by biogeographical region (when positive, the coverage of the groups is better than the mean coverage of a region) **b** mean coverage by biogeographical region and **c** relative frequency of threatened species with a coverage below 10% by biogeographical regions. The number on top of the bars indicates the total number of threatened species studied per biogeographical region. The Pannonian region has no species falling in this category.

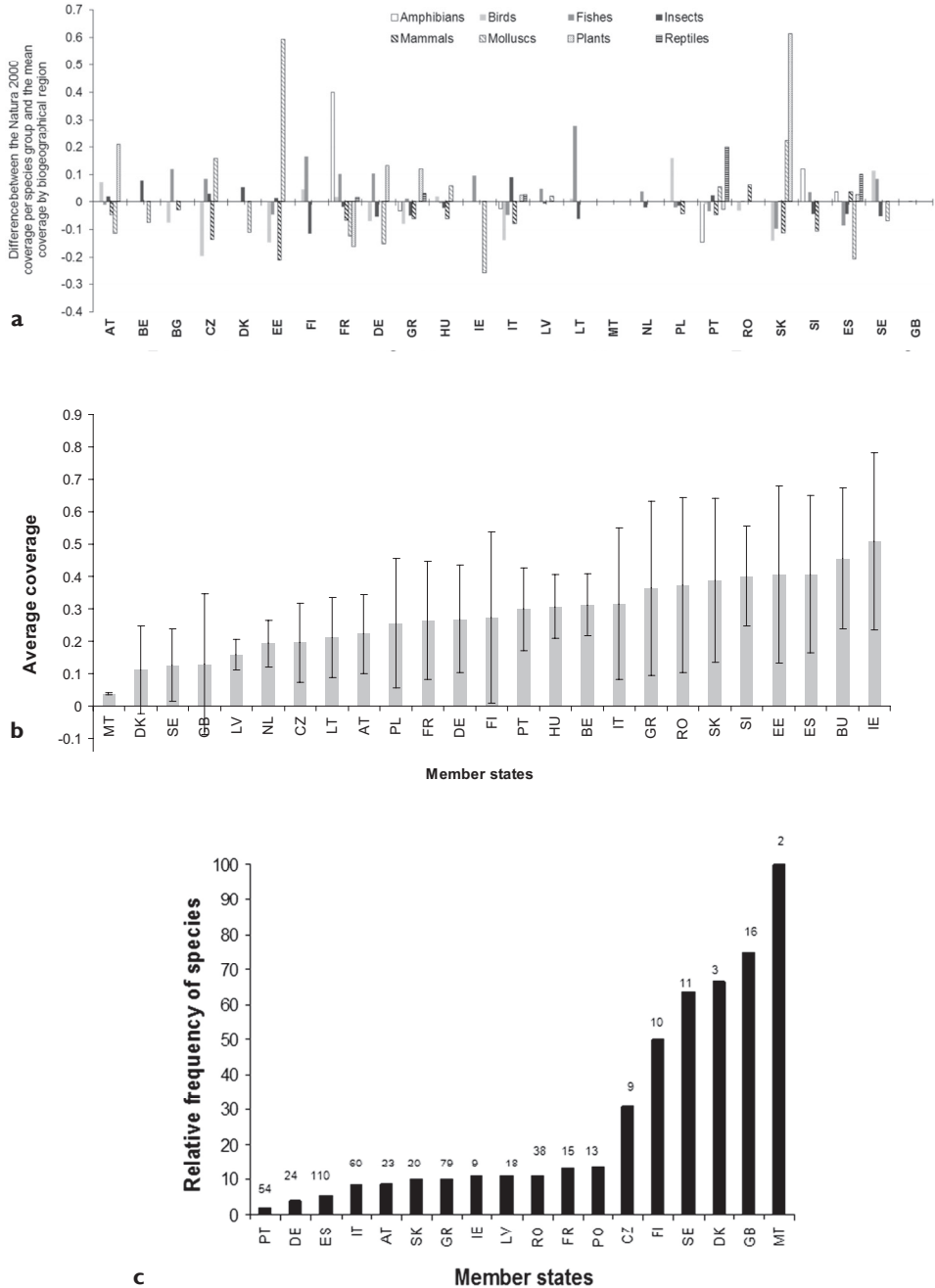


Figure 3. a Difference between ratio per species group and mean ratio by Member State (when positive, the coverage of the groups is better than the mean coverage of a country) **b** mean coverage of threatened species and **c** relative frequency of threatened species with a coverage below 10% by Member State. The number on top of each bar represents the total number of studied threatened species by Member State. The Natura 2000 network from non represented countries had no threatened species with a coverage of less than 10%.

Across species groups, the average coverage of threatened species by country showed significant differences ($F_{24,567} = 3.9596$, $P < 0.001$). Notably, the United Kingdom (0.131 ± 0.218 ; median = 0.031) and Sweden (0.127 ± 0.113 ; median = 0.072) had a low coverage compared to the mean across countries, while Spain (0.407 ± 0.244 ; median = 0.333; Tukey's HSD tests: $P < 0.001$) and Bulgaria (0.457 ± 0.218 ; median = 0.402; Tukey's HSD tests: $P < 0.001$ and $P = 0.014$ respectively) generally showed a high coverage of threatened species. In the United Kingdom, 11 of 16 threatened species, all of them fish species and three listed on the annex II of the Habitats Directive, have less than 10% of their distribution covered by the Natura 2000 network (Fig. 3c). The Greek Natura 2000 sites cover 8 of 79 threatened species (1 amphibian, 7 fishes) with less than 10% (i.e. low coverage), while two of these species (2 fish species) are listed on the annex II of the Habitat Directive (Fig. 3c). In Spain only 5% of threatened species had a coverage of their distributions of less than 10% by the Natura 2000 network.

Relationship between coverage and national indicators

The best model explaining the mean national coverage of threatened species (dependent variable 'coverage') consisted of the variable population density (PD) and the national network surface compared to total national surface (%size; $F_{2,22} = 13.12$; $P < 0.001$; $AICc = -46.7160$; Table 2, Table 3 and Appendix 2). The variable %size had a higher explanatory power ($t_{22} = 3.924$; $P < 0.001$) than PD ($t_{22} = -2.334$; $P = 0.029$). Another model explaining the mean national coverage of threatened species included the percentage of national urban population (UP) and %size ($F_{2,22} = 11.75$; $P < 0.001$; $AICc = -45.2501$; Table 2 and Table 3).

The best model explaining the mean national coverage of species with a low coverage (dependent variable 'Nspecies<10%') included the national network surface compared to total national surface (%size), the gross domestic product (GDP) and the weighted emissions of greenhouse gas emissions (CO_2 ; $F_{1,13} = 13.92$; $P < 0.001$; $AICc = -99.3453$). The mean national coverage of species with a low coverage was negatively correlated to CO_2 ($t_{13} = -3.901$; $P = 0.002$) and positively correlated to %size ($t_{13} = 3.268$; $P = 0.006$) and GDP ($t_{13} = 2.452$; $P = 0.029$). Another good model explaining the mean national coverage of species with a low coverage included TP (total population) instead of GDP ($F_{3,13} = 12.7$; $P < 0.001$; $AICc = -98.1737$; Table 2 and Table 3).

With only fish species and after model selection, 6 different models were retained. Within these 6 models, the percent of total Natura 2000 surface (%size), the population density (PD) and the percent of urban population (UP) have a significant effect on the coverage. As the retained explicative predictors were the same than in the global model (with all species) demonstrating a similar analysis, we did not show this specific result.

Table 2. Results of the linear models showing the relationship between ratios of coverage and the extent national and Natura2000 indicators. Models with the dependent variable ‘coverage’ are models taken into account all threatened species. Models with the dependent variable ‘Nspecies10%’ are models taken into account only threatened species with a coverage <10%. All models with a $\Delta AICc < 2$ are represented.

Variables	RSS	R ²	AICc	$\Delta AICc$
Coverage ~ %size + PD	0.1514	0.5025	-46.7160	0.000
Coverage ~ %size + UP	0.1606	0.4725	-45.2501	1.4659
Nspecies10% ~ CO2 + GDP + %size	0.0012	0.7078	-99.3453	0.000
Nspecies10% ~ CO2 + %size + TP	0.0012	0.6869	-98.1737	1.1716

Table 3. Results of the best linear models selected by AICc values. (a) models with the independent variable ‘coverage’ and (b) models with the dependent variable ‘Nspecies10%’.

Variables	Estimates for variables				
(a) Models with the dependent variable ‘coverage’					
Model 1	Estimate	DF	F	P	AdjR ²
Int.	0.1465 **	2, 22	13.12	< 0.001	0.5025
%size	9.286x10 ⁻³ ***				
PD	-1.627x10 ⁻⁴ *				
Model 2	Estimate	DF	F	P	AdjR ²
Int.	0.3807 *	2, 22	11.75	< 0.001	0.4725
%size	0.0075 *				
UP	-0.0033				
(b) Models with the dependent variable ‘Nspecies10%’					
Variable	Estimate	DF	F	P	AdjR ²
Int.	0.0335 ***	3, 13	13.92	< 0.001	0.7078
CO2	-1.0381x10 ⁻⁴ **				
GDP	1.73x10 ⁻⁸ *				
%size	1.381x10 ⁻³ **				
Variable	Estimate	DF	F	P	AdjR ²
Int.	0.0371 ***	3, 13	12.7	< 0.001	0.6869
CO2	-1.384x10 ⁻⁴ **				
TP	1.017x10 ⁻⁹ *				
%size	9.679x10 ⁻⁴ *				

Discussion

Here, we analysed the coverage of 300 threatened IUCN red listed species by the European Natura 2000 network. Our analysis showed that a large proportion of threatened species of mammals, birds and reptiles showed a high coverage ($\geq 90\%$) by the current Natura 2000 network. Hence, the Natura 2000 network also covers species not listed in the annexes of the Nature Directives. However, our results revealed that a large proportion of threatened species, some of them even listed on the annexes of the Habitats Directive and especially fishes are currently poorly covered ($\leq 10\%$) by the Natura 2000 network. Factors explaining the coverage of threatened species included national

network surface compared to total national surface (%size), national proportion of urban population (UP), national population density (PD), gross domestic product (GDP) and weighted emissions of greenhouse gas emissions (CO₂).

Our analysis showed an especially low coverage of threatened fish species by the Natura 2000 network (especially in the United Kingdom). Of the 124 fish species considered, 22 species had a range coverage of less than 10% by the Natura 2000 network, despite the fact that seven are listed in the annexes (II, IV and/or V) of the Habitats Directive [in United Kingdom: *Coregonus clupeoides* (La Cepède 1803), *Coregonus pennantii* (Valenciennes 1848) and *Coregonus stigmaticus* (Regan 1908); in Austria: *Coregonus danneri* (Vogt 1908); in Greece: *Barbus euboicus* (Stephanidis 1950), *Eudontomyzon hellenicus* (Vladykov, Renaud, Kott & Economidis, 1982) and *Acipenser naccarii* (Bonaparte 1836); see Appendix 1]. Concerning amphibians, three species (*Speleomantes flavus*, *Rana latastei*, *Pelophylax epeiroticus*) are weakly covered by the Natura 2000 network, of which two are listed in annex II and annex IV of the Habitats Directive. *S. flavus* (Stefani 1969) is endemic to Sardinia (Italy). This species is also listed in Appendix II of the Bern Convention but no conservation program is known at the moment, despite a need for close monitoring of the population status of this species (Lecis et al. 2008). *R. latastei* (Boulenger 1879) has a low coverage by the Natura 2000 network, but benefits from national protection in Italy, Switzerland and Slovenia. However, generally we did not find any difference between the coverage of IUCN threatened species and species listed on the European annexes.

Our analysis also showed that the region with the best average coverage of threatened species was the Black Sea region, covering 58.7% of the distribution ranges of threatened species. Although we did not find a size effect between the mean coverage and the surface of biogeographical regions, we want to stress that the Black Sea region is the smallest European biogeographical region (9705 km²) and has only 21 threatened species (7% of all species analysed here), while the total area of all Natura 2000 sites in this region represent 71.8% of the terrestrial surface (negative relationship between the surface of biogeographical region and the proportion of Natura 2000 network per region; see EEA 2010). In addition, the Black Sea region contains a low proportion of poorly covered species (4.76%; Fig. 2c). In contrast, the Alpine region is the region with the lowest proportion of poorly covered species (1.64%, or 1 of 61 species) and also the region with the highest proportion of terrestrial surface cover by Natura sites. In contrast, the Atlantic, Boreal and Continental biogeographical regions have a high proportion of poorly covered species (31.48%, 26.67% and 14.71% respectively; Fig. 2c). Our analysis suggests that the difference between biogeographical regions with good coverage and the ones with poor coverage could be resulting from industrial occupation, with industrial areas invoking difficulties for Natura 2000 site establishment. Moreover, in these large biogeographical regions, the increase in urbanisation and tourism development have generated fragmentation and habitat loss (especially in the Mediterranean region; EEA 2010). Further, our analysis on the national scale showed that the mean coverage of the species with a Natura 2000 coverage of less than 10% and an overall low national mean coverage of all species was largely explained by

a high population density and a low Natura 2000 surface. That result suggests that the establishment of new sites within urbanized countries was difficult and an adaptation of the site designation process and conservation policy might be needed in the future.

Globally, our analysis confirmed that the Natura 2000 network, despite the huge efforts of the EU Member States, may have shortcomings in protecting some of the threatened species, also suggested by earlier studies on national (Dimitrakopoulos et al. 2004, Maiorano et al. 2007) and European scales (Jantke et al. 2011). Based on a gap analysis using modelling tools for conservation planning, Jantke et al. (2011) recommended significantly increasing the Natura 2000 area to achieve complete coverage of all considered species. Instead, we recommend to increase the number of Natura 2000 sites, because we also tested that an increasing of Natura 2000 site's surface did not significantly increased the coverage of threatened species, even with an increasing of 10% of surface (data not shown). For the Greek island of Crete, the network was characterised as an inadequate protection for endangered species (Dimitrakopoulos et al. 2004). In addition, Maiorano et al. (2007) have shown that despite significant efforts in establishing new sites and an annual expansion of the Italian network, some areas with high species richness currently have no coverage. These areas contain endemic and rare species with limited distribution ranges. The same authors outlined that objectives and measures proposed for site designation were clearly insufficient to safeguard the many species and habitats present within the network. This was further supported by the European Commission assessment (European Commission 2007) that 16.4% of the 712 annex II species were not represented at all in Natura 2000 sites. However, despite implementation problems, conservation programs such as Natura 2000 do bring measurable benefits to wildlife (Donald et al. 2007).

Recommendations and perspectives

In order to improve the management of Natura 2000 sites (with a high efficiency), a common and standardized management of the Natura 2000 network with a uniform framework among member states needs to be established. Natura 2000 sites should be under continuous observation and evaluation, to determine their importance for the conservation of biodiversity in a biogeographical region, either using site selection algorithms as implemented in the programs ZONATION (Moilanen et al. 2005) or MARXAN (Watts et al. 2009) or by determining the international importance of the sites for the global survival of a species (Schmeller et al. 2008a, b). Such an approach would improve efficiency and create importance categories for each Natura 2000 site, as well as providing a basis on which to determine appropriate resource allocation. These approaches will help with the selection process, and may decrease the impact of the political agenda, as currently observed (Mathevet and Mauchamp 2005). We further recommend developing public awareness and participation to increase the ecological conscience (Stoll-Kleemann et al. 2010). The involvement of local people in conservation strategies has been shown to be highly efficient (e.g. Schmeller et al. 2012). For example, in the United States, bird protection has been recently modified and improved by

crediting landowners who have adapted their land to migratory and threatened species (recovery credit trading), and by establishing a Farm Bill, a law encouraging farmers and ranchers to protect important habitats through the Conservation Reserve Program (U.S. Department of Agriculture, Farm Service Agency 1997). Our recommendations should increase the efficiency of Natura 2000 network by avoiding the establishment of ineffective sites (with a low number of protected species), as observed in several member states with a high Natura 2000 surface coverage but with a low number of protected species.

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Appendix I

Summary of all threatened species studied with their IUCN status and their global ratio of coverage (next to each taxonomic group: mean + SD). (doi: 10.3897/nature-conservation.4.3626.app1). File format: MS Word Document (doc).

Explanation note: The species listed on the annex of the Habitats Directive or Birds Directive have across in the relevant columns. An asterisk (*) before the species names indicates that the species is a priority species.

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Appendix II

Principal component analysis of socio-economic, national indicators and parameters describing the Natura2000 network (see also Table 1). (doi: 10.3897/natureconservation.4.3626.app2). File format: MS Word Document (doc).

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Investigation of fungal root colonizers of the invasive plant *Vincetoxicum rossicum* and co-occurring local native plants in a field and woodland area in Southern Ontario

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Abstract

Fungal communities forming associations with plant roots have generally been described as ranging from symbiotic to parasitic. Disruptions to these associations consequently can have significant impacts on native plant communities. We examined how invasion by *Vincetoxicum rossicum*, a plant native to Europe, can alter both the arbuscular mycorrhizal fungi, as well as the general fungal communities associating with native plant roots in both field and woodland sites in Southern Ontario. In two different sites in the Greater Toronto Area, we took advantage of invasion by *V. rossicum* and neighbouring uninvaded sites to investigate the fungal communities associating with local plant roots, including goldenrod (*Solidago spp.*), wild red raspberry (*Rubus idaeus*), Canada anemone (*Anemone canadensis*), meadow rue (*Thalictrum dioicum*), and wild ginger (*Asarum canadense*). Fungi colonizing roots were characterized with terminal restriction fragment length polymorphism (T-RFLP) analysis of amplified total fungal (TF) and arbuscular mycorrhizal fungal (AMF) ribosomal fragments. We saw a significant effect of the presence of this invader on the diversity of TF phylotypes colonizing native plant roots, and a composition shift of both the TF and AMF community in native roots in both sites. In native communities invaded by *V. rossicum*, a significant increase in richness and colonization density of TF suggests that invaders such as *V. rossicum* may be able to influence the composition of soil fungi available to natives, possibly via mechanisms such as increased carbon provision or antibiosis attributable to unique root exudates.

Keywords

Plant invasions, *Vincetoxicum rossicum*, arbuscular mycorrhizal fungi (AMF), molecular identification, diversity, T-RFLP

Introduction

Plant invasions have resulted in observable shifts in the above ground plant communities, and there is evidence that soil fungi are also being significantly altered as the invasion process progresses (Hallett 2006, Mummey and Rillig 2006, Stinson et al. 2006, Zhang et al. 2010). Arbuscular mycorrhizal plant-fungal symbioses form highly specialized ‘nutrient-exchange’ structures (vesicles and arbuscules) at the plant-fungus interface and are recognized as being a particularly beneficial group of root endophytes. Plants associating with arbuscular mycorrhizal fungi are typically more competitive and better able to tolerate environmental stresses than are non-mycorrhizal or poorly colonized plants (Biermann and Linderman 1983, Daniell et al. 2001, Bianciotto and Bonfante 2002, Vandenkoornhuysen et al. 2002, Bonfante 2003, Brundrett 2004, DeBellis and Widden 2006, Greipsson and DiTommaso 2006, Parniske 2008, Shah et al. 2010). AMF are also known to provide protection from a variety of microbial pathogens to their host plants (Newsham et al. 1994, Borowicz 2001, Klironomos 2002, Pozo and Azcón-Aguilar 2007, Van der Putten et al. 2007, Appoloni et al. 2008, Mogg et al. 2008, Krüger et al. 2009, Wehner et al. 2009).

AMF species richness has been shown to affect plant productivity, and observed shifts in plant-AMF associations could impact plant community structure (Simberloff and Von Holle 1999, Maherali and Klironomos 2007). Given that AMF are functionally diverse and confer differential benefits to plants (Sanders and Fitter 1992, Bever et al. 1996, Bever 2002, Klironomos 2003), responses of AMF communities to invaders would be potentially highly disadvantageous to established native species (Helgason et al. 2002, Kourtev et al. 2002, Greipsson and DiTommaso 2006, Hawkes et al. 2006, Stinson et al. 2006), and would cause shifts in native plant communities (van der Heijden et al. 1998). Thus, disruptions to community plant-AMF associations in local ecosystems may support system dominance by invaders (van der Heijden et al. 1998, Daniell et al. 2001, Helgason et al. 2002, Kourtev et al. 2002, Greipsson and DiTommaso 2006, Hawkes et al. 2006, Bastias et al. 2007, Curlevski et al. 2010).

We compared both total fungal (TF) and AMF associations of the non-native vine *Vincetoxicum rossicum* or dog-strangling vine (DSV), several field and woodland native plants growing in dense patches of DSV, and the same natives growing separately from DSV. DSV is widely distributed in Eastern North America and has established virtual monocultures, consequently reducing diversity in local native ecosystems (Pimm et al. 1995, Ernst and Cappuccino 2005). DSV has been shown to be more densely colonized by arbuscular mycorrhizal fungi than co-occurring native plants in Hen-

derson Harbour, New York (Greipsson and DiTommaso 2006, Smith et al 2008), and extracts of DSV tissue demonstrated antifungal activity against plant pathogenic yeast-like and filamentous fungi as well as broad-host-range plant pathogens such as *Fusarium spp.* (Mogg et al. 2008).

In this study, we investigated the fungal communities associating with the roots of DSV and native plants in two proximal watersheds where dramatic invasions of DSV have occurred, taking advantage of the natural invasion of DSV into native field and woodland areas. We hypothesized that fungal root colonizers of native plants growing within dense patches of DSV would increase significantly in both richness and density relative to those growing separately from DSV. These findings are novel with respect to the use of molecular techniques to characterize the fungal community associating with DSV and natives persisting in DSV patches. The alternative, more traditional methods, including soil spore analysis or root-piece microscopy, are not able to produce similarly reliable and replicable results.

Materials and methods

Study sites and sample collection

Root samples of DSV and co-occurring native plant species were collected from field and woodland plots in two main sites that are in permanent neighbouring watersheds in Southern Ontario: East Highland Creek at the University of Toronto at Scarborough (UTSC: 43.7803°N, 79.1886°W), and the Rouge River at the Toronto Zoo (Zoo: 43.8208°N, 79.1852° W). Both sites provide access to DSV patches that are well established, as well as occurrences of plants in both field and woodland areas; site details are shown in Table 1. Within a given plot, three replicates of DSV or each native plant commonly co-occurring with DSV were taken from field and woodland areas in spring and fall; the distance between individual plant samples collected ranged from approximately 1–4 m. Plot selections were based on sufficient representation by DSV in field and woodland environments (densely populated areas), a distance of at least 5 m away from field or woodland edge, and representation by field and woodland natives in i) the absence of DSV (visually) as well as ii) the presence of densely populated patches of DSV. Collections were made from eight Zoo plots and eight UTSC plots: $n = 2$ DSV plots per site, $n = 3$ natives growing separately from DSV ('natives') plots per site, and $n = 3$ natives growing in dense DSV patches ('natives-in-dsv') plots per site. A cumulative total of 36 samples per site (18 field and 18 woodland) were collected from the plots in May and October 2009, or 72 samples available for analyses (Table 2). Soil samples from two invaded and two uninvaded plots per site were sent to the University of Guelph Laboratory Services Division (Guelph, Ontario) for analysis, including both field and woodland samples. Soil sampling depth was approximately 15–20 cm. Details of the soil analysis are listed in Table 3.

Table 1. Site locations, prevailing plants, and soil descriptions for plots in study sites.

Site (Including field and woodland plots)	Coordinates/ location	Soil	Well-represented native/naturalized plants
Highland Creek Ravine at UTSC Campus	43.7803°N, 79.1886°W (Morningside Ave. and Ellesmere Rd., Toronto)	Mostly Sandy-silts, sands, clays (http://www.trca.on.ca/dotAsset/37550.pdf)	Staghorn sumac (<i>Rhus typhina</i>), solomon's seal (<i>Polygonatum pubescens</i>), goldenrod (<i>Solidago spp.</i>), poison ivy (<i>Rhus radicans</i>), grasses, white + purple aster (<i>Symphotrichum spp.</i>), queen Anne's lace (<i>Daucus carota</i>), vetch (<i>Vicia cracca</i>), riverbank grape (<i>Vitis riparia</i>), dandelion (<i>Taraxacum spp.</i>), yellow goatsbeard (<i>Tragopogon dubius</i>), sweet white clover (<i>Melilotus alba</i>), chicory (<i>Cichorium intybus</i>), mullein (<i>Verbascum thapsus</i>), jewelweed (<i>Impatiens capensis</i>), phlox (<i>Phlox spp.</i>), avens (<i>Geum aleppicum</i>), lopseed (<i>Phryma leptostachya</i>), bedstraw (<i>Galium mollugo</i>), red raspberry (<i>Rubus idaeus</i>)
Toronto Zoo (near Rouge River)	43.8208°N, 79.1852°W (Meadowvale Rd. and Old finch Rd., Toronto)	Sandy loam, silty clay (http://www.trca.on.ca)	Meadow rue (<i>Thalictrum dioicum</i>), dandelion (<i>Taraxacum spp.</i>), goldenrod (<i>Solidago spp.</i>), grasses, vetch (<i>Vicia cracca</i>), milkweed (<i>Asclepias syriaca</i>), staghorn sumac (<i>Rhus typhina</i>), wild ginger (<i>Asarum canadensis</i>), bloodroot (<i>Sanguinaria Canadensis</i>), dutchman's breeches (<i>Dicentra cucullaria</i>), wild leek (<i>Allium tricoccum</i>), baneberry (<i>Actaea rubra</i>) and (<i>Actaea pachypoda</i>), may apple (<i>Podophyllum peltatum</i>), aster (<i>Aster spp.</i>), queen Anne's lace (<i>Daucus carota</i>), cinquefoil (<i>Potentilla simplex</i>), dames rocket (<i>Hesperis matronalis</i>), jewelweed (<i>Impatiens capensis</i>), sweet white clover (<i>Melilotus alba</i>), dogbane (<i>Apocynum androsaemifolium</i>)

Table 2. Plant collections from 2 sites: UTSC and ZOO. Natives growing in DSV patches denoted by '-dsv' post script.

Site	Environment	Native plant species	Fall (October)	Plot	Spring (May)	Plot
UTSC 43.7803°N, 79.1886°W	Field Woodland	<i>Solidago spp.</i>	3 goldenrod	U1	3 goldenrod	U1
			3 goldenrod-dsv	U2	3 goldenrod-dsv	U2
			3 DSV	U3	3 DSV	U3
		<i>Rubus idaeus</i> <i>Anemone canadensis</i>	3 raspberry	U4	3 anemone	U7
			3 raspberry-dsv	U5	3 anemone-dsv	U8
			3 DSV	U6	3 DSV	U6
ZOO 43.8208°N, 79.1852°W	Field Woodland	<i>Solidago spp.</i>	3 goldenrod	Z1	3 goldenrod	Z1
			3 golderod-dsv	Z2	3 goldenrod-dsv	Z2
			3 DSV	Z3	3 DSV	Z3
		<i>Asarum canadense</i> <i>Thalictrum dioicum</i>	3 wild ginger	Z4	3 meadow rue	Z7
			3 wild ginger-dsv	Z5	3 meadow rue-dsv	Z8
			3 DSV	Z6	3 DSV	Z6

Table 3. University of Guelph Soil analysis report shows P, K, and Mg levels for four samples collected from each of the two main study sites. F = field, W = woodland, Z = Zoo, U = UTSC, -dsv = grown in DSV, -no = no/little DSV present; *t*-test shows comparisons between all DSV and non-DSV samples for each soil component analyzed (i.e. samples 1-4 and 5-8); *significant results.

Sample/	Moist	NH ₄	NO ₃	Tot carb	Inorg Carb	Org Carb	P	Mg	K	pH
Env-Site	% dry	mg/ kg	mg/ kg	% dry	% dry	% dry	mg/ L	mg/ L	mg/ L	
1 FZ-dsv	17.57	1.95	0.49	3.83	2.46	1.37	4	100	57	7.9
2 FU-dsv	23.74	0.94	0.49	3.12	1.72	1.4	3.3	75	78	7.9
3 WZ-dsv	24.34	1.72	1.22	6.25	3.74	2.51	14	140	170	7.7
4 WU-dsv	27.72	1.49	5.94	5.03	1.05	3.98	9.4	88	44	7.7
Ave	23.3	1.5	2.0	4.6	2.2	2.3	7.7	100.8	87.3	7.8
5 FZ-no	26.52	1.79	4.28	2.56	0.25	2.31	13	56	120	7.7
6 FU-no	29.37	1.25	4.31	3.47	1.85	1.62	2.9	52	20	7.9
7 WZ-no	34.84	1.54	0.97	3.98	0	3.98	5.5	100	45	6.6
8 WU-no	40.49	2.34	3.8	5.14	0.08	5.06	3.8	290	120	6.9
Ave	32.8	1.7	3.3	3.8	0.5	3.2	6.3	124.5	76.3	7.3
<i>t</i> -test p-value	0.02*	0.27	0.22	0.21	0.03*	0.19	0.35	0.35	0.39	0.07

Fungal Community Comparisons

Root Sampling

The plants were dug up carefully to protect root hairs and to ensure that the entire root ball was extracted from the ground. Samples were transported to the lab within a few hours of collection, where they were stored at 4°C until processing the following day. The roots were then washed extensively in distilled water, and then root sections were removed for subsequent DNA extractions. Only fine roots attached to the plant were used.

DNA extraction

FastDNA[®] SPIN Kit (Q-Biogene, Carlsbad California) was used for all DNA extractions. Genomic DNA was extracted from approximately 200 mg of root tissue in accordance with the manufacturer's protocols, using a fungal specific lysis buffer included in the kit (CLS-Y). After extraction, total DNA concentrations from all samples were estimated spectrophotometrically using the NanoDrop[™] ND-1000 V3.7.0 (Thermo Fisher Scientific Inc., Wilmington, USA), and visualized by gel electrophoresis on a 0.8% (wt/vol) agarose gel containing ethidium bromide in 0.5× Tris/Borate/EDTA buffer and quantified using a DNA ladder (GeneRuler[™] 1kb DNA Ladder Plus, Fermentas, Burlington, Ontario); gels were run at 100 V for 0.5 hour intervals until distinct bands were resolved. The average yield of DNA was 6.5 mg of DNA per root sample.

Amplification and digestion of total fungal (TF) fragments

Primers designed to specifically amplify fungal sequences from the inter-transcribed spacer (ITS) region of the ribosomal operon (ITS1F 5'-CTT GGT CAT TTA GAG GAA GTA A-3' forward and ITS4 5'-TCC TCC GCT TAT TGA TAT GC-3' reverse) were used for detecting general fungal colonizers as they were putatively more selective for fungal DNA than the alternative ITS1-ITS4 primer pair (Gardes and Bruns 1993, Dickie and FitzJohn 2007, Manter and Vivanco 2007). Genomic DNA was diluted 1:10 prior to amplification. 20 µl amplification reactions consisted of 10 µl of HotStar Taq Plus Master mix, 2x (Qiagen, Canada), 0.4 µl of each primer at 50 µM, 7.8 µl of RNase-free water, 0.4 µl bovine serum albumin (albumin solution from bovine serum, 20 mg/mL in H₂O, Sigma-Aldrich Canada, Oakville, Ontario) and 1 µl of DNA template. Reactions were incubated in a PTC-100™ thermal cycler (MJ Research Inc., Waltham, Massachusetts) using the following conditions: DNA polymerase initialization at 95°C for 5 minutes, followed by 34 cycles at 94°C for 50 seconds, 51°C for 1 minute, 72°C for 1 minute, ending with a final extension step at 72°C for 10 minutes. A negative control of 15 µl PCR water was run instead of root DNA for amplifications. Amplicons were digested following the manufacturer's instructions using restriction enzymes *EcoRII* and *FspBI* for 2 hours at 37°C (Fermentas Canada Inc., Burlington, Ontario) (isoschizomers for *MaeII* and *BstNI* respectively. Alvarado and Manjón (2009) performed an in silico study of terminal fragment size distributions to test primer-enzyme pairs, and recommended these enzymes for T-RFLP using ITS1F-ITS1 primers based on their effectiveness in producing differential T-RFLP cuts reflective of fungal diversity. Digests contained 15 µl of PCR product, 2U each *EcoRII* and *FspBI*, 2 µl Tango™ 1X buffer, and 2.6 µl sterile water. All digest products and a positive control of typed sample were visualized against a Fermentas GeneRuler 1 kb Plus DNA ladder on a 2.5% (wt/vol) agarose gel stained with ethidium bromide.

Amplification and digestion of AMF fragments

The small subunit (SSU) primers AML1 and AML2 (AML1 5'-ATC AAC TTT CGA TGG TAG GAT AGA-3' forward and AML2 5'-GAA CCC AAA CAC TTT GGT TTC C-3' reverse) designed by Lee et al. (2008) were used to amplify arbuscular mycorrhizal fungi exclusively. 20 µl amplification reactions were carried out as above. Denaturation was at 95°C for 5 minutes, followed by 34 cycles at 94°C for 50 sec., 55.5 °C for 50 sec., 72°C for 60 sec., followed by a final extension step at 72°C for 10 minutes. A negative control of 15 µl PCR water was run instead of root DNA for amplifications. Amplicons were digested using restriction enzymes *AluI* and *HinfI* for 2 hours at 37°C (Fermentas Canada Inc., Burlington, Ontario). Querejeta et al. (2009) described these enzymes as producing the greatest number of diagnostic restriction fragment patterns while analyzing AMF. Digests contained 15 µl of PCR product,

2U each *AluI* and *HinfI*, 2 µl Tango™ 1X buffer, and 2.6 µl sterile water. All digest products and a positive control of typed sample were visualized against a Fermentas GeneRuler 1 kb Plus DNA ladder on a 2.5% (wt/vol) agarose gel stained with ethidium bromide.

Terminal restriction fragment (TRF) analysis of TF and AMF

All primers were labeled with fluorescent dyes phosphoramidite 6-FAM and HEX (forward and reverse label respectively, labeled at the 5' end; Invitrogen Canada) and PCR products were visualized using a 1.0% (wt/vol) agarose gel, as above. 10 µl of each restricted sample was analyzed on a 3730 DNA sequencer (Applied Biosystems Inc., Fremont, California) for sizes and intensities (peak height) of the 5'-terminal fragment at the Laboratory Services Division at the University of Guelph (Guelph, Ontario). T-RFLP data were displayed graphically as individual fluorescence peaks that represent individual phlotypes. Fragment sizes ranged from 50–900 base pairs (bp) and included a range of fluorescence intensities. Replicate, independent PCRs and endonuclease restrictions, as well as T-RFLP analysis were tested for several samples, which confirmed reproducibility. The total number of TRFs is treated as an estimate of the fungal community complexity or diversity.

Molecular cloning and sequencing

To analyze amplified PCR products from both ITS and AML primers, selected amplicons were cloned with TOPO TA Cloning® Kit for Sequencing (Invitrogen, United States) according to manufacturer's protocols. pCR®4-TOPO® plasmids were added to a mixture of PCR products, dilute salt solution and RNase free water at room temperature. Ligated plasmids were transformed into chemically competent One Shot® TOP10 or Mach-1™ – T1R *Escherichia coli* cells. Transformed cells were selected on kanamycin [50 ng/µl] containing LB plates. Single colonies were transferred to liquid culture medium containing selective agent kanamycin [50 ng/µl] and grown for plasmid extraction using QIAprep® Miniprep kit (Qiagen, Canada). Genomic DNA, proteins and cellular debris were precipitated and removed by centrifugation. Plasmids were captured by QIAprep spin column and eluted using Tris-based buffer. PCR amplification (using ITS1F-ITS4 and AML1-AML2 primer pairs) or restriction enzyme digestion with *EcoRI* was carried out to verify the presence of inserted amplicons. Plasmids containing inserts of interest were sequenced using M13 (-27) reverse primer by The Center for Applied Genomics (SickKids Hospital, Canada).

Clone sequences were analyzed using nucleotide BLAST program from NCBI (<http://blast.ncbi.nlm.nih.gov/>). The AML1-AML2 primer pair showed high AMF specificity, amplifying only species belonging to the *Glomus* or *Paraglomus* genus (phylum of Glomeromycota) from all plant samples.

Data analyses

Peak height data for each individual TRF or phylotype were normalized to a percentage of total fluorescence per sample to account for the differences in the amount of amplicon sent for analysis. The normalized terminal restriction fragment data were then aligned using a macro created by Christopher Walsh (Rees et al. 2004) in Excel 2007 (Microsoft Corporation), and then subsequently used in downstream analyses. TRFs were also manually aligned such that single base pair differences were binned together when warranted by obvious patterns in the data. TRFs less than 50 base pairs (bp) were not analyzed. The normalized dataset was exported to *R* open source statistical analysis software, v 2.12.0 (*R* Development Core Team 2010) using the *Adonis* function in *vegan* (Oksanen et al. 2012) and NMDS in *MASS* (Venables and Ripley 2002) packages. *Statistica* (StatSoft Inc., v 7.1) was used for multivariate analyses.

NMDS (based on Bray-Curtis distances) and *Adonis* (non-parametric permutation MANOVA or analysis of difference in *R*, based on 99 permutations; analogous to redundancy analysis, Legendre and Anderson 1999) analyses were used as a means of data reduction, and to investigate the underlying structure of the fungal communities in each sample. Samples were grouped as types ‘DSV’, ‘natives-in-dsv’, or ‘natives’ to represent DSV, native plants growing in dense DSV patches, and native plants growing separately from DSV, respectively. NMDS is a common ordination method for T-RFLP community data that preserves the rank ordering of original distances among observations, and uses these ranks to map the objects in two-dimensional ordination space (Ramette 2007). The stress value (Kruskal’s) looks at the rank-order between distances and dissimilarities of the NMDS analysis: as the agreement between distances and dissimilarities improves, the stress is lower. Stress values of 0.20 or less indicate a biologically relevant result (Rees et al. 2004). NMDS and *Adonis* are free from the assumption of normality (Rees et al. 2004). The *Adonis* function (analysis of difference) in *R* was used to determine the significance of differences between samples or plant types (i.e. DSV, natives-in-dsv, or natives).

To determine significant TRFs contributing to the different analyses, principal component analysis (PCA) was performed in *R* to reduce the TRF data dimensionality prior to performing discriminant analysis (DA); principal components with eigenvalues > 1 were used in the DA. Significant loadings (TRFs) from the principal components were used in the DA to produce graphs of sample cluster patterns based on the variables that best discriminated among the different defined groups. Differences in fungal phylotype density and richness levels between samples were tested using Student’s *t*-tests or ANOVA.

Results

TF amplicons of approximately 700–900 bp and AMF amplicons of 500–800 bp were successfully obtained from root tip DNA. TRFs were successfully obtained from

the samples using both FAM- and HEX-labeled primers and both sets of restriction enzymes. Replicate T-RFLP analyses for both TF and AMF produced highly similar results with regard to both TRF phylotypes and peak heights. Based on sequencing results, all TF sequences were fungal, and all AMF sequences were Glomeromycota.

The community composition of the TF and AMF communities for DSV, natives-in-dsv, and natives was investigated using multivariate analyses of the normalized FAM and HEX TRF data. Given the tendency of the data to sort significantly by season in initial analyses (data not shown), all data were grouped by season (spring or fall) for subsequent fungal community comparisons.

TF phylotypes showed a tendency to sort by site initially and to a lesser extent environment when woodland and field samples were analyzed collectively. However, once the data were parsed into field and woodland sets, site was no longer a significant factor in either the spring or fall data analyses. Sample type remained significant throughout the analyses, with FAM and HEX data showing similar results, indicating where TF community patterns were strongly affected by the presence of DSV. Ordination results for spring and fall TF sample sets are shown in Table 4. The NMDS stress values for all analyses are < 0.20 , indicating that the ordination results for the data sets are biologically relevant (Rees et al. 2004). The R^2 results were determined using data grouped by the terms type (DSV, natives-in-dsv, and natives), plant (different plant species), environment (woodland or field), and site (UTSC or Zoo), with F -tests used to report p -values showing the tendency of data to sort according to the terms defined in the model.

For the AMF data, site was not a significant factor affecting sample type group association, even prior to taking environment into consideration for either the spring or fall data. Sample type remained significant throughout the analyses, indicating where DSV, natives-in-dsv, and natives support different AMF phylotype patterns. Ordination results for fall and spring AMF sample sets are shown in Table 4.

For discriminant analysis, loadings (FAM-labeled TRFs) used in the analyses were derived from principal components determined using the Kaiser (1960) criterion (eigenvalues > 1) for PCA in *R*. The plots for all woodland data showed separation of fungal community by sample 'type' in the normalized TRF patterns for DSV, natives-in-dsv, and natives for both the TF and AMF data (Figures 1 and 2 respectively), significant loadings are indicated where relevant.

Using the ratio of the average FAM peak height values to the extracted root DNA concentration per sample as a measure of fungal colonization density, we observed that the TF colonization of roots differed significantly between the natives and the natives-in-dsv, with the natives-in-dsv collectively averaging 1.9 times greater TF colonization densities (Student's t -test, $p = 0.00003$; Appendix 1). As well, AMF colonization of roots differed significantly between the natives and the natives-in-dsv, with the natives-in-dsv showing a difference of 3.9 times greater average colonization densities ($p = 0.002$).

Collectively, there were significantly fewer FAM AMF phylotype variants in all samples (mean of 6.5 ± 2.3) than TF variants (mean of 27.4 ± 13.0), $p = 0.0001$. There was a significant difference in the number of TF phylotypes between DSV and natives,

Table 4. Multivariate results comparing DSV, natives-in-dsv, and natives TF and AMF sample sets where data are included from 2 sites + 2 environments (env) (field versus woodland) and subsequently reduced to only woodland environment; results from both FAM- and HEX-labeled primers are shown.

	All fall samples		Fall wood only (FAM)		Fall wood only (HEX)		All spring samples		Spring wood only (FAM)		Spring wood only (HEX)	
	TF	AMF	TF	AMF	TF	AMF	TF	AMF	TF	AMF	TF	AMF
NMDS Stress	0.19	0.15	0.12	0.006	0.15	0.001	0.19	0.12	0.15	0.12	0.19	0.10
Adonis R^2 (type)	0.16	0.24	0.24	0.38	0.12	0.17	0.07	0.25	0.13	0.30	0.16	0.25
Adonis R^2 (plant)	0.26	0.28	0.33	0.29	0.25	0.38	0.27	0.06	0.30	0.10	0.22	0.31
Adonis p -value (site)	0.01	0.19	0.12	0.97	0.06	1.0	0.01	0.06	0.82	0.72	0.11	0.97
Adonis p -value (env)	0.04	0.86	n/a	n/a	n/a	n/a	0.05	0.86	n/a	n/a	n/a	n/a
Adonis p -value (type)	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.03	0.01	0.05
Adonis p -value (plant)	0.01	0.01	0.01	0.02	0.01	0.07	0.01	0.81	0.01	0.96	0.01	0.14

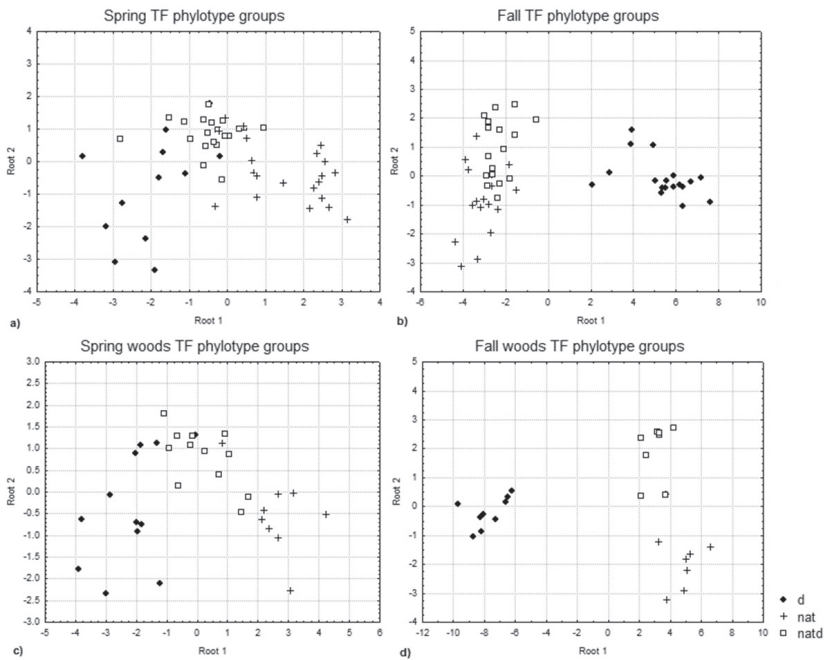


Figure 1. TF phylotype discriminant analysis plots for **a** spring (field and woodland), $p = 0.0024$, significant loadings = 150 basepairs (bp); includes dsv, goldenrod, anemone and meadow rue **b** fall (field and woodland), $p = 0.0000$, significant loadings = 142, 158, 321; includes dsv, goldenrod, wild ginger and raspberry **c** spring woods, $p = 0.0045$, significant loadings = 138 bp; includes dsv, anemone and meadow rue; and **d** fall woods, $p = 0.0064$, significant loadings = 138, 158 bp; includes dsv, wild ginger and raspberry. Distinct groups can be seen for DSV (d), natives growing in DSV (natd), and natives (nat) in the woodland data.

as well as between natives and natives-in-dsv ($p = 0.0014$ and $p = 0.016$, respectively using ANOVA, with Fisher’s LSD). The average number of TF phylotypes for DSV, natives-in-dsv, and natives were 31 ± 11.6 , 29 ± 12.4 , and 19 ± 11.9 respectively (Table 5).

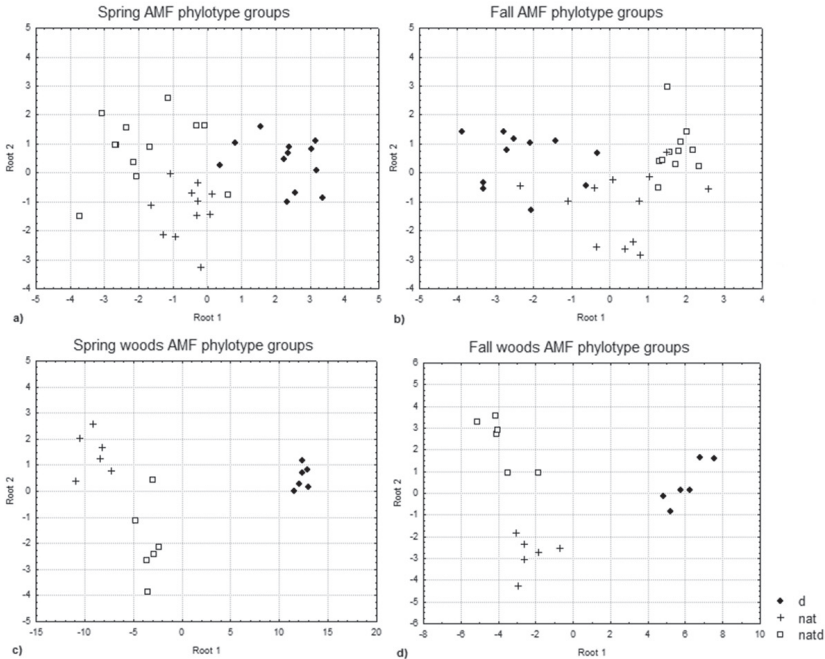


Figure 2. AMF phylotype discriminant analysis plots for **a** spring (field and woodland), $p = 0.0049$, significant loadings = 54 basepairs (bp) **b** fall (field and woodland), $p = 0.0012$, no significant loadings **c** spring woods, $p = 0.0009$, significant loadings = 54, 56, 65, 81, 85, 116, 272 bp; and **d** fall woods, $p = 0.0036$, significant loadings = 54bp. Distinct groups can be seen for DSV (**d**), natives growing in DSV (natd), and natives (nat) in the woodland data. Plants as in Figure 1.

Table 5. ANOVA analysis results showing difference in number of TF phylotypes for the groups DSV, natives-in-dsv (Nat-d), and natives growing separately from DSV (Natives).

Host plant type	No. of root samples	Mean no. of TRFs	SD	p-values using Fisher's LSD		
				DSV	Nat-d	Natives
DSV	24	31.4	11.6	-		0.001374
Nat-d	24	28.5	12.4	0.39551	-	0.015563
Natives	24	19.4	11.9	0.001374	0.015563	-
All groups	72	26.6	12.6			

There was no significant difference in the number of AMF phylotypes among the DSV, natives-in-dsv, and native TRFs ($p = 0.95$). However, there were observed differences in the phylotype occurrences between groups/plants (Appendices 2 and 3).

The dominant FAM TF and AMF phylotypes for individual plant species is shown in Appendices 2 and 3, respectively. For the TF data, the spring goldenrod-in-dsv had greater or equal phylotype representation for all TRFs considered relative to goldenrod growing separately from DSV, while the fall goldenrod-in-dsv had greater or equal phylotype representation in all twelve dominant TRFs relative to

goldenrod. For woodland plants, results were mixed with wild ginger-in-dsv showing greater phylotype representation in seven out of ten TRFs, meadow rue-in-dsv showing greater or the same phylotype representation in five out of eleven TRFs, raspberry-in-dsv showing greater or the same phylotype representation in six out of eleven TRFs, and anemone growing in DSV showing greater phylotype representation in seven out of eleven TRFs. Anemone-in-dsv had considerably more rare phylotypes (average of 61) relative to anemone (average of 9), the greatest difference between any native-in-dsv/native plant sampled. All TRFs found collectively in anemone-in-dsv samples (136 in total) were also found in the DSV samples except TRFs 236, 285, and 253bp. Six out of eight of the most dominant phylotypes in DSV (67bp, 134bp, 150bp, 224bp, 321bp, and 324bp) were significantly greater in natives-in-dsv relative to their native counterparts (Student's *t*-test $p = 0.04, 0.03, 0.003, 0.045, 0.03,$ and $0.05,$ respectively).

TRF 67bp was present in 88% of the total DSV samples, and TRF 324bp was present in 96% of the DSV samples. There was an increase in TRF 67bp representation in all natives-in-dsv relative to natives except anemone, which remained unchanged, and TRF 324bp representation in all natives-in-dsv groups except meadow rue (that showed a decrease in 324bp), suggesting that these phylotypes may be universally affected by DSV invasion. As well, 72% of the TF phylotypes that were dominant in DSV showed increased representation in the natives-in-dsv relative to natives (by varying amounts), and 23% of TF phylotypes not observed in DSV showed reduced representation in natives-in-dsv relative to natives. TRF 321bp, which was present in > 50% of the DSV samples, was only present in natives-in-dsv, with the exception of goldenrod. BLAST sequences were obtained for FAM TRFs 67bp, 138bp, 222–224bp, and 324bp (Bongard et al. 2013), suggesting colonization by uncultured Ascomycota, *Tetracladium spp.*, *Glomus spp.*, and uncultured Ascomycota respectively. BLAST sequences for HEX TRFs 99bp and 142bp were uncultured Ascomycota and *Fusarium spp.* respectively.

For the AMF data, while significantly greater colonization densities were observed in the natives-in-dsv relative to the natives (as mentioned earlier), phylotype representation of the dominant TRFs in the invaded samples generally decreased relative to uninvaded samples (Appendix 3). The goldenrod-in-dsv had the same or reduced phylotype representation in six/seven out of nine TRFs (spring/fall respectively) relative to the goldenrod (growing separately from DSV). Woodland plants had mixed results with wild ginger-in-dsv showing reduced phylotype representation in seven out of nine TRFs, raspberry showing the same or reduced phylotype representation in five out of seven TRFs, anemone showing the same or reduced phylotype representation in six out of nine TRFs, and meadow rue showing the same or increased phylotype representation in five out of eight TRFs. TRF 54bp was present in only 29% of the total DSV samples, and TRF 81bp was present in 50% of the DSV samples. There was a reduced or maintained representation by TRF 54bp in all natives-in-dsv groups, suggesting that this phylotype is universally affected by DSV invasion. Similarly, reduced representation by TRF 81bp occurred in all natives-in-dsv relative to natives

except fall goldenrod (increased levels). Only 22% of the TRFs that were dominant in DSV showed increased representation in the natives-in-dsv over natives for the AMF phylotypes, and 33% of TRFs not observed in DSV showed reduced representation in natives-in-dsv. BLAST sequences obtained for AMF were *Glomus spp.*

Discussion

Disruption or alteration of soil fungi is one mechanism by which introduced plants are able to preferentially succeed in a novel environment (Hawkes et al. 2006, Stinson et al. 2006, Vogelsang and Bever 2009). AMF may either be disrupted or harnessed by aggressive invasive plant species, resulting in altered native fungal communities in the soil as the invader attains dominance in the system (van der Heijden et al. 1998, Daniell et al. 2001, Greipsson and DiTommaso 2006, Bastias et al. 2007, Curlevski et al. 2010). Mummey et al. (2005) used T-RFLP and multivariate analyses to show that AMF communities associating with a common forage grass species *Dactylis glomerata*, naturalized in mid-western US, shifted to reflect the community composition associated with a noxious weed native to eastern Europe, *Centaurea maculosa*, post invasion. Our research supports this mechanism as a possible contributor to DSV success by demonstrating that there are significant shifts in both the total and arbuscular mycorrhizal fungal communities associating with native plant roots in invaded relative to non-invaded sites.

We were able to show that native plants living in close association with DSV differ from those living distantly from DSV in that they have higher TF and AMF colonization densities, higher TF phylotype richness levels, and different TF and AMF community structures overall. We found that the AMF colonization density increased in native plants invaded by DSV collectively relative to uninvaded natives, an observation that has been supported by other researchers (Greipsson and DiTommaso 2006, Smith et al. 2008). There was also a shift in the AMF phylotypes themselves. We observed a decrease in the quantity of significant AMF phylotypes (determined by PCA) colonizing natives in the invaded sites. The explanation for this decrease in AMF phylotypes could be varied; however, fungal infection loci in roots, which are limited in number, may be dominated by relatively few AMF species such as *Glomus intraradices* which can propagate via fungal fragments in mechanically disrupted sites (unlike many AMF) and preferentially colonize invasives (Daniell et al. 2001, Greipsson and DiTommaso 2006).

The majority of dominant TF phylotypes in DSV showed significantly increased representation in the natives-in-dsv over native counterparts. While the fungal species have not been elucidated in this investigation, there is a reasonable likelihood that a component of the observed increases in the general fungal phylotypes would include pathogenic or harmful fungi. We compared significant FAM- and HEX-labeled TRFs to BLAST sequences (Bongard et al. 2013), and the TF species with BLAST matches > 90% similarity included predominantly *Tetracladium spp.*, *Fusarium spp.*, or uncul-

tured Ascomycota. These fungi have been described as generalists, and also include important plant pathogens (Roldán et al. 1989, Priest et al. 2003). Though invaders can accumulate pathogens in their introduced range, the Enemy Release hypothesis suggests that they may avoid deleterious effects of novel pathogens (Callaway et al. 2008), and local generalist pathogens may have a more negative effect on the native plant species than on the invader (Eppinga et al. 2006, Mangla et al. 2008). Eppinga et al. (2006) hypothesized that while the accumulation of pathogens may initially limit invader abundance in the novel environment, it might feed back more negatively to the native plant community.

The use of relative fluorescence units:DNA concentration ratio as a proxy measurement of colonization density in this study is based on the assumption that DNA extraction efficiency across samples is comparable. Only the wild red raspberry may not be supported by this assumption as woody species are known to have tannins that can make DNA extraction more challenging. There is no assumption that this proxy for colonization density infers that the relative biomass of the various root samples is consistent. However, given that the same approximate amount of root tissue was used for the initial DNA extractions (i.e. 200 mg), and that there was high fidelity between replicates of both PCR amplification and T-RFLP runs, it is reasonable to conclude that normalized peak heights/DNA concentration provides an adequate proxy for colonization density. Though bias may result from differences among fungal types in the copy number of the targeted gene and differences in extraction efficiency (Nagashima et al. 2003), Burke et al. (2006) found T-RFLP to be a reliable method of microbial abundance analysis across natural communities relative to traditional methods. Peak height was chosen over integrated peak area for relative colonization density analysis in this study due to the overlap of some peaks (Stephouskas et al. 2003).

It would be expected that the TF community in natives would be less diverse/abundant relative to natives-in-dsv if diverse fungi associated with invaders (that are not present in natives) are being introduced subsequent to invasion (Brundrett 2004). Our ability to differentiate natives from natives-in-dsv based on phylotype colonization patterns suggests that there is a generalizable phenomenon at play. While in some cases the individual plant species accounted for more of the variance in the fungal communities than the plant 'type' in the present study, our investigation was focused on comparing the uninvaded and invaded natives species collectively as a first step. It would be important to examine each native species independently, with an increased sample size.

Soil fungi are an important consideration in plant fitness, plant community composition, and larger ecosystem processes, and as such, alterations to fungal communities is one likely means to successful invasion by introduced plant species. Elucidating invader fungal association dynamics could support strategies used by restoration ecologists in reclaimed sites where fungal inoculation is being considered. Given the findings of this and other related studies, further investigations into the general fungal and AMF association dynamics pre- and post-invasion are strongly warranted.

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Appendix 1

Data for the genomic DNA concentrations of root samples and relative fluorescence unit intensities (RFUs). (doi: 10.3897/natureconservation.4.3578.app1). File format: MS Word Document (doc).

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Appendix 2

TF phylotype occurrences in natives and natives growing in DSV. Natives are depicted with dark bars and natives-in-dsv are depicted with light bars. The x-axis shows the dominant phylotypes occurring in all plants (basepair length), and the y-axis shows the % of samples with representation by a particular TRF. (doi: 10.3897/natureconservation.4.3578.app2). Tagged Image File Format (tiff).

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Appendix 3

AMF phylotype occurrences in natives and natives growing in DSV; details as in Appendix 2. (doi: 10.3897/natureconservation.4.3578.app3). File format: Tagged Image File Format (tiff).

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