

Implications of reserve size and forest connectivity for the conservation of wood-inhabiting fungi in Europe

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ABSTRACT

Among the factors that affect the conservation efficiency of protected areas, lack of connectivity is considered as one of the main problems. In this study, we assessed the influence of connectivity of European beech forest reserves on wood-inhabiting fungal communities, compared to the influence of local factors. To address this topic, we used a data matrix consisting of 344 fungal species on 1571 resource units (i.e. fallen beech logs, including their standing snags) sampled in 42 European beech forest reserves. Our results show that connectivity has significant effects on wood-inhabiting fungal communities in European beech forest reserves, and that the effectiveness of reserves for maintaining the wood-inhabiting fungal diversity is compromised by habitat fragmentation. Connectivity at small scales (measured as the area of the reserve) had a strong influence on the occurrence of indicator species and was also critical for the number of species at a resource. Connectivity at larger scales (connectivity to surrounding beech forests) seemed to be particularly critical for the community composition both at resource and reserve levels. In line with previous research, we found other covariates such as size of the resource units and annual temperature range to positively influence wood-inhabiting fungal species richness. The effects of habitat fragmentation were especially strong in western and northern European regions where the smallest and more isolated reserves were located. We propose that an effective conservation strategy for wood-inhabiting fungi should focus on increasing the areas of the present reserves as well as conserving new reserves in the proximity of the existing ones.

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1. Introduction

Protected areas are a basal tool for biodiversity conservation. However, the selection and design of reserve networks and the assessment of their effectiveness remain highly debated (Gaston et al., 2006; Geldmann et al., 2013; Brooks, 2014). Little is known on how to set appropriate targets, or of the extent to which the current global protected area network fulfils its goal of conserving biodiversity (Margules and Pressey, 2000; Rodrigues et al., 2004; Geldmann et al., 2013). Among the factors that affect the conservation efficiency of protected areas, lack of connectivity is considered as one of the main problems (Margules and Pressey, 2000).

As the consequence of habitat loss and fragmentation, continuous natural landscapes are converted into habitat patches isolated from each other by a matrix of unsuitable habitats. Several studies have demonstrated that systematic conservation planning applications based on

protecting areas where the species are currently present are not sufficient to ensure their persistence in fragmented landscapes (Carroll et al., 2003; van Teeffelen et al., 2006; Cerdeira et al., 2010). Indeed, species extinctions can be expected within protected areas if the surrounding unprotected areas of habitat are lost or heavily altered (Cabeza, 2003; DeFries et al., 2005), as it is difficult for populations to persist in regions where patches are small and scattered (Hanski, 1999). Similarly, there is empirical evidence that increased isolation reduces the likelihood of persistence of certain species (e.g. Davies et al., 2000; Nordin et al., 2013).

Many conservation studies have focused on the efficiency of large protected natural or semi-natural areas as a tool to counteract habitat fragmentation effects (Simberloff and Abele, 1982; Desmet and Cowling, 2004). This conservation strategy has mainly been based on the species–area relationship concept (SAR), which can be used to relate the number of species extinctions to habitat loss (MacArthur and Wilson, 1967; Connor and McCoy, 1979). The validity of SAR-based predictions in cases of habitat loss has been heavily debated, because SAR can either overestimate (He and Hubbell, 2011) or underestimate (Rybicki and Hanski, 2013) extinctions,

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depending on the time scale and spatial context evaluated, and because they consider only the total amount of habitat and not fragmentation effects (Hanski et al., 2013). Nevertheless, SAR predictions still provide a valuable tool for assessing the impacts that habitat loss causes on biodiversity, especially in broad-scale studies (Hanski et al., 2013).

Previous research has highlighted the importance of maintaining suitable habitats outside protected areas, as an efficient landscape-level conservation strategy that avoids isolation problems (Lindenmayer and Franklin, 2002). In this way, matrix composition and management is receiving increased attention in conservation biology (Franklin and Lindenmayer, 2009). The magnitude of isolation effects can depend on the surrounding matrix type. For instance, Prugh et al. (2008) showed that the strongest isolation effects occur in forest-dominated landscapes when the surrounding forest matrix is managed by clear-cutting.

Beech forests (*Fagus sylvatica*) constitute a major forest type in Europe (Brunet et al., 2010), and are one of the principal natural vegetation types of temperate Europe (Pott, 2000). Nevertheless, the long history of land use in the European beech forest zone has led to a highly fragmented beech forest landscape, where remaining old-growth fragments (most of them under protection status) are small and highly scattered (Parviainen, 2005).

Wood-inhabiting fungi are confined to ephemeral habitats (decomposing dead wood units) and organized as habitat-tracking metacommunities (Halme et al., 2013), which makes them especially sensitive to habitat loss and fragmentation (Stokland et al., 2012). For wood-inhabiting organisms, habitat loss and fragmentation can be assessed at two levels; at the overall habitat level corresponding to the forest area and at the resource level, corresponding to the amount of dead wood present in the forest. In managed beech forests the amount of dead wood is typically reduced to less than 10% of the natural level (Christensen et al., 2005), and hence resources available for wood-inhabiting organisms may be scarce and scattered even in landscapes with high forest connectivity if the management intensity is high.

So far the effects of habitat loss and fragmentation on wood-inhabiting fungi have mainly been studied in European boreal forests, especially in Fennoscandia. Considerable changes in fungal biodiversity have been reported, with several species declining in modified forests and landscapes (e.g. Penttilä et al., 2006; Nordén et al., 2013). The main traits that characterize species that have declined in managed landscapes are related to high habitat specialization level and low innate population abundances (Berglund et al., 2011; Nordén et al., 2013). Human impact is rather recent in the boreal forest zone, while forest fragmentation was initiated thousands of years before present in more temperate parts of Europe (Hannah et al., 1995). Hence, the forest landscape of continental Europe provides an interesting case to investigate the long-term effects of habitat fragmentation on wood-inhabiting fungi. Nevertheless, only few large-scale studies have been conducted in this area (Ódor et al., 2006; Halme et al., 2013; Heilmann-Clausen et al., 2014a), and none of these have focused specifically on the effects of habitat loss and fragmentation. Furthermore, a previous study which attempted to measure isolation effects in a beech forest landscape, failed to do so conclusively because of the small scale of the study (Abrego and Salcedo, 2014).

The objective of the current study was to assess the relative influence of connectivity compared to influence of resource availability and climatic conditions on wood-inhabiting fungal communities in European beech forest reserves. To address this aim, we use a resource-unit level survey data to examine how 1) species richness, 2) the presence of indicator species, and 3) community composition are influenced by connectivity (reserve size and presence of beech forests in the proximity of the reserve), in relation to resource availability and climatic variables.

2. Material and methods

2.1. Study area and site selection

The study included 41 European beech forest reserves located in 8 countries (see Table A1 for the names and locations of sampled reserves). The surveyed beech forests represent ca. 0.02% of all European beech forest area. The main criteria for site selection was that sites should represent as far as possible the best reference for natural beech forests for each country, and that altogether, the locations of the reserves should represent the European beech forest distribution.

All forest reserves were dominated by beech (*F. sylvatica*) but in several reserves also other tree species were present. In reserves located at high altitudes or northern latitudes spruce (*Picea abies*) or silver-fir (*Abies alba*) was abundant, while oak (*Quercus robur*) and ash (*Fraxinus excelsior*) were common intermixed species in the Atlantic regions. A more detailed description of the sampled reserves is given in Table A1.

2.2. Field data inventories

In each reserve, the sampling units were fallen beech logs (of at least 10 cm in diameter), including their standing snag if the snag was still present at the time of the survey (henceforth named resource units). The sampling design was based on a stratified random design that aimed to cover a balanced representation of the different resource unit types in each reserve: it was attempted to sample an equal number of resource units from the main five decay stages (same method as in Christensen et al., 2005). Nevertheless, this was not always possible as some reserves did not hold a balanced representation of all decay classes (this was especially the case with small reserves).

The fungal samplings were carried out between the years 2001–2012, during the main fruiting seasons in each European region (from late August to early December), surveying each resource unit one to three times. The presence of wood-inhabiting fungal species (polyporoids, agaricoids, some corticoids and larger discomycetes, stromatic pyrenomycetes) was registered at resource unit level. Most species were identified in the field, and when microscopic identification was necessary, the specimens were collected for further identification in the laboratory. We classified the species into four groups: (1) specialists on beech wood, (2) species with a strong preference for deciduous wood, (3) species with a strong preference for conifer wood (but occasionally occurring on beech), (4) species that grow equally well on deciduous and conifer wood, henceforth named generalists (the classification of the species is given in Table A2). The classification was based primarily on Hansen and Knudsen (1997), Hansen et al. (2000) and Knudsen and Vesterholt (2012).

2.3. Measured variables

The environmental variables measured for each resource unit and reserve are described in Table 1. At resource unit level, average decay stage, diameter at breast height, moss cover percentage and death cause of the tree were recorded in the field. At reserve level, reserve area, average altitude, vegetation type (according to the dominant intermixed tree species), connectivity to surrounding beech forests, annual temperature range and annual precipitation were measured, calculated or extrapolated. The reserves were grouped in five regional categories according to their geographical location (see Fig. A1 and Table A1).

We used the tree species distribution maps of Europe produced by EFI-Alterra (Brus et al., 2012) to calculate the connectivity of each reserve to European beech forests. The EFI-Alterra maps are based on data measured at irregularly located plots, modelled to yield predicted probabilities of tree species presence at 1 km × 1 km resolution. The maps describe for each 1 km² grid cell i the probability p_i that the grid

Table 1

Descriptions of measured variables in this study, and variables that were considered for different models (last two columns). The sources and measurement procedures of the variables are explained in the methods section.

		Units	Data type	Models 1, 3, 5 and 7	Models 2, 4, 6 and 8
Connectivity 1	Connectivity value at 1 km spatial scale	Unitless	Log transformed, continuous	X	X
Connectivity 10	Connectivity value at 10 km spatial scale	Unitless	Log transformed, continuous	X	X
Connectivity 100	Connectivity value at 100 km spatial scale	Unitless	Log transformed, continuous	X	X
Area	Area of the reserve	ha	Log transformed, continuous	X	X
Average altitude	Average altitude of the reserve	m (msl)	Log transformed, continuous	X	X
Vegetation type	Dominant tree species apart from beech: 1) <i>Fraxinus</i> and <i>Quercus</i> ; 2) <i>Picea</i> ; 3) <i>Betula</i> , <i>Pinus</i> , <i>Picea</i> and <i>Quercus</i> ; 4) <i>Abies</i> and <i>Picea</i> ; 5) <i>Quercus</i> ; 6) Only <i>Fagus</i> ; 7) <i>Carpinus</i> and <i>Quercus</i> ; 8) <i>Pinus</i> and <i>Quercus</i> ; 9) <i>Acer</i> and <i>Tilia</i> ; 10) <i>Abies</i> ; 11) <i>Carpinus</i> ; 12) <i>Acer</i> and <i>Fraxinus</i> ; 13) <i>Fraxinus</i> , <i>Quercus</i> and <i>Ulmus</i> ; 14) <i>Acer</i> ; 15) <i>Carpinus</i> , <i>Fraxinus</i> and <i>Quercus</i>	15 classes	Ordinal	X	X
Annual temperature range	The difference between the mean temperatures of the warmest and coldest months of the year	°C	Continuous	X	X
Annual precipitation	Sum of the monthly precipitation values within a year	mm	Log transformed, continuous	X	X
Average moss percentage in resource units	Average moss cover of sampled resource units in each reserve	%	Continuous	X	X
Average decay stage of the resource units	Average decay class of sampled resource units in each reserve	Numerical index from 1 to 5	Continuous	X	X
Average diameter of the resource units	Average diameter of sampled resource units in each reserve	cm	Continuous	X	X
Number of sampled resource units	Number of sampled resource units in each reserve	Unitless	Log transformed, continuous		X
<i>Resource unit</i>					
Diameter	Diameter at breast height of the resource unit	cm	Continuous	X	
Decay stage	Decay stage of the resource unit	Numerical index from 1 to 5	Ordinal	X	
Moss cover		%	Continuous	X	
Death cause	Whether the log (resource unit) was fallen with the plate or broken	Classes (fallen/broken)	Ordinal	X	
Times sampled	Times that each log were sampled	Classes (A = once sampled, B = three times sampled)	Ordinal	X	X

cell consists of beech forest. We calculated the connectivity of sampling plot located in grid cell i to the neighbouring beech forests j with the formula $S_i = \sum_j e^{-\alpha d_{ij}} p_j$. Here d_{ij} is the distance (in kms) between the focal grid cell i and the grid cell j . We set the spatial scale parameter of the exponential weighting to $\alpha_1 = 1/\text{km}$, $\alpha_2 = 0.1/\text{km}$ or $\alpha_3 = 0.01/\text{km}$, so that we calculated the connectivity effectively at $\frac{1}{\alpha_1} = 1 \text{ km}$, $\frac{1}{\alpha_2} = 10 \text{ km}$ and $\frac{1}{\alpha_3} = 100 \text{ km}$ spatial scales. The numerical computation of connectivity values was made with the Zonation Conservation Planning Software (Lehtomäki and Moilanen, 2013). Note that the connectivity variables describe the overall habitat availability at the indicated spatial scales, while reserve size is a better proxy of local resource availability, since dead wood amounts in reserves are generally at least ten times higher than in managed beech stands (Christensen et al., 2005).

We used the climatic GIS layers BIO7 and BIO12 by Hijmans et al. (2005) for characterizing respectively the annual temperature range and the annual precipitation in the reserves.

2.4. Models for assessing the relative effect of connectivity on wood-inhabiting fungal communities

The variables considered in each model are shown in Table 1. All statistical analyses were carried out using the programme R version 3.1.3 (R Development Core Team, 2013).

We first modelled the number of species found per resource unit in relation to resource unit and reserve level covariates. We used Poisson regression in which we included region and reserve (nested within region) as random effects. Subsequently we modelled how the mean number of species per resource unit related to the reserve level covariates. We used linear regression, in which we included region as a random effect. In order to control for the survey effort, we included the times (1–3) that each resource unit was sampled as an explanatory variable. Thirdly, we modelled how the total number of species, and the

respective numbers of beech forest specialists, deciduous forest specialists, conifer forest specialists and generalist species, related to variables describing the properties of the reserve. For these models we applied Poisson regression including region as a random effect. In order to control for the effect of the sampling effort, we included the number of sampled resource units as a reserve level covariate.

Fourthly, we modelled the number of indicator species per reserve using the same set up, and additionally the probability of indicator species presence at resource unit using logistic regression. In this analysis we included resource-unit and reserve level covariates, with reserves nested within regions as a random effect. As indicator species, we used the list of wood-inhabiting fungi proposed to indicate conservation value in European beech forests by Christensen et al. (2004).

Finally, we examined how fungal community compositions at the resource unit and reserve levels depended on resource-unit and reserve level covariates. We calculated the Bray–Curtis similarity index from fungal presence absence data, and related variation in community composition to the covariates using distance-based redundancy analysis (db-RDA) with the Vegan package (Oksanen et al., 2015).

All models were analyzed separately with four different connectivity measures, i.e. with the area of the reserve and with connectivity to beech forests, measured at each of the three spatial scales.

The linear regression models were fitted with the function *lmer* and the Poisson and logistic regression models with the function *glmer* from the lme4 package (Bates et al., 2015). For all models, backward and forward variable selection with AIC criteria was used to find and fit the most parsimonious models. To avoid collinearity problems, we assessed correlations among the explanatory variables using Spearman correlations, and we used the rms package to calculate the variance inflation factors (VIFs) for the variables that were retained after model selection.

We assessed the explanatory powers of the linear regression models by their R^2 values, of the db-RDA models by their constrained variation, and of the logistic regression models by their Tjur R^2 values (Tjur, 2009).

In the univariate models we partitioned the explained variation among the explanatory variables by computing the proportion of sum of squares associated to each variable. In the multivariate models (db-RDA) we calculated the proportion of constrained variation captured by each variable.

3. Results

3.1. Raw data exploration

Altogether, the full data consisted of 16,996 records of 344 fungal species (out of which 17 were suggested as indicator species, 21 were classified as beech wood specialists, 270 as deciduous wood specialists, 19 as conifer wood specialists, and 34 as generalists) on 1571 resource units sampled in 42 beech forest reserves. The minimum (respectively maximum) number of logs sampled in each reserve was 5 (125) and the average 38. The mean number of species per resource unit was 11 and the mean diameter of the sampled resource units was 58 cm.

The minimum (respectively maximum) area of the sampled reserves was 3 ha (1600 ha) and the average was 102 ha. The altitude of the sampled beech reserves ranged from 2 to 1760 m above sea level. According to the connectivity to surrounding beech forests, the beech reserves from northern European countries (Valaklitt, Bjurkärr, Åskemossen and N Kroksjön) presented the lowest connectivity values, while the reserves from central European countries (Krokar, Rajhenav, Klenovski Vepor and Dobroc) showed the highest connectivity values (Fig. A2, panel A). Likewise, the smallest reserves (Amelisweerd, Oostbroek, Wulperhorst and Drie) were located in northern European countries and the biggest ones (Boatin, Stuzica, Silkosia and Havesova) in central and southeast European countries. These results reflect an overall gradient in beech forests size and fragmentation from central to northern Europe (Fig. 1). All connectivity values (area of the reserve and connectivity values at 1, 10 and 100 km spatial scales) were positively correlated (Fig. A2, panel B).

3.2. Species richness

The number of species recorded per resource unit was not related to either of the connectivity measures (area of the reserve or connectivity to surrounding forests), and was mainly explained by resource availability related variables (mainly diameter and decay stage), with only a minor contribution from the annual temperature range ($p < 0.01$ in all the cases, Table 2, Fig. A3A–D). Also the average number of species per resource unit was unrelated to any of the connectivity measures, and was mainly explained by the average diameter of the resource units within each reserve (positive effect), and to a lesser extent by the annual temperature range (negative effect) and average moss cover (positive effect) ($p < 0.05$ in all the cases, Table 2, Fig. A4A–C).

The total number of species per reserve was positively related to reserve size ($p < 0.05$, Table 2), so that fewer species were recorded from small compared to larger reserves ($\rho = 0.3$, Fig. 2B), while connectivity to the surrounding forests had no significant effect. The total number of species recorded per reserve was also positively influenced by the number of the sampled resource units as well as the average diameter of the resource units ($p < 0.05$ in both cases, Table 2, Fig. 2A & C).

The separate analyses for species with different levels of habitat specializations, showed that the number of beech wood specialist species at reserve level was equally positively related to reserve area and to the average diameter of the resources ($p < 0.05$ in both cases, Table 2). The average diameter of the resource units contributed positively to explaining species richness also in the other fungal groups considered (except conifer specialists), while reserve area or connectivity had no effect. Richness of deciduous wood specialists was mainly explained by temperature range while moss cover contributed to explaining the number of host generalist species recorded per reserve. The only variable that proved to significantly influence the number of conifer wood

specialist species was the times that the resources were sampled ($p < 0.05$ in all the cases, Table 2).

3.3. Indicator species

The area of the reserves strongly affected the occurrences of indicator species. The logistic regression model predicted the presence of indicator species to be positively influenced mostly by the area of the reserve, and to a lesser extent by the diameter of the resource units ($p < 0.05$ in both cases, Table 2, Fig. 4). The same variables explained the total number of indicator species recorded per reserve, with only a minor contribution from the number of sampled resource units ($p < 0.05$ in all the cases, see Table 2).

3.4. Community composition

Community composition, both at resource and reserve levels, was significantly related to connectivity at the broad scale (at 10 km spatial scale). Additional covariates that significantly influenced fungal community composition at resource level were (with decreasing contributions) decay stage, annual temperature range, diameter of the resource and moss cover, ($p < 0.05$ in all the cases, Table 2). As can be seen in Fig. 3A, the angle of the vectors for diameter of the resource unit and moss cover were correlated in relation to the community composition, as were also connectivity and annual temperature range. More or less the same variables described community composition at reserve level, but with larger relative contributions from annual temperature range, and smaller from average decay stage and diameter of resource units. In addition, and somewhat spuriously, even the number of sampled resource units was found to have an effect on reserve level community composition, ($p < 0.05$ in all the cases, Table 2, Fig. 3B).

The final model outputs (including AIC values) are given in Table A3.

4. Discussion

4.1. Effects of connectivity

In this study we show that the highly fragmented nature of the current network of protected beech forests in Europe may be insufficient for maintaining intact the fungal biodiversity connected to dead wood. Even though we investigated some of the best protected beech reserves across Europe, we found that the total species richness at reserve level and in particular the frequency and richness of beech wood specialists and indicator species was strongly negatively affected by the small area of the reserves. We interpret this as reflecting that specialized wood-inhabiting fungi confined to beech wood, or suggested as indicators of conservation value, depend on amounts and densities of dead wood that are not present in small and isolated reserves. This is in concordance with earlier findings from boreal forests that, in particular, red-listed species suffer from the loss of connectivity (Nordén et al., 2013), possibly because they are resource specialists and thus particularly vulnerable to fragmentation (see Henle et al., 2004). On top of that, microclimatic stress close to forest edges (Crockatt and Bebbber, 2015) may constitute an additional threat to species depending on stable humid conditions, given the much larger edge to area ratio in small forest patches.

In addition to the effect of reserve area, we found that connectivity at the 10 km scale significantly influenced community composition both at the resource and reserve levels. In boreal forests changes in community composition have been found to be driven by a decline of rare and resource specialist species and the corresponding increase of resource generalist species (Berglund et al., 2011; Nordén et al., 2013). Another factor which affects wood-inhabiting fungal composition in fragmented landscapes is their colonization capability. Even if wood-inhabiting fungi with airborne dispersal can disperse their spores globally, they are dispersal limited in the sense that their colonization and occurrence

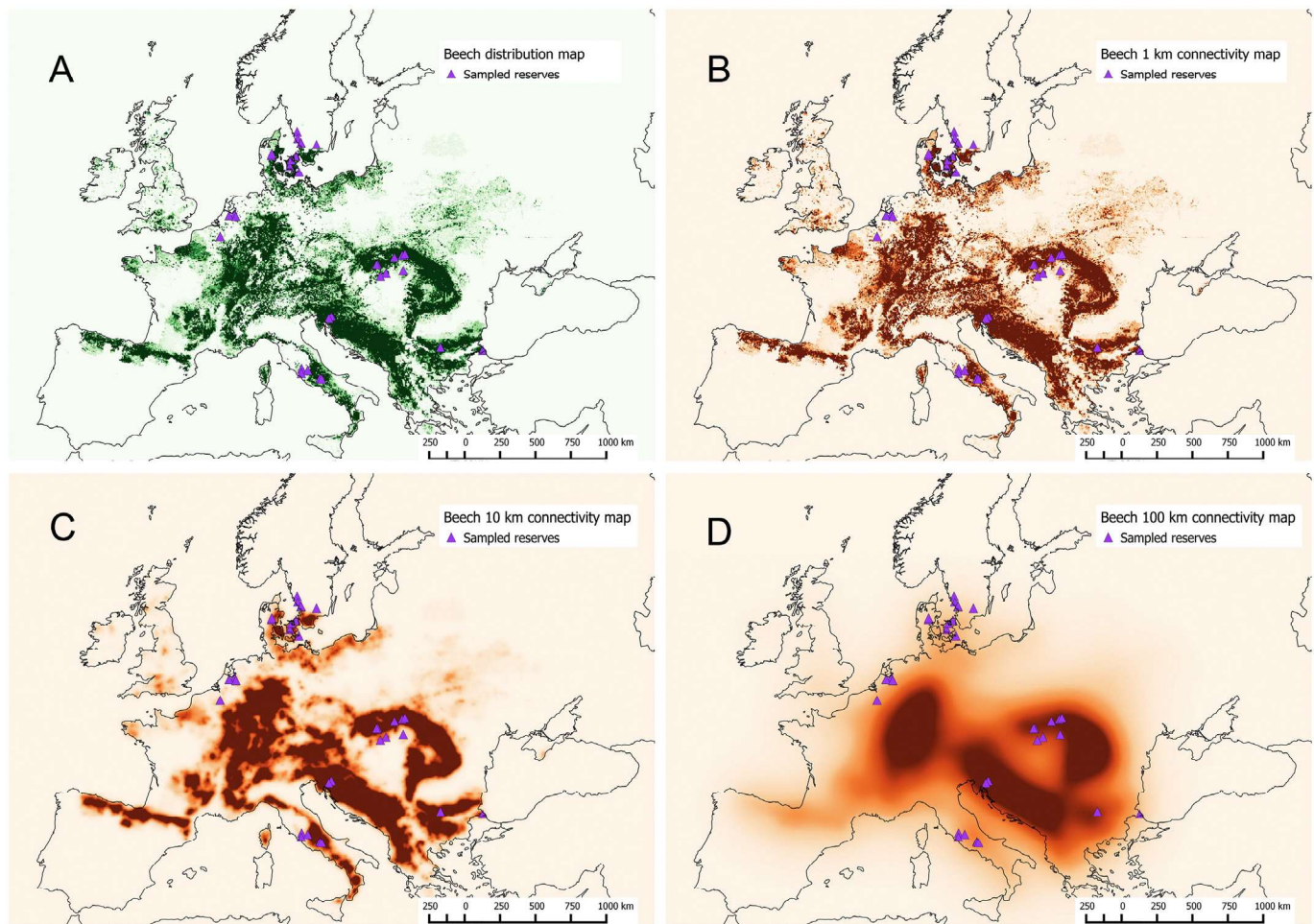


Fig. 1. Distribution (panel A) and connectivity (panels B, C, D) maps of beech forests across Europe, and the geographical location of sampled reserves in Europe (purple triangles). The beech distribution map corresponds to the map produced by EFI-Alterra (Brus et al., 2012). The connectivity map was obtained using the spatial scale parameter of the exponential weighting to $\alpha = 1$ (panel B), $\alpha = 0.1$ (panel C) and $\alpha = 0.01$ (panel D) in the Zonation Conservation Planning Software (Lehtomäki and Moilanen, 2013).

Table 2

Variation partitioning of different models in this study. The first column shows the proportion of the total variance explained by each model (Total R^2). The remaining columns show the relative proportions explained by each of the explanatory variables.

Model	Total R^2	Area (ha)	Connectivity (10 km)	Diameter of the resource* (cm)	Decay stage*	Moss cover* (%)	Death cause	Number of sampled logs	Temperature range (°C)	Times sampled
Model 1	48			74	20	4	1		1	
Number of species at resource level										
Model 2	80	6		6				88		
Number of species at reserve level										
Model 3	46			61		9			30	
Mean number of species at resource level										
Model 4a, Number of beech wood specialist species at reserve level	52	50		50						
Model 4b, Number of broadleaved wood specialist species at reserve level	43			15					85	
Model 4c, Number of host generalist species at reserve level	66			56		44				
Model 4d, Number of conifer wood specialist species at reserve level	3									100
Model 5	12	73		27						
Presence of indicator species at resource level										
Model 6	75	86		10				4		
Number of indicator species at reserve level										
Model 7	10		17	17	42	8			17	
Community composition at resource level										
Model 8	36		17	9	11	17		14	23	
Community composition at reserve level										

* Diameter and decay stage values at resource unit in Models 1, 2, 3, 5 and 7, and average diameter and decay stage of the resource units at reserve level in Models 4, 6 and 8 (see Table 1).

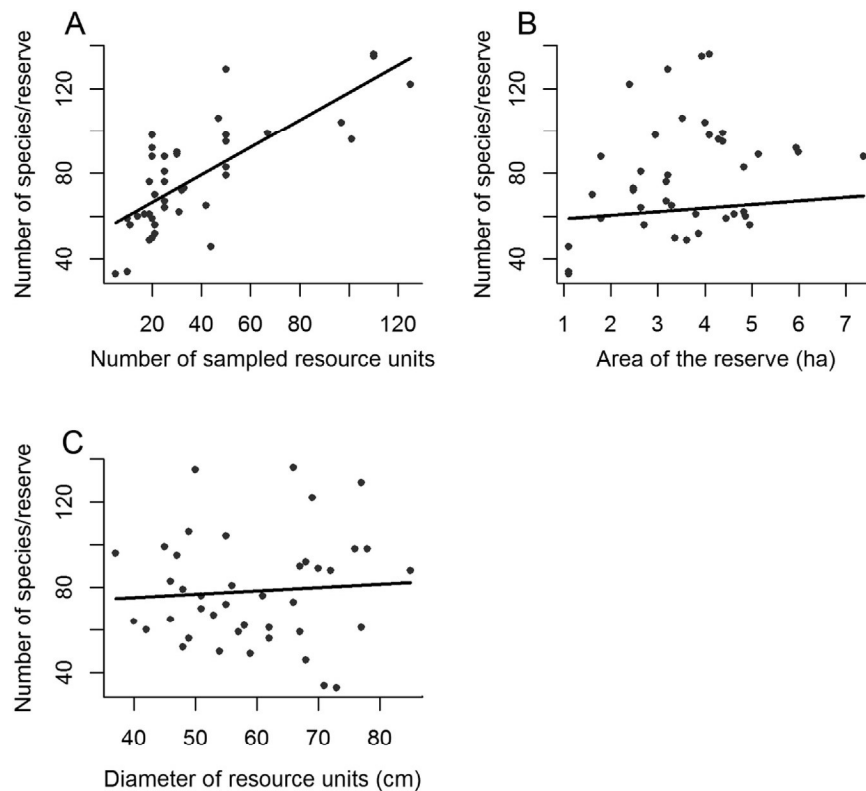


Fig. 2. Relation between the explanatory variables (x axes) and the number of species per reserve (y axes). The significant variables for explaining the number of species within reserves were the number of sampled resource units (panel A), area of the reserve (panel B), and the average diameter of the resource units in the reserve (panel C).

probabilities decrease rapidly with distance to the nearest source (Norros et al., 2014). Edman et al. (2004) showed that the poor colonization capability of some of these species limits their ability to colonize isolated habitat patches even if they are of high quality. In the same line, Norros et al. (2012) demonstrated that dispersal can limit the establishment of specialist species even at small scales, and thus their occurrence in fragmented landscapes.

As previously emphasized, we measured the degree of habitat fragmentation (or conversely, connectivity) in two ways: as the area of the

strict forest reserve and as connectivity to surrounding forests. These measures provide information about connectivity at different levels: reserve area provides an estimate of dead wood abundance and connectivity at the local scale, while connectivity measures the connectivity to surrounding forests habitats at a more regional scale (Moilanen and Nieminen, 2002), irrespective of the actual amounts of dead wood present in these forests. In most of the studied reserves, the surrounding beech forest area is managed for timber production, and thus is of limited relevance for specialized wood inhabiting fungi (Bässler et al.,

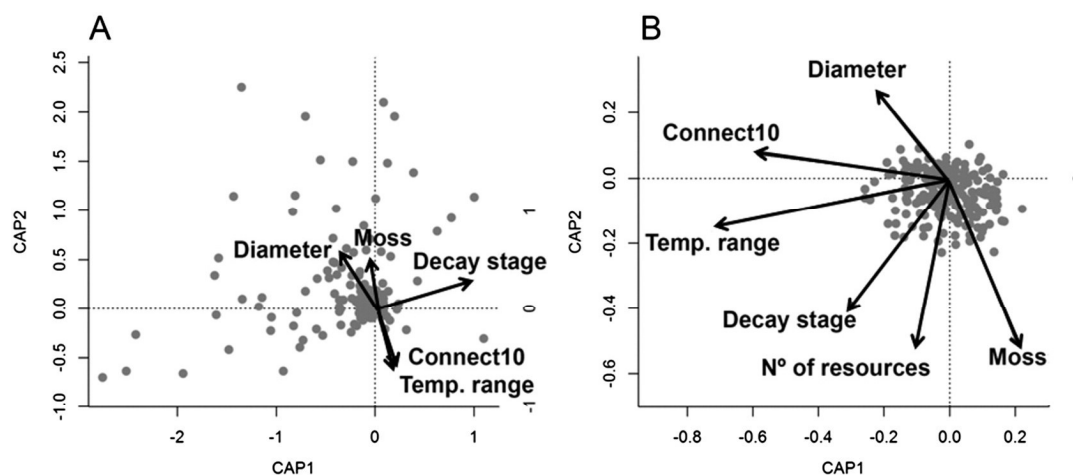


Fig. 3. db-RDA triplots, based on the Bray–Curtis similarity matrix calculated from the presence/absence data of species per resource unit (Panel A) and reserve level (Panel B), constrained by the significant environmental variables. Resource unit: diameter of the resource unit (Diameter), moss cover percentage (Moss), average decay stage (Decay stage), connectivity of the reserve (Connect10) and the annual temperature range (Temp. range). Reserve: average diameter of the resource units (Diameter), connectivity of the reserve (Connect10), temperature range of the reserve (Temp. range), average decay stage of the resource units (Decay stage), number of sampled resource units (No. of resources), average moss cover of the resource units (Moss). The grey circles correspond to the species and the arrows to the significant explanatory variables.

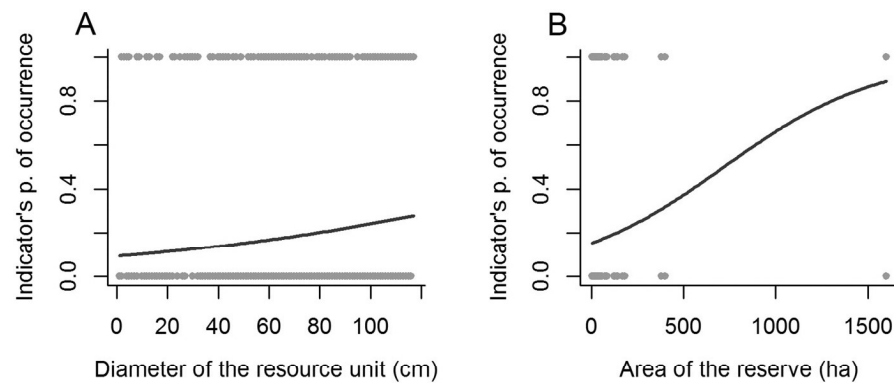


Fig. 4. Predictions made for the probability of occurrence of indicator species in the reserves (x axes) in relation to the significant explanatory variables in the logistic regression model (y axes). Panel A shows the prediction of the probability of occurrence of indicator species according to the diameter of the resource unit and Panel B to the area of the reserve.

2010; Abrego et al., 2014). This fact may explain why our analyses failed in pinpointing the importance of connectivity in relation to species richness or the presence of indicator species. We note that while it is straightforwardly expected that the total number of species increases with increasing reserve area, the more subtle question that we addressed here is whether species density is affected by the area of the reserve (Connor et al., 2000; Nordén et al., 2013).

Only a few studies have previously detected effects of regional-scale connectivity for wood-inhabiting fungi (Penttilä et al., 2006; Nordén et al., 2013), partly because many studies have been carried out on a too small spatial scale for detecting connectivity effects (e.g. in Abrego and Salcedo, 2014). While the whole European scale of our study enabled us to detect connectivity effects at the 10 km scale, detecting connectivity effects at larger spatial scales would be challenging with the current study design. This is due to the unavoidable fact that connectivity shows spatial autocorrelation at the spatial scale in which it is measured. For example, the beech forest reserves that we sampled showed a clear geographical pattern, with large and well connected reserves being located in central and southeastern regions, and small and isolated ones in western and northern regions. This pattern reflects an overall gradient in the fragmentation level of beech forests in Europe, with western Europe being characterized by a higher human density and a longer-history of land-usage than eastern Europe (Kaplan et al., 2009). Thus our 100 km scale connectivity measure showed high values for virtually all central and southeastern European reserves, and small values for virtually all northern and western European reserves, making conclusive statistical inference impossible. In order to account for the less evident but still existing spatial autocorrelation in our analyses conducted with the 10 km scale connectivity measure, we grouped the reserves in five regional categories, included as random effects in the models. A more powerful solution would be a study design in which both connected and unconnected reserves would have been sampled across the different regions in Europe, but the configuration of beech forests and the locations of existing reserves does not allow for such a design. Similar problems have been found in earlier studies in which the longitudinal gradient of anthropogenic pressure and climate across Europe have challenged attempts to disentangle the effects of these factors (Ódor et al., 2006; Heilmann-Clausen et al., 2014a).

4.2. Effects of resource quality and climate

Apart from the effects of reserve area and beech forest connectivity we found that environmental covariates relating to resource quality and climate significantly influenced wood-inhabiting fungal communities. Among these variables, size and the decay stage were the main factors affecting the occurrences of species at resource unit level and the temperature range was the principal factor affecting the fungal species at reserve level. Most of these effects are in concordance with the results of many previous studies (Heilmann-Clausen, 2001; Heilmann-Clausen and

Christensen, 2004, 2005; Stokland et al., 2012), and will not be evaluated further here. However, the effects of micro- and macroclimatic drivers are still sparsely investigated in fungal community ecology (but see Bässler et al., 2010; Heilmann-Clausen et al., 2014a). Moss cover of the resource units showed a positive effect on species number, suggesting that the microclimatic conditions provided by the moss layer increase the suitability of the wood for fungal establishment and fruiting (see Heilmann-Clausen and Christensen, 2005; Boddy and Heilmann-Clausen, 2008). Similarly to analyses conducted on a part of the present dataset (Heilmann-Clausen et al., 2014a), we found that the annual temperature range had a strong effect on community composition. Additionally, the present results show a strong negative effect also on fungal species richness. The annual temperature range is related to continentality, which is suggested by various authors (e.g. Ódor et al., 2006; Boddy and Heilmann-Clausen, 2008) to favour the occurrence of stress tolerant species. It is important to emphasize that our study deals only with fruiting macrofungi, and our results cannot be translated directly to patterns at the mycelial level. Nevertheless, fruiting body based survey methods are still a very relevant method in a conservation context, as it provides significant information on the occurrences and distribution of fungi.

4.3. Conservation implications and reserve selection

Our results indicate that fragmentation of beech forests and especially the small size of many temperate forest reserves in Europe (e.g. Götmark and Thorell, 2003; Johansson et al., 2013) is a threat for the conservation of wood-inhabiting fungi. Therefore, we propose that an effective conservation strategy for wood-inhabiting fungi should focus on increasing the areas of the present reserves as well as conserving new reserves in the proximity of existing ones. This result is in agreement with previous studies in boreal forests. Berglund and Jonsson (2005), found that the extinction risk of wood-inhabiting fungal species increased in small old growth forest remnants, and concluded that if conservation efforts are focused on small forest remnants only, a considerable portion of the wood-inhabiting fungal diversity is lost. Based on similar results, Nordén et al. (2013) suggested that protecting big areas that are well connected is more effective for conservation than protecting small fragments distributed across the landscape. Previous studies conducted on beech forests at much smaller scales have also suggested that the protection of large areas is important, especially for rare wood-inhabiting fungal species (Abrego et al., 2014). Our results thus parallel the findings of many population viability studies which have pinpointed the importance of connectivity for the persistence of populations, and thus proposed the implementation of connectivity measures in reserve selection procedures (e.g. Araújo et al., 2002; van Teeffelen et al., 2006).

Especially in Fennoscandia the use of indicator species for assessing the conservation value of forests is well-recognized (Heilmann-Clausen et al., 2014b), but still most indicator species are suggested based on

field experience and lack scientific validation (but see Penttilä et al., 2006). Our study showed that the presence and richness of the indicators suggested by Christensen et al. (2004) was strongly and positively related to reserve size. This does not imply that these indicator species do not occur in smaller reserves or in unprotected forests. Rather, our results suggest that a rich presence of indicators in smaller old growth forest fragments indicate past or present processes that has supported the persistence of species that generally depend on large natural forest areas. Such fragments are likely to be important life-boats also for other aspects of old growth forest biodiversity in fragmented landscapes, and are hence crucial to consider in conservation planning. The establishment of a complete indicator list for forest integrity in temperate Europe is no small challenge. However, the present study represent a step further in the creation of a suitable species list for the evaluation of forests conservation value, as our results indicate that the indicators species proposed by Christensen et al. (2004) are indeed highly sensitive to local connectivity of beech forest reserves, and hence they provide an useful tool to identify and monitor conservation value of old growth beech forest.

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