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A novel comparative research platform designed to determine the functional significance of tree species diversity in European forests

V1.0

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Abstract: This paper first reviews the current knowledge on suitable approaches to study the functional importance of forest tree diversity. Then, we present a new research platform with comparative study sites varying in tree diversity, replicated at a continental scale, and outline how it addresses many of the deficiencies in existing research. The paper further specifies the design and implementation of the platform, synthesises the specific hypotheses that can be tested and provides an outlook for scientists and managers. This report was adapted from **Baeten et al. (2013) Perspectives in Plant Ecology, Evolution and Systematics. http://dx.doi.org/10.1016/j.ppees.2013.07.002**

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1   Introduction

The past two decades of research on biodiversity and ecosystem functioning (BDEF) have shown that biodiversity is not only a passive consequence of the environmental conditions and interactions between species (Naeem, 2002; Loreau, 2010), but that differences in biodiversity can largely affect ecosystem functioning. Several hundreds of published effects have provided ample evidence that plant biodiversity can influence key ecosystem processes such as biomass production, nutrient cycling and pest regulation (Cardinale et al., 2012). While it is now generally accepted that plant diversity can affect function, the importance of BDEF relationships in complex natural ecosystems and their relevance to ecosystem management and conservation have been debated (Srivastava and Vellend, 2005; Duffy, 2009; Hillebrand and Matthiessen, 2009). Many of these concerns have arisen because early experiments and models on functional biodiversity lacked environmental and biological complexity and did not consider ecosystem multifunctionality, i.e., the maintenance of multiple functions (Hillebrand and Matthiessen, 2009). A new generation of studies therefore attempts to investigate BDEF relationships in real-world settings (Solan et al., 2009). Short-lived test systems such as microcosms, mesocosms and grasslands have dominated previous research (Balvanera et al., 2006), but much greater emphasis is now being placed on structurally more complex systems containing long-lived plants, including forests (Scherer-Lorenzen et al., 2005a).

Trees are text book examples of ecosystem engineers capable of modifying aspects of their environment, yet surprisingly little is known about the functional significance of tree species diversity in forests (Nadrowski et al., 2010). Experimental forestry trials comparing monocultures with two-species mixtures have existed for many decades (Pretzsch, 2005), but large-scale experiments with more species richness levels were only installed during the past decade (e.g., Scherer-Lorenzen et al., 2005b; www.treedivnet.ugent.be), meaning that these systems are still far from maturity. While important diversity-functioning patterns have already been reported from these experiments (Vehviläinen and Koricheva, 2006; Potvin et al., 2011; Lei et al., 2012), the results might differ considerably from those found in mature forests, when trees are older and the species diversity affected the environment for several decades. Furthermore, experimental tree communities face several specific limitations including small plot size, simplified age distributions and simplified stand structure compared with mature forest (reviews: Scherer-Lorenzen et al., 2005b; Leuschner et al., 2009). Studying existing, mature forests in real landscapes would therefore complement the results from these experiments. To this end, two approaches seem to be promising. First, regional or national forest inventory databases are ready-to-use and have now also been explored to examine BDEF relationships in mature forest (Caspersen and Pacala, 2001; Vilà et al., 2005; Paquette and Messier, 2011; Gamfeldt et al., 2013). However, they suffer from the disadvantages that tree diversity is generally confounded with many environmental variables and that usually only a few functions

are measured. The second approach, comparative observational plots set up along tree diversity gradients in mature forests while maximally controlling other environmental factors, would allow to better isolate potential diversity effects from confounding environmental conditions. This approach would complement the existing networks of experimental research sites and inventories.

A first case study in a central European beech forest compared forest patches on similar soils, in which past ownership and forest use had created a small-scale mosaic of tree species diversity (Leuschner et al., 2009). However, this study used a complete dilution design (Nadrowski et al., 2010; see Glossary) where the only monocultures were the ones of *Fagus sylvatica* and this tree species was present in all mixtures. This does not allow for separating effects of increasing species diversity from the confounding effects of decreasing dominance of *F. sylvatica*. At present few other local-scale comparative research platforms exist and most do not allow for disentangling the diversity signal from confounding factors such as environmental gradients and species identity (see review by Nadrowski et al., 2010). A more extensive comparative approach, which goes considerably beyond typical observational studies, was recently adopted in the German Biodiversity Exploratories, which focus on the effects of land-use change on biodiversity and ecosystem processes in forest and grassland (Fischer et al., 2010). A similar comparative approach could be used to allow for comparisons of stands of different tree species diversity in mature forest, in analogy to the land-use comparisons in the German Biodiversity Exploratories.

A comparative approach with study sites varying in tree species diversity and replicated at the continental scale would address many of the deficiencies in forest functional biodiversity research outlined above. Here we present the design and implementation of the FunDivEUROPE Exploratory Platform, a network of forest plots along tree species diversity gradients in six major European forest types (FunDivEUROPE: Functional significance of forest biodiversity; www.fundiveurope.eu). With the additional asset of studying forest types of very different European regions, the platform will contribute to answering some of the most important unsolved questions in BDEF research: does tree species diversity affect ecosystem functioning and the provisioning of ecosystem services? Do the effects of differences in resource use and facilitation among species (i.e., complementarity effects) vary along broad environmental (climatic, soil) gradients? Using forest inventory data bases, Paquette and Messier (2011) showed that complementarity may be more important for tree productivity in boreal compared with temperate forests, while Zhang et al. (2012) found similar complementarity effects across biomes. Furthermore, there are virtually no data available on ecosystem processes other than productivity. In the FunDivEUROPE project we will therefore measure a large and comprehensive spectrum of different functions and related services in each plot of our platform. The Exploratory Platform provides further added value because it is

combined with two complementary platforms that are studied in parallel (Fig. 1): (i) a network of European tree species diversity experiments (Experimental Platform) and (ii) a compilation of national and regional forest inventories (Inventory Platform). As the Exploratory Platform was set up as a hypothesis-driven network of plots, we begin this contribution with an overview of the specific research questions and hypotheses we are aiming to test. The rationale of the platform and the design are subsequently discussed.

Fig. 1 Locations of the study sites that form the different research platforms in the FunDivEUROPE project: the Exploratory Platform (filled circles), the Experimental Platform (stars) and Inventory Platform (dark grey countries). The six exploratory regions were selected to represent major European forest types (see Table 1) and are described in detail in this paper. Some of the experiments were in fact established in several localities within a region (FORBIO, BIOTREE) and the figure indicates their approximate location.

2 Guiding research questions and hypotheses

The research platform was designed to test five general hypotheses that have been highlighted as unresolved in recent review and opinion papers (e.g., Hillebrand and Matthiessen, 2009; Nadrowski et al., 2010; Cardinale et al., 2012). The first four hypotheses have been explored extensively in other systems but need further attention in forest ecosystems, while the fifth is especially relevant to trees and forests:

(1) Tree species mixtures outperform ecosystem functioning of monocultures, including the stocks and fluxes of energy and materials and their stability over time (Pacala and Kinzig, 2002). Mixtures have a higher probability of containing key species with a large impact on ecosystem functioning (i.e., identity and sampling/selection effect) and resource partitioning and positive interactions among heterospecific neighbouring trees further influence function (i.e., complementarity effect).

(2) The relative importance of species identity effects and complementarity effects is not consistent for forests at different positions along extensive environmental gradients. For instance, the strength of BDEF relationships is expected to increase with environmental stress (Paquette and Messier, 2011; Jucker and Coomes, 2012).

(3) Mixtures of tree species enhance associational resistance to pests and diseases via reduced host colonization and increased top down regulation by natural enemies. Likewise, their resistance and resilience towards abiotic environmental stressors and disturbances such as drought are increased compared with monocultures (the relative importance of the potential mechanisms are debated; Griffin et al., 2009).

(4) The number of species needed to support a single ecosystem function is lower than the number of species needed to support multiple functions simultaneously. The significance of forest tree species diversity for ecosystem functioning increases when multiple functions are considered.

(5) Mixtures create larger environmental heterogeneity at the scale of several individual trees compared with monospecific stands because trees are autogenic ecosystem engineers and individuals may modify their immediate environment (e.g., via root processes, litter input, light penetration and rain interception). Diversity effects are therefore not only expressed in terms of mean values, but also in terms of variability within forest stands.

A test of this set of hypotheses will provide the most comprehensive assessment of BDEF relationships in forests to date. Furthermore, interactions among coexisting species are at the heart of any diversity effect on ecosystem functioning. Testing the importance of species interactions therefore requires recognizing individuals, not only species, as a relevant level of observation. The size of trees makes them preferred study objects to explicitly explore the interactions between individuals. We are thus also interested in how tree performance and a tree's impact on its immediate environment are influenced by the diversity and structure of its neighbourhood.

Glossary

Complementarity effect The complementarity effect quantifies the combined effects of species interactions on mixture performance after accounting for changes in the relative abundance of species (selection effect – see below). Positive values result when mixtures do better than expected based on the relative abundance of species and their monoculture performances and are consistent with 'niche differentiation' in terms of resource partitioning, facilitation or diversity-dependent effects of natural enemies (although other explanations are possible – see Turnbull et al., 2013). Negative values are thought to equate to interference competition.

Comprehensiveness The spectrum of ecosystem functions and services quantified in a study (Nadrowski et al., 2010).

Dilution gradient A design with monoculture stands of only one species, combined with a species diversity gradient including this monoculture species in all mixtures (Nadrowski et al., 2010). Increasing species diversity is confounded with decreasing dominance of this species.

Species identity effect The expected effect of a component species on mixture performance as derived from its monoculture performance, i.e., purely additive effects as opposed to complementarity effects (Kirwan et al., 2009). A strong identity effect may cause the sampling/selection effect if this species becomes dominant at the expense of others, although particular species may be associated with positive complementarity too. Identity effects are due to the (binary) presence/absence of particular species across mixtures and are distinct from species composition effects which distinguish the effects between different species combinations (Hector et al., 2011).

Orthogonality of species diversity Orthogonality means the statistical independence of species diversity from other factors. Only orthogonal designs allow the effect of species diversity to be completely uncorrelated with that of other variables and covariates (e.g., species identity, environment, management) (Nadrowski et al., 2010). Unfortunately, it is often not possible to design studies where diversity is completely orthogonal from other variables due to unavoidable biological correlations, which can be seen as either 'hidden treatments' (Huston, 1997), or the mechanisms by which diversity has its effects.

Representativeness The degree to which the units of a study represent the larger population, such as randomly drawn forest stands. High representativeness allows for generalization of results and increases the relevance of the results for the study system as we find it in the real-world landscapes (Nadrowski et al., 2010).

Sampling/selection effect The sampling effect recognizes that more diverse communities are more likely to contain high-performing species, which can generate a positive BDEF relationship if they then dominate the community (Huston, 1997; Tilman et al., 1997). The selection effect is similar, but allows for negative as well as positive effects. Selection occurs when changes in the relative abundances of species covary with their monoculture performances (Loreau and Hector, 2001). Dominance of species with high (or low) monoculture performance generate positive (or negative) BDEF relationships.

3   Rationale: maximizing three fundamental design criteria

A research platform designed to answer the questions above should comprise a systematic network of research plots in existing forests that maximizes three fundamental criteria: comprehensiveness, representativeness and orthogonality (Nadrowski et al., 2010; see Glossary). First, comprehensiveness refers to the spectrum of ecosystem functions and services that can be quantified. Ecosystems inherently exhibit a multitude of functions, so increasing the relevance of BDEF studies requires a multifunctional perspective (Gamfeldt et al., 2008; Hillebrand and Matthiessen, 2009). Since functions are expressed at different spatial scales, the challenge is to establish plots with a layout matching the particular measurement requirements of a diverse set of functions, some of which need a certain number of trees while others need particular plot sizes. Low comprehensiveness is generally a limitation of existing observational (inventory) networks, which focus on a limited number of ecosystem processes such as productivity. The second criterion, representativeness, is achieved for plots established in characteristic forest types as we find them in landscapes, allowing a credible translation of results to the real world. Representativeness thus relates to the relevance of the results for managers and policy makers. This is an important design element and is the fundamental reason for complementing planned experiments with comparative studies in existing forest. Representativeness in our platform is maximized in three directions: (i) the forests are at least historically managed for forestry and are currently in the mid to late stem exclusion, understory reinitiation or old-growth development stage (i.e., excluding very young stands; Oliver and Larson 1996), (ii) six major European forest types (EEA, 2007) are studied and (iii) focus is on sets of target tree species that are regionally common and/or important from a silvicultural point of view. The third criterion, an orthogonal design, permits separating diversity effects from other variables that influence ecosystem functioning. The general idea is to include plots that primarily differ in (stochastic or management driven) tree species diversity while keeping the variation in confounding factors (topography, soil, disturbances) at a minimum. For example, a design where the more diverse species mixtures are, by chance, situated on the most productive soils would be problematic (Vilà et al., 2005). Along this species diversity gradient, the compositional variation among plots should include true species turnover, not species dilution. These three fundamental criteria were fully integrated in the design and implementation of the platform and will emerge in the subsequent sections. This rationale is also reflected in the term "exploratory" (coined for the German Biodiversity Exploratories; Fischer et al., 2010), which was explicitly chosen to illustrate the hybrid nature of the platform, combining strengths of true observatories (representativeness) and experiments (comprehensiveness and orthogonality).

4 Design and implementation of the diversity oriented Exploratory Platform

4.1 Six European forest types

The Exploratory Platform covers the major forest regions of Europe that extend from southern Mediterranean Europe ('Alto Tajo' in Spain and 'Colline Metallifere' in Italy) to the northern Boreal (subarctic) region 'North Karelia' in Finland (Fig. 1). The European Environment Agency classification, which is based on the distribution and classification of the natural vegetation and ecological site conditions, was used to select the different types (EEA, 2007; Table 1). The selected focal regions cover large soil and climatic gradients as they occur across Europe. The mean annual temperatures range from *ca.* 2 °C in North Karelia (Finland) at 62.6 °N, to *ca.* 13 °C in Colline Metallifere (Italy) at 43.2 °N (Table 1). Together, the regions host many important European broadleaved (e.g. *Fagus sylvatica*, *Quercus petraea/robur*, *Quercus ilex*, *Betula pendula/pubescens*) and coniferous tree species (e.g. *Pinus sylvestris*, *Picea abies*), with most tree species occurring in several exploratory regions. Certain less representative forest types such as mire, swamp and floodplain forests and alpine coniferous forests were excluded, but they may be included later on.

4.2 A nested platform design

An appropriate design for the Exploratory Platform that allows determining how species identity and species interactions influence ecosystem functioning should control for the variation in species composition along the tree species diversity gradient. If every species in the pool is not present in about the same number of plots at every species richness level – with a complete dilution design being the most extreme example – there is the risk that the effect of the presence of a particular species cannot be separated from the effect of species diversity. Similarly, testing for tree species diversity effects has to be done against different community compositions, i.e., including different species combinations at every species richness level, to allow distinguishing the effects of particular species combinations from species diversity (Schmid et al., 2002). To create a sound design, the basic setup of experiments in terms of the compositional variation between the plots was mimicked in the Exploratory Platform. This means we aimed to include the monocultures of all the tree species, replicate each species richness level with different mixtures and have every species represented at each richness level, preferably in similar frequencies (ORPHEE experiment: Castagneyrol et al., 2013; FORBIO experiment: Verheyen et al., 2013; BIOTREE experiment: Scherer-Lorenzen et al., 2007). Such a design avoids complete dilution, but comprises a series of dilution gradients that can be used, among others, to look at pathogen or herbivore effects on focal species. For the majority of species combinations we included two or more "realizations" (not strict replicates, because species abundances may differ), which will allow comparing the importance of species diversity with that of species composition for this subset of plots (e.g., model by Hector et al., 2011). The

strength of the interactions among particular species can be quantified for the full design, i.e., also including the species combinations with only one realization (e.g., model by Kirwan et al., 2009).

Table 1 Description of the six exploratory regions and region-specific design of the new research platform, including the pool of species used to create a gradient in species diversity. [1]Categories of the European Environment Agency (EEA, 2007). [2]MAT: mean annual temperature, MAP: mean annual precipitation. [3]Altitude in metres above sea level. [4]Stand developmental stages according to Oliver and Larson (1996).

	North Karelia	Białowieża	Hainich	Râșca	Colline Metallifere	Alto Tajo
(a) Site description						
Country	Finland	Poland	Germany	Romania	Italy	Spain
Latitude/longitude (degree)	62.6, 29.9	52.7, 23.9	51.1, 10.5	47.3, 26.0	43.2, 11.2	40.7, -1.9
Forest type[1]	Boreal	Hemiboreal, nemoral coniferous, mixed broadleaved-coniferous	Beech	Mountainous beech	Thermophilous deciduous	Mediterranean mixed
Ownership	State, large private forest companies	State	State	State	State	State
MAT, MAP[2]	2.1 °C, 700 mm	6.9 °C, 627 mm	6.8 °C, 775 mm	6.8 °C, 800 mm	13 °C, 850 mm	10.2 °C, 499 mm
Topography, altitude[3]	Flat, 80-200 m	Flat, 135-185 m	Mainly flat, 500-600 m	Medium-steep slopes, 600-1000 m	Medium-steep slopes, 260-525 m	Flat-medium slopes, 960-1400 m
Study area size (km × km)	150 × 150	30 × 40	15 × 10	5 × 5	50 × 50	50 × 50
Responsible site manager	L. Finér	B. Jaroszewicz	H. Bruelheide	O. Bouriaud	F. Bussotti	F. Valladares
Stand developmental stage:[4]						
(1) Mid/late stem exclusion	x	x		x	x	x
(2) Understory reinitiation	x	x	x	x		x
(3) Old growth			x			
Tree cohorts	Single	Multiple	Multiple	Single	Multiple	Multiple
(b) Platform design						
Species richness levels	3	5	4	4	5	4
Number of plots	28	43	38	28	36	36
Plots per richness level	11/14/3	6/11/13/11/2	6/14/14/4	8/10/8/2	9/10/9/7/1	11/18/4/3
Species pool:						
(1) Coniferous						
Abies alba				×		
Picea abies	×	×	×	×		
Pinus nigra						×
Pinus sylvestris	×	×				×
(2) Broadleaved						
Acer pseudoplatanus			×	×		
Betula pendula/pubescens	×	×				
Carpinus betulus		×				
Castanea sativa					×	
Fagus sylvatica			×	×		
Fraxinus excelsior			×			
Ostrya carpinifolia					×	
Quercus robur/petraea		×	×		×	
Quercus cerris					×	
Quercus faginea						×
Quercus ilex (evergreen)					×	×

A fixed pool of target species needed to be selected for each region to establish the species diversity gradient. With the design that was put forward, the maximum size of this species pool was constrained by the total sample size. Biological and logistic realities impose a natural limit to the number of plots that can be measured within each of the six regions and desired time window (e.g., phenological period versus the 3,200 km traveling distance between the Finnish and Spanish region). We proposed *ca.* 40 plots per region as a feasible sample size. Preliminary power analyses using simulated data (Gelman and Hill, 2007) were performed to determine the maximum number of species richness levels that still allows for the detection of a diversity effect with about 40 plots, i.e., a longer gradient would have less realizations at each richness level, reducing the statistical power. For instance, using the 'species identity model' from Kirwan et al. (2009) and realistic productivity differences of ± 20 % between five species (coefficient of variation of 15 %), the probability to detect the species identity effects was > 95 %. Designs with larger species pools would require even stronger species effects and not all of the other ecosystem properties and processes we will measure are expected to vary that much between species. Furthermore, similar analyses using 'species interactions models' showed that the diversity effects become small and indiscernible at very unequal relative abundances of the species in the community, i.e., at low evenness (Kirwan et al., 2007; Kirwan et al., 2009; see below).

A pool of five regionally important species co-occurring in similar forest habitats were selected in three regions (Białowieża, Hainich and Colline Metallifere; Table 1). The naturally restricted species pool in North Karelia limited the pool to three species, while in Alto Tajo and Râșca four species were included as we would have needed to move to quite different environmental conditions if a fifth species was to be included. Some species are represented in several regions (e.g., 3 × *Pinus sylvestris*, 3 × *Quercus robur/petraea*, 4 × *Picea abies*), so their identity and interaction effects can be compared between bioclimatic regions. Furthermore, each region except Colline Metallifere combines one or two evergreen coniferous species with broadleaved species. To allow for direct comparisons between the six regions differing in the size of their species pool, we opted for a nested platform design. The rationale is that the design of a region with a smaller species pool is a hierarchically nested subset of the more species rich regions, i.e., the design (not the species combinations) of the regions with large species pools can always be reduced to the low-species regions simply by leaving out certain plots. This was put into practice by searching for each possible species combination at each species richness level. For North Karelia this means for instance that the three monocultures, the three different two-species combinations and the full three-species mixture were included. The optimal number of realizations per combination was adjusted to have adequate sample sizes in the more species poor regions (e.g., four in North Karelia, two or three in the other regions) and balance sample sizes between species richness levels (Table 1; Appendix 1). While for experiments it is initially no problem to

establish and maintain all possible mixtures (Scherer-Lorenzen et al., 2007), the challenge of working in existing forests is to find rare combinations (e.g. *Castanea sativa/Ostrya carpinifolia* mixtures in the Italian study area) and, at the same time, to meet all other selection criteria. Ultimately, we selected 209 plots across Europe.

4.3 Selection criteria

Focus of this research platform is on forest stands that were at least historically managed for forestry and are currently either managed by low frequency thinning or minimal intervention. They are at least in the late to mid stem exclusion stage, the understory reinitiation or old-growth stage (Oliver and Larson, 1996). All sites are considered ancient forest, i.e., they have been continuously forested at least since the oldest available land-use maps. The implementation of the platform further required a rigorous set of criteria to evaluate the suitability of forest stands in the field. The central principle for selection was to have silvicultural management and/or pure random species assembly to be the key drivers of the diversity gradient. Covariation between an environmental factor (e.g., soil variation, topography) and species richness levels was strictly avoided, whereas covariation with species composition was avoided as much as possible. Because the spatial clustering of particular species richness levels and species combinations would increase the probability of covariation, no clustering was allowed.

There were three important criteria. First, we have put much emphasis on the evenness of the community composition, which expresses the distribution of the relative abundances of the tree species in a mixed stand. Few experiments have investigated the importance of evenness for ecosystem processes directly and the outcomes are still inconclusive (Mulder et al., 2004; Kirwan et al., 2007; Hillebrand et al., 2008). Zhang et al. (2012) showed in a meta-analysis that forests productivity increases with evenness, but the specific mechanisms still need to be tested. Evenness is considered an important design issue here because tree species that are present in low numbers or as small individuals probably do not influence the ecosystem processes much either directly or via interspecific interactions (Mulder et al., 2004). Mixtures strongly dominated by a single species rather exhibit a monoculture signature, which would be especially problematic if a species with high monoculture performance also tends to be the dominant in most mixtures (i.e., a high selection effect). Whereas experiments can manipulate relative species abundances quite easily, one or few tree species usually dominate forest stands. This is why we did not apply random or grid-based plot selection, but particularly looked for sites where the target species had similar abundances, setting a lower limit of 60 % of maximum evenness based on basal area (Fig. 2). The species also needed to be represented by at least two trees in the mixtures allowing measurements that use individuals as the level of observation to have replicates of 'species identity'.
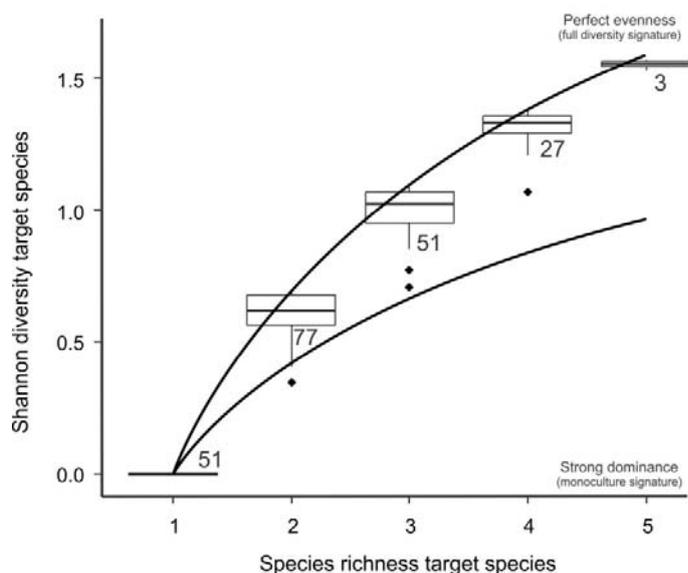
Fig. 2 Boxplots of the Shannon index of diversity for each of five species richness levels in the 209 selected plots. The number of plots in each species richness level is shown for each box. Here we used the total basal area of each species as a measure of abundance and omitted (low abundance) admixed species. The upper line shows the maximum theoretical Shannon diversity for each richness level attained at perfect evenness (i.e., basal areas of the species are equal); the lower line delineates 60 % of this maximum. At low Shannon values one of the species dominates and the mixture rather has a monoculture signature. Evenness was a central selection criterion during plot selection.

The second criterion related to the presence of non-target species. Admixture of such species was unavoidable, but we aimed to keep the summed basal area of the admixed species below 5 % of the total basal area (with a maximum of *ca.* 10 %). Across the regions, only 18 out of the 209 plots slightly exceeded the 10 % admixture criterion and 43 exceeded the 5 % criterion.

The third criterion was to ensure that the effect of tree species diversity was not confounded with site-, soil- and stand-related factors. These factors were operationalized in a list of basic descriptors, which was used as a checklist during field exploration (Appendix 2). Regarding the stand-related criteria, shrubs lower than 1.3 m were considered to be response variables, i.e., not contributing to the desired mixtures, whereas larger individuals followed the same rules of admixed species outlined above. The age distribution and forest structure were allowed to covary to some degree with the species diversity gradient, e.g., more uneven-aged and multi-layered sites at the higher species richness levels. We consider this covariation as an integral part of the diversity effect. Yet, along the diversity gradient, the tree populations had to be in the same developmental stage.

4.4 Stepwise selection and establishment of research plots

An important issue in the implementation of a research platform is plot size. Relatively small plots (e.g., < 2000 m²) are criticized for containing few trees of each species at high levels of diversity, may represent atypical habitat islands in the larger forest matrix creating edge effects and may poorly represent particular larger-scale ecosystem properties such as resilience after disturbance (Leuschner et al., 2009). Large plots are on the other hand more likely to be environmentally heterogeneous and to contain the dominant species in every plot. We performed a preliminary test with a circular plot shifted at 10 m intervals over the tree maps of two full forest inventories (Hainich National Park, Germany, 28 ha; Liedekerke, Belgium, 9 ha).

The tested plot sizes were 500 m², 1000 m² and 2000 m², corresponding to plot radii of 12.6 m, 17.8 m and 25.2 m. At a plot size of 2000 m², it was impossible to avoid a complete dilution design, with the dominant species present in every potential site (Hainich: *Fagus sylvatica*; Liedekerke: *Betula pendula*). We decided to use a 900 m² core plot size (30 m × 30 m) for the Exploratory Platform, i.e., small enough to avoid a complete dilution design and at least the minimum size for several measurements to be ecologically meaningful (e.g., herbivory, litter input, water quality). To account for potential edge effects, a 10 m wide buffer zone around each plot was requested during plot selection (selected zone 50 × 50 m). This buffer zone had to be similar to the core plot in terms of the forest type, structure and composition, but the evenness and admixture criteria were less strict.

The selection of plots basically occurred in two steps, each of which also included part of the plot characterization. Once the design and selection criteria were fixed, the species pool was selected for each exploratory region and the focal regions were screened to delineate subareas with a good probability to find those species in all the desired combinations (Fig. 3). This screening was done using regionally available data such as forest management plans and soil maps, complemented with exploratory field visits. Then, local teams searched several potentially suitable sites within these subareas for each of the desired species combinations during intensive field campaigns. The idea was to initially have more sites than actually needed in the final design ("oversample") and select the final set of sites randomly from this larger pool. This approach introduced an important randomization step into the design. In some cases (e.g., rare species combinations) only one site was ultimately available for selection, of course. The suitability of each site was checked in the field using a standardized list of quick descriptors of site conditions, soil properties and forest stand structure that could be estimated without establishing plots (Appendix 2). Actual dendrometric measurements were performed where needed to verify the suitability of the mixture in terms of the evenness or the degree of admixture of non-target species.

After this first selection phase, we analysed the variation among the selected sites based on the three groups of descriptors (site, soil, stand) to identify undesired covariation with species richness levels and to check for clearly deviating sites (e.g., one plot on calcareous bedrock when all others were on sandstone) (Fig. 4). The final set of sites was selected after removing potentially problematic ones. In a second field campaign, 900 m² plots (30 m × 30 m), subdivided into nine 100 m² subplots, were established in the selected sites using wooden poles (Fig. 5a). The position (± 0.5 m) and diameter at breast height (dbh, ± 0.5 cm) of each tree > 7.5 cm dbh was measured in these plots. Based on these tree position data, plot-specific plans were drawn allocating each measurement to particular locations within the plot, including strict no-go areas and preferred walking tracks (Fig. 5b).
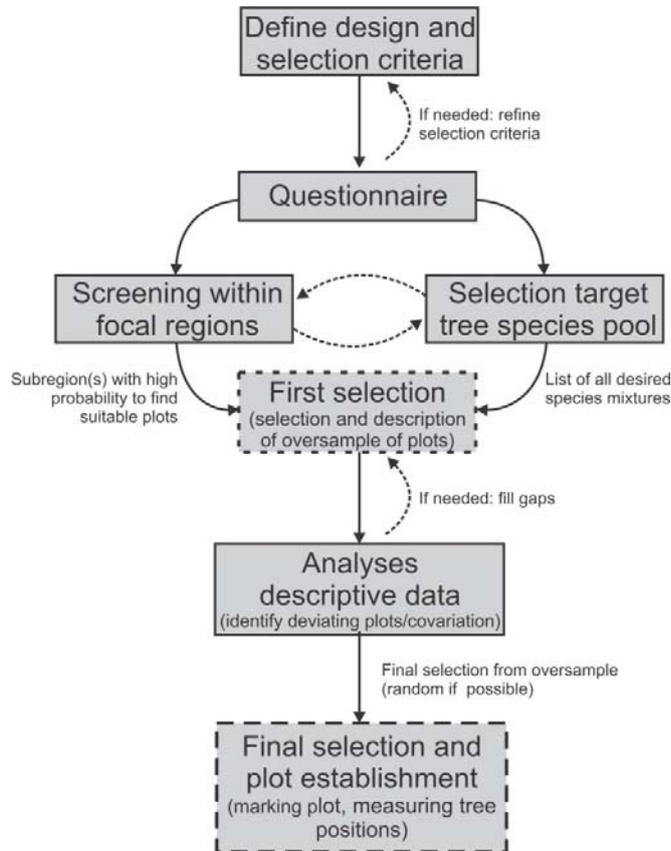
Fig. 3 Schematic overview of the plot selection procedure. Dashed lines show iterative steps and the two dashed boxes indicate plot characterization phases. After designing the research platform and defining the plot selection criteria, a questionnaire was sent to every local manager to verify potential regional incompatibilities and make an inventory of available data for plot selection. The target tree species pool to create a diversity gradient was defined and the six focal regions were explored to find potentially suitable sites (e.g. based on land-use maps, forest management plans, field visits). In the first selection phase, we searched for many more sites in the field than were actually needed and characterized those sites with quick descriptors (e.g., stand structural features, soil properties; Appendix 2). After the analyses of the site descriptors and filtering out the deviating sites, we performed a final randomized subsetting from the larger pool of sites. Plots were further characterized during plot establishment.
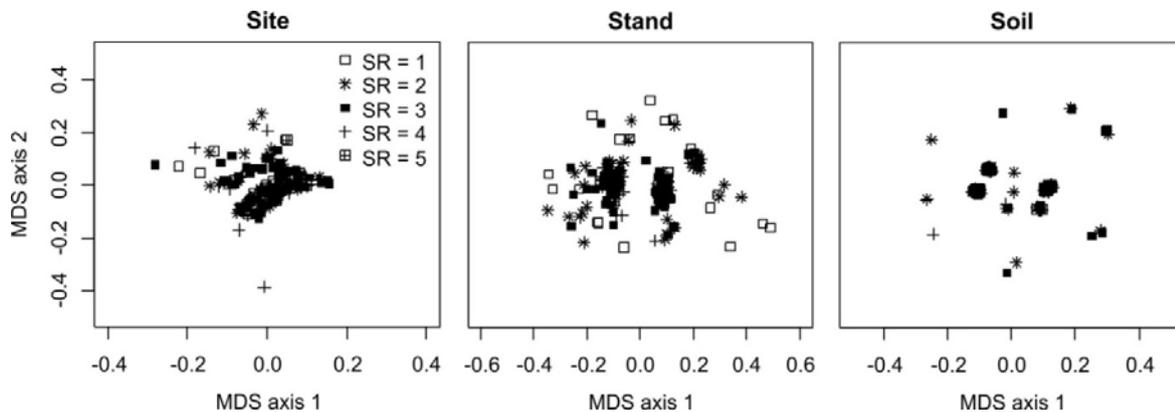

Fig. 4 Result of a non-metric multidimensional scaling ordination on soil, stand and site descriptors of 164 preselected sites in Białowieża (Poland) (descriptors in Appendix 2). We used Gower dissimilarities with the Podani (1999) extension to ordinal variables. Permutational analysis of variance (PERMANOVA; Anderson 2001) showed that soil and site conditions did not significantly differ between the species richness levels (F = 0.36, P = 0.91 and F = 1.53, P = 0.20). Stand descriptors differed between species richness levels (F = 2.85, P = 0.003), mainly due to differences in forest structure and current management between monocultures and two species mixtures on the one hand (generally single/double layered canopies, no management) and the higher diversity levels on the other hand (generally multi layered, low frequency thinning).
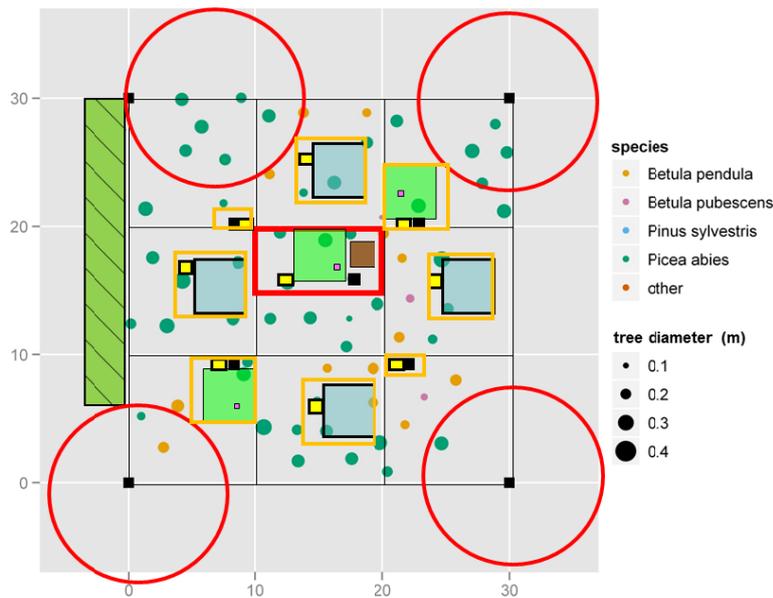
16

(a)

(b)

Fig. 5 (a) Photograph of an established plot in North Karelia, Finland (two-species mixture with Picea abies and Betula pendula/pubescens). The photo was taken from a corner post and shows a plot border and a plot diagonal; the wooden poles are at every 10 m (Photograph by Timo Domisch). (b) Example of a detailed plot scheme for this 30 m × 30 m plot. Dots represent trees, with dot sizes proportional to the tree diameter at breast height. Shapes with different colors indicate the various locations for sampling and setting up experiments. The large red circles are for instance the areas for dead wood sampling and the small yellow squares indicate the sites for root biomass and soil sampling. The green shaded area left of the plot is used to plant small trees with different provenances.

5 Outlook for scientists and managers

With this Exploratory Platform we move away from earlier research on the effects of environmental factors on forest biodiversity to a new research paradigm focussing on the effects of biodiversity on ecosystem functioning. Together with the experiments and inventory data, the Exploratory Platform provides an important new European research infrastructure for long-

term monitoring of the effects of tree species diversity on forest ecosystem functions and the ecosystem services provided by forests. With its diversity-oriented design, the Exploratory Platform definitely complements and provides added value to existing networks that were based on a systematic sampling grid such as national and regional forest inventories or the pan-European network of forest monitoring plots (ICP Forests Level I and II; www.icp-forests.org), originally designed to monitor air pollution effects on forest vitality. We focussed particularly on a design that allows separating tree species identity from complementarity effects, improving on more common dilution schemes. With mixtures up to five species, the species diversity gradient is longer than common two-species mixture trials, and the special attention to community evenness ensures that no single species dominates the mixtures.

The platform uses the same methodological approach in contrasting forest types along an extensive environmental gradient and offers a common set of suitable research plots to groups of researchers from very different disciplines (cf. Fischer et al., 2010). Various measurements characterizing a wide array of ecosystem properties, processes and functions will be performed and, in turn, related to the provisioning of ecosystem services. Measurements related to supporting and provisioning services include the quantification of nutrient stocks and cycling, net above and belowground productivity, photosynthesis parameters and the water balance. Measurements related to regulating services include the quantification of the load of insect pests, mammal herbivory, fungal pathogens and invasive plants. Comprehensive syntheses across many response variables and modelling efforts to forecast BDEF relationships under changing environmental conditions such as climate change heavily rely on such data sets collected on common plots. It is therefore the platform's philosophy to make sure that all measurements are performed on all plots. This philosophy calls for indicators and proxies that can be measured quickly and at low cost, instead of relying on the monitoring of only few processes in great detail, which is the core activity of other networks (e.g., CarboEUROPE, ICP Forests Level II). Only few labour intensive and expensive measurements (e.g., water quality, sapflow) are carried out on a subset of Highly Instrumented Plots (HIPs). The lifetime of the platform is intended to be stretched far beyond the duration of the project funding (2014), e.g., by continuing and improving the research in close collaboration with the further development of the LTER Europe network (www.lter-europe.net). In this way, it can be continued as a long-term network open to other scientists ready to work within the same philosophy.

The knowledge generated within the Exploratory Platform will contribute to new views on sustainable forest management and nature conservation. This input is coming at a critical time, when strategies to adapt European forests to global changes, such as climate change (Kolström et al., 2011) and the transition towards more biobased economies (Schulze et al.,2012), are required. It is key that these strategies are based on an in-depth understanding of the relationships between forest structure, composition and function. Since most of the economically

important European timber tree species are represented in the Exploratory Platform, it has great potential for translating the forest diversity-functioning relationships into guidelines that are of real interest for forest management. These guidelines will, among others, list which forest types, tree species or tree species combinations will lead to the optimization of multiple ecosystem services or create important trade-offs between services (Gamfeldt et al., 2013) . Finally, knowledge on the resistance of different forest types to perturbations as well as their adaptive capacity will be generated, which is crucial in an era of global change.

6   Acknowledgements

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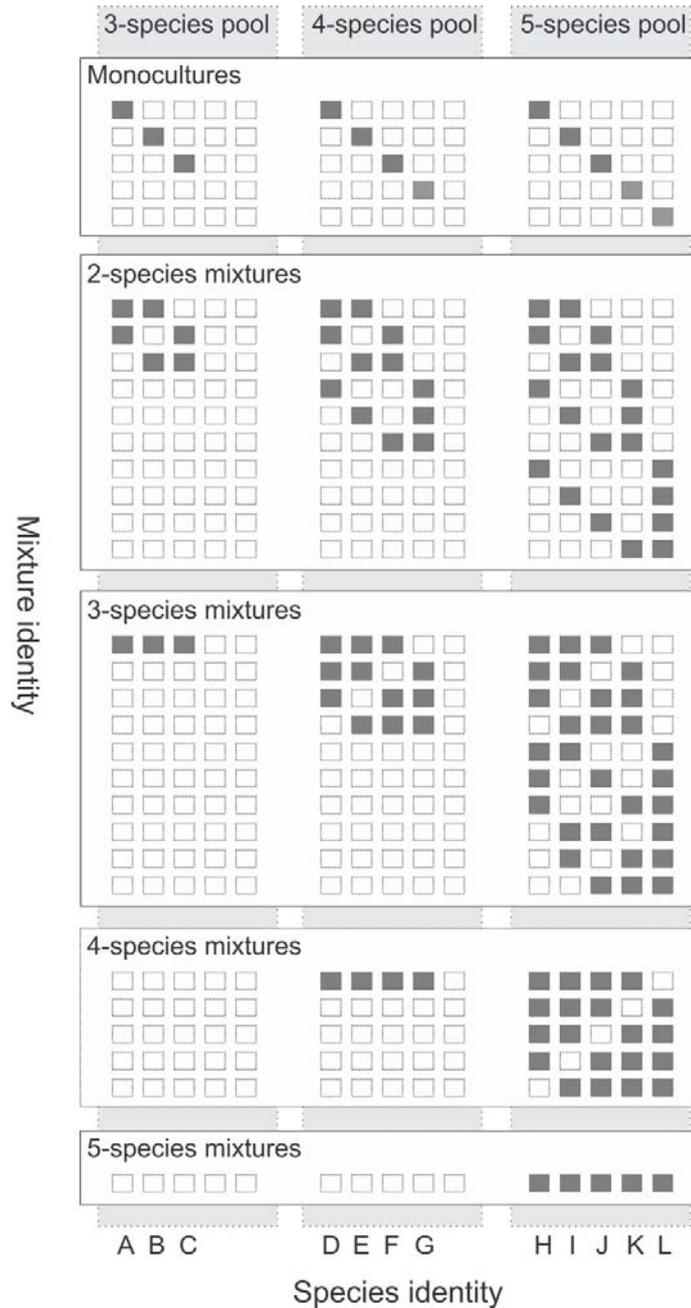
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8   Appendices

Appendix 1 Scheme summarizing the Exploratory Platform design, representing study regions with a species pool of three, four or five species as grey pillars. Columns represent different species A:E; a species may be present (filled square) or absent (open square). Rows represent different species combinations (mixture identities). The three-species pool design is fully nested within the four species pool design, although with other species, and both are fully nested within the five species design. Every combination is realized 2 – 4 times in the design, except the two- and three-species mixtures in the regions with a pool of five species (it already required 10 plots to represent all combinations for these levels).

Appendix 2 Basic plot descriptors that were estimated in the field during the first plot characterization phase. The descriptors are classified in three groups: site-, soil- and stand-related variables. Type: C continuous; O ordinal; N nominal; B binary variable. For each of the study regions, symbols show whether the variable was estimated and differed between plots (×), was estimated but equal for every plot (=) or not estimated (-). Only the estimated variables that differed between the plots were used in the multivariate analyses.

Descriptor	Type	North Karelia	Białowieża	Hainich	Râșca	Colline Metallifere	Alto Tajo
(a) Site descriptors							
Ownership	N	×	=	=	=	×	=
Accessibility	C	×	×	=	×	×	×
Altitude	C	×	×	×	×	×	×
Slope	O	×	=	×	×	×	×
(b) Soil descriptors							
Rocks/boulders cover	C	×	×	×	×	×	×
Bedrock type	N	×	=	=	×	×	=
Calcareous bedrock	B	=	=	=	=	×	=
Sand	O	×	×	=	×	×	=
Silt	O	=	×	=	×	×	×
Clay	O	=	×	=	×	×	×
Soil drainage	O	=	=	=	×	×	×
Soil depth	C	×	=	=	×	×	×
Soil type	N	×	×	-	=	=	=
(c) Stand descriptors							
Stand origin	N	=	×	=	×	×	-
Current management	N	=	×	=	×	×	=
Forest structure	N	=	×	=	×	×	×
Age distribution	N	=	=	=	×	=	×
Age canopy trees	C	×	×	-	-	×	-
Number of canopy trees	C	×	×	×	×	×	×
Minimum DBH[1]	C	×	×	×	×	×	×
Maximum DBH	C		×	×	×	×	×
Canopy cover	C	×	×	×	×	×	×
Shrub layer cover	C	×	×	×	-	×	×

[1]Mean DBH for North Karelia