

Assessment of the functional role of tree diversity: the multi-site FORBIO experiment

Kris Verheyen^{1,*}, Kris Ceunen¹, Evy Ampoorter¹, Lander Baeten^{1,2}, Bernard Bosman³,
Etienne Branquart⁴, Monique Carnol³, Hans De Wandeler⁵, Jean-Claude Grégoire⁶,
Pierre Lhoir⁷, Bart Muys⁵, Nuri Nurlaila Setiawan¹, Margot Vanhellefont¹ & Quentin Ponette⁷

¹Ghent University, Department of Forest and Water Management, Forest & Nature Lab, Geraardsbergsesteenweg 267, BE - 9090 Melle-Gontrode, Belgium

²Ghent University, Department of Biology, Terrestrial Ecology Unit, K. L. Ledeganckstraat 35, BE - 9000 Ghent, Belgium

³University of Liège, Department of Biology, Ecology, Evolution, Laboratory of Plant and Microbial Ecology, Institute of Botany Bât. 22, Boulevard du Rectorat 27, BE - 4000 Liège, Belgium

⁴Belgian Biodiversity Platform, Avenue de la Faculté d'Agronomie 22, BE - 5030 Gembloux, Belgium

⁵KU Leuven, Division of Forest, Nature and Landscape, Celestijnenlaan 200 E, Box 2411, BE - 3001 Heverlee, Belgium

⁶Université Libre de Bruxelles, Faculté des Sciences, Campus du Solbosch, CP160/12, avenue F.D. Roosevelt 50, BE - 1050 Bruxelles, Belgium

⁷Université Catholique de Louvain, Environmental Sciences, Earth and Life Institute, Croix du Sud 2 - Box L7.05.09, BE - 1348 Louvain-la-Neuve, Belgium

*Author for correspondence: kris.verheyen@ugent.be

Context – During the last two decades, functional biodiversity research has provided strong support for the hypothesis that more biodiverse ecosystems have the potential to deliver more and better services. However, most empirical support for this hypothesis comes from simple structured communities that are relatively easy to manipulate. The impact of forest biodiversity on forest ecosystem functioning has been far less studied.

Experiment design – In this paper, we present the recently established, large-scale FORBIO experiment (FORest BIODiversity and Ecosystem Functioning), specifically designed to test the effects of tree species diversity on forest ecosystem functioning. FORBIO's design matches with that of the few other tree diversity experiments worldwide, but at the same time, the FORBIO experiment is unique as it consists of a similar experimental set-up at three sites in Belgium (Zedelgem, Hechtel-Eksel and Gedinne) with contrasting edaphic and climatological characteristics. This design will help to provide answers to one of the most interesting unresolved questions in functional biodiversity research, notably whether the effects of complementarity on ecosystem functioning decrease in less stressful and more productive environments. At each site, FORBIO consists of 41 to 44 plots (127 plots in total) planted with monocultures and mixtures up to four species, selected from a pool of five site-adapted, functionally different tree species. When allocating the treatments to the plots, we maximally avoided any possible covariation between environmental factors. Monitoring of ecosystem functioning already started at the Zedelgem and Gedinne sites and will start soon in Hechtel-Eksel. Multiple processes are being measured and as the trees grow older, we plan to add even more processes.

Expected results – Not only basic science, but also forest management will benefit from the results coming from the FORBIO experiment, as FORBIO is, for instance, also a test case for uncommon, not well-known tree species mixtures. To conclude, FORBIO is an important ecosystem experiment that has the potential to deliver badly needed insights into the multiple relationships between biodiversity and ecosystem functioning, which will be valuable for both science and practice.

Key words – forest ecology, forest management, biodiversity-ecosystem functioning research, tree diversity, mixed forest, field experiment.

INTRODUCTION

Forests are among the most complex ecosystems on earth. They harbour a large part of the terrestrial biodiversity and provide human society with a large range of services, from the supply of wood and energy, over the regulation of climate and water flows to the provision of a recreation space and aesthetic and spiritual values (Millennium Ecosystem Assessment 2005). However, human society strongly altered the extent and the structure and composition of forests in large parts of the world. It is estimated that the global extent of forest cover decreased by 50% during the Holocene, and today, the global forest cover is still decreasing at a rate of c. 5.2 million ha per year (FAO 2010), mainly in the tropics and subtropics. Not only have forests disappeared, but many of the forests still existing today have a strongly simplified structure and composition. It is estimated that only 36% of the present forest area consists of natural forests; the other 64% has been converted to semi-natural or plantation forests, often composed of a limited number of tree species (FAO 2010).

The depletion of natural resources at an ever increasing rate (e.g. www.wri.org), combined with an unprecedented impact of the human enterprise on the globe (Rockström et al. 2009), will reinforce the need for ecosystems that can provide a wide range of services and that exhibit a high resistance and resilience to disturbances. During the last two decades, functional biodiversity research has provided strong support for the hypothesis that more biodiverse systems have the potential to deliver more and better services (Biodiversity and Ecosystem Functioning hypothesis or BEF hypothesis; for reviews see: Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2011, Hooper et al. 2012). The positive impact of biodiversity on ecosystem functioning has been attributed to two types of mechanisms, which are not mutually exclusive. In the ‘selection effect’, dominance by species with particular traits affects ecosystem processes, whereas in the ‘complementarity effect’, resource partitioning or positive interactions lead to increased total resource use and better functioning (Loreau & Hector 2001). Recently, the accumulation of soil pathogens, and the negative feedbacks associated with them, has been shown to represent an alternative mechanism why monocultures and low-diversity mixtures exhibit a decreased functionality (e.g. Maron et al. 2011, Schnitzer et al. 2011). However, most empirical support for the BEF hypothesis comes from communities that are relatively easy to manipulate, such as algal and grassland communities. The impact of biodiversity on forest functioning has been far less studied, despite the global importance of forests.

Until now, most empirical tests of the BEF hypothesis in forests have looked at the relationship between tree species diversity (mostly richness) and ecosystem functioning using one of three complementary approaches. These approaches differ in orthogonality, comprehensiveness and representativity (cf. Nadrowski et al. 2010). The first approach, i.e. data mining of large forest inventory databases (e.g. Vilà et al. 2007), has a high degree of representativity. In general, however, data for the quantification of only a few functions are available, and confounding factors may mask the BEF signal

in the data, i.e. the comprehensiveness and orthogonality of the approach are limited. Others have used a network of observational plots in existing forests that vary in tree species diversity (e.g. Leuschner et al. 2009). These observational plots should be carefully selected to optimize orthogonality, which generally reduces the representativity. The logistical constraints of working in mature tree stands can limit the comprehensiveness of this approach. The third approach is the experimental creation of a tree diversity gradient, the so-called synthetic community approach. In grasslands, this type of experiments has proven its merits and significantly deepened our knowledge on BEF relationships (e.g. BIO-DEPTH: Hector et al. 1999; Cedar Creek experiment: Tilman et al. 2001; Jena-experiment: Scherber et al. 2010). While this approach is the least representative of the three, the establishment of synthetic communities allows maximizing the orthogonality and comprehensiveness of the design. Approximately a decade ago, the first tree diversity experiments have been set up in Finland (1999, e.g. Vehviläinen & Koricheva 2006) and Germany (2003, Scherer-Lorenzen et al. 2007). Today, a global network of tree diversity experiments (TreeDivNet, www.treedivnet.ugent.be) is developing.

In this paper, we present the recently established FORBIO experiment (FORest BIODiversity and Ecosystem Functioning, <http://forbio.biodiversity.be/>). FORBIO is a tree diversity experiment of which the basic design features are similar to the other experiments in TreeDivNet. At the same time, the FORBIO experiment is unique as it consists of a similar experimental set-up at three sites with contrasting edaphic and climatological characteristics. Paquette & Messier (2011) stated that one of the most interesting unresolved questions in BEF research is whether the effects of complementarity on ecosystem functioning decrease in favour of competitive exclusions in less stressful and more productive environments (cf. stress-gradient hypothesis of Bertness & Callaway 1994). Using productivity data from a large forest survey database in Québec (Canada), these authors indeed demonstrated that complementarity may be less important in temperate forests growing in relatively stable and productive environments where competitive exclusion is the most probable outcome of species interactions, whereas in the more stressful environments of boreal forests, beneficial interactions between species are assumed to be more important. Vilà et al. (2007) came to the same conclusion, using forest inventory data from Catalonia (NE Spain). They found that the high productivity of deciduous forests is not affected by the addition of new functional groups to the community, but that the low productivity of sclerophyllous forests increases with the presence of other functional groups. Running a simulation model calibrated on eleven forest sites in Switzerland along a broad environmental gradient, Morin et al. (2011) were able to further refine the hypothesis. These authors demonstrated that the slope of the richness-complementarity relationship was steeper at the less fertile sites, but that the absolute complementarity values, calculated following the additive partitioning approach proposed by Loreau & Hector (2001), are larger at the most fertile sites. Based on a literature review and experiments with microalgae, Steudel et al. (2012) also came to the conclusion that biodiversity enhances ecosystem functioning under stressful environmental

conditions in relative, but not in absolute, terms. However, empirical support for the changing BEF relationship along environmental stress gradients in real world situations is largely confined to inventory studies with a low orthogonality and comprehensiveness, i.e. covarying factors are present and the studies only focus on aspects of productivity. Hence, there is a clear need to build variation in environmental conditions directly into the design of field experiments (cf. Gessner et al. 2010) and to measure multiple ecosystem processes.

The specific hypotheses we would like to test with the FORBIO experiment are:

- (1) Ecosystem functioning, including stocks and fluxes of energy and materials and their stability over time (cf. Pacala & Kinzig 2002), increases with increasing diversity, but the strength of the BEF relationship depends on the function under consideration;
- (2) More species are needed when multiple functions need to be optimized simultaneously;
- (3) Across all functions, the complementarity effect is more important than the selection effect and the relative importance of the complementarity effect is most pronounced in more stressful environments.

In this paper, we will first introduce the characteristics of the three FORBIO sites. Then we will explain the experimental design, the planting and the management of the sites. We end with an outlook on the ongoing and planned research.

FORBIO EXPERIMENT

Site characteristics

The three FORBIO sites are distributed across Belgium (fig. 1) and have contrasting site conditions (table 1). The Zedelgem site is located in the Cuesta ecoregion (sensu Sevenant et al. 2002), close to the North Sea. The site has a very mild, temperate climate. The soil parent material varies from sand to loamy sand, and the soil moisture regime varies between dry (minimum water table depth 90–120 cm) to moderately wet (minimum water table depth 40–60 cm). Until 2008, the site was in agricultural use and both arable crops (potatoes, maize) and grass were grown. The Hechtel-Eksel site is located in the northeastern part of Belgium, in the Campine ecoregion. The climate is somewhat less mild with higher temperatures in summer and lower temperatures in winter. The soil consists of coarse sand and is dry (minimum water table depth 90–120 cm). The site was converted

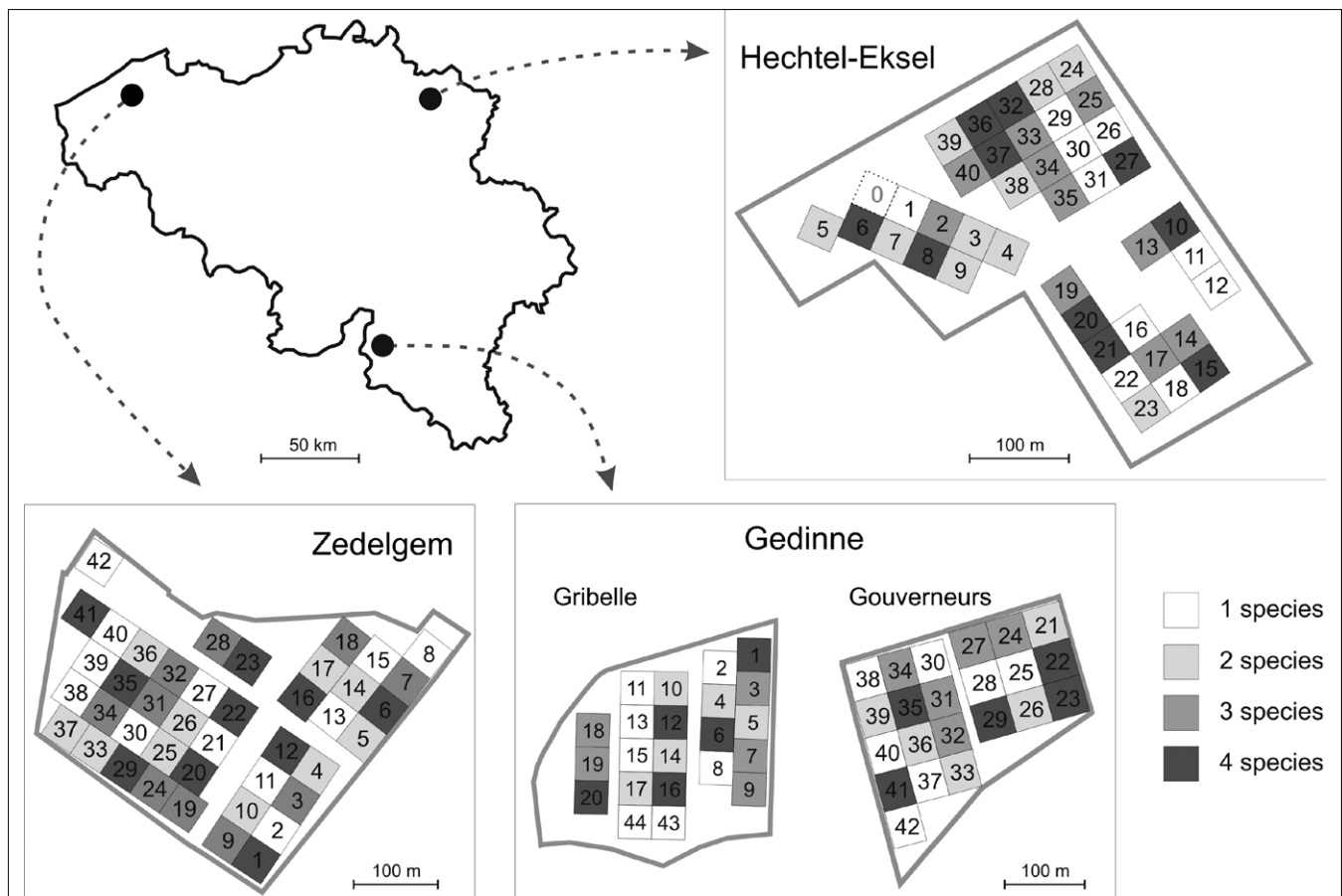


Figure 1 – Map of Belgium with the three FORBIO sites and the experimental set up at each site. The tree species diversity per plot ranges from one species (white) to four species (dark grey). In Zedelgem, three provenances of oak were used in the series of plots with numbers 21–42. In Gedinne, three provenances of beech were used in the plot series 21–44. In the plots numbered 1–20 at these sites, only one of the provenances of oak (Zedelgem) or beech (Gedinne) was used. In Hechtel-Eksel, plot 0 was not planted and was left for spontaneous succession.

Table 1 – General characteristics of the three FORBIO sites.

*P: mean annual precipitation, MAT: mean annual temperature, Tc: mean temperature of the coldest month, Tw: mean temperature of the warmest month. Data from nearby meteo stations (www.meteo.be): Koksijde (30 km to Zedelgem), Kleine Brogel (12 km to Hechtel-Eksel), Saint-Hubert (30 km to Gedinne).

see Van Ranst & Sys (2000) and Bock (2007) for Belgian Soil Map codes and IUSS Working Group WRB (2006) for WRB codes.

Site (town)	Zedelgem	Hechtel-Eksel	Gedinne
Local name	Vloethemveld	Pijnven	Gribelle (Gr) & Gouverneurs (Go)
Coordinates	51°9'N 3°7'E	51°10'N 5°19'E	49°60'N 4°59'E (Gr) 49°59'N 4°59'E (Go)
Climate*	P: 687 mm MAT: 9.4°C Tc (Jan): 2.9°C Tw (Aug): 16.3°C	P: 799 mm MAT: 9.0°C Tc (Jan): 1.4°C Tw (Jul): 16.7°C	P: 1021 mm MAT: 6.9 °C Tc (Jan): -1.0 °C Tw (Jul): 14.4°C
Size	c. 9.5 ha	c. 8 ha	2 × c. 4.5 ha
Altitude (m a.s.l.)	11–16	55–56	367–376 (Gr) 421–426 (Go)
Soil type (Belgian Soil Map code/WRB code)#	Relatively dry sandy soil (Zbh/Podzol) to moderately wet loamy sand soil (SdP/Gleysol)	Dry sandy soil with gravel substrate (tZbg/Podzol)	Moderately dry stony loam soils (Gbb/Cambisol; both Gr & Go)
Former land use	Agriculture (mainly arable)	Heathland converted to pine plantation in 1908	Forest: spruce plantation clearcut in 2005 and installed c. 1920, broadleaved forest before
Owner	Flemish Region	Flemish Region	Town of Gedinne
Local manager	Agentschap Natuur en Bos (ANB)	Agentschap Natuur en Bos (ANB)	Division de la Nature et des Forêts (DNF)

from heathland to pine plantation in 1908. At the time of the clearcut in late 2010, stands were composed of *Pinus sylvestris* and *Pinus nigra* subsp. *laricio* with an age varying between 72 and 96 years. The Gedinne site is located in the Ardennes ecoregion and consists of two subsites c. 2 km apart. The climate is colder and harsher compared to the other two sites (table 1). The relatively shallow (c. 60 cm) soil has developed in a stony solifluxion sheet in which the weathering products of the bedrock (sandstone and schist) were mixed with periglacial loess. In contrast to the Zedelgem and Hechtel-Eksel sites, the Gedinne site has had a forest land use at least since the oldest available map (de Ferraris 1771–1778), and it is not unlikely that, even in the more distant past, it has never been reclaimed for agriculture. Hence, it is considered to be a so-called ancient forest site (cf. Hermy et al. 1999). The original broadleaved forest has been converted into a spruce plantation in the 1920s (or earlier; exact date not known). This c. 85 year old plantation has been clearcut in 2005.

Prior to the establishment of the experiment, the intra-site variability in soil characteristics was assessed to avoid any coincidental covariation between diversity levels or tree species presence and soil characteristics. Hence, a large number of soil samples were taken at the three sites and analysed for pH, total phosphorus (P_{tot}), total nitrogen (N) and total carbon (C) content (electronic appendix 1). The values at the Zedelgem site still reflect the agricultural history. The pH and P_{tot} were relatively high, whereas the % C, % N and C/N were relatively low. Fairly strong spatial patterns occurred: % C and pH were higher on the more elevated northeastern part of the site, whereas P_{tot} was clearly lower at the southeastern

part of the site, which is probably related to the fact that this part of the site has known a longer use as (less intensively fertilized) grassland. The pH and P_{tot} values at the Gedinne site were clearly lower than at the Zedelgem site, whereas % C, % N and C/N were higher. The Gribelle subsite at c. 373 m altitude had a significantly higher pH and P_{tot} and a lower C/N compared to the Gouverneurs subsite (one-way ANOVA, $p < 0.05$), located at c. 424 m altitude. At the Hechtel-Eksel site, the average C/N value is a factor two to three higher and the average P_{tot} value is a factor three to ten lower compared to the Gedinne and Zedelgem site, respectively.

Summarizing, it can be assumed that the overall growing conditions are most favourable at the Zedelgem site because of its favourable climatic conditions and the higher availability of water and, especially, soil nutrients. Water availability and soil fertility are most limiting at the Hechtel-Eksel site, whereas the climatic conditions are less favourable at the Gedinne site.

Experimental design

The experimental design of the three sites is similar and takes into account the numerous considerations for setting up a proper diversity experiment (cf. Scherer-Lorenzen et al. 2005). At each site, a pool of five tree species was used (table 2). The species were selected based on the following criteria (ranked in decreasing order of importance): (1) the species must be well-adapted to the local site conditions; (2) the species must be (economically) important for forest management in Belgium and (3) the trait diversity in the site-specific species pool should be as large as possible. The lat-

Table 2 – Overview of the functional traits of the tree species used in the FORBIO experiment.

Data compiled by Scherer-Lorenzen et al. 2007, except Litter C/N, which is taken from Hobbie et al. 2006. Underlined species are non-native in Belgium.

* Ze: Zedelgem; Ge: Gedinne; H-E: Hechtel-Eksel. # Mono: Monopodial growth; Symp – S: Sympodial growth with a narrow crown; Symp – W: Sympodial growth with a wide crown

Species	Site			Functional trait						
	Ze*	Ge	H-E	Leaf phenology	Light requirement young trees	Crown form #	Root system type	Max. tree height	Maximum mean annual increment (m ³ ha ⁻¹ yr ⁻¹)	Litter C/N
<i>Acer pseudoplatanus</i>		X		Deciduous	Intermediate	Symp – W	Heart	High	Intermediate	49.1
<i>Betula pendula</i>	X		X	Deciduous	Very high	Symp – S	Surface	Intermediate	Low	38.5
<i>Fagus sylvatica</i>	X	X		Deciduous	Low	Symp – W	Heart	Very high	High	55.9
<u><i>Larix x eurolepis</i> (e)</u>		X (e)		Deciduous	Very high	Mono	Heart	Very high	Intermediate	64.9
<u><i>Larix kaempferi</i> (k)</u>										
<i>Pinus sylvestris</i>	X		X	Evergreen	Very high	Mono	Tap	High	Intermediate	81.9
<u><i>Pseudotsuga menziesii</i></u>		X	X	Evergreen	Intermediate	Mono	Heart	Very high	Very high	72.0
<i>Quercus robur</i> (r)	X (r)	X (p)	X (p)	Deciduous	High	Symp – W	Tap	High	Intermediate	37.7
<i>Quercus petraea</i> (p)										
<i>Tilia cordata</i>	X			Deciduous	Low	Symp – W	Heart	High	Intermediate	37.0

ter criterion was added since species traits link community composition with ecosystem functioning (cf. Suding et al. 2008), and since it can be assumed that communities exhibiting a higher functional trait diversity will display a higher degree of ecosystem multifunctionality (Hector & Bagchi 2007, Gamfeldt et al. 2008) and will deliver a larger range of ecosystem services. It should be noted that we have selected two non-native tree species at both the Gedinne and Hechtel-Eksel sites (table 2). Recently, it has been shown that non-native species may show a different behaviour than native species in biodiversity experiments, among others due to the less pronounced plant-soil feedbacks (Wilsey et al. 2009).

The selected species were used to create a diversity gradient from monocultures up to four-species mixtures. In total, twenty treatments were established, including five monocultures, all five possible four-species combinations and a random selection of five two- and five three-species combinations. Two replicates of each treatment was realized, which resulted in $20 \times 2 = 40$ plots per site. This will allow testing the significance of particular species combinations and estimating the variation within treatment combinations. When selecting the two- and three-species combinations, it was assured that species were equally represented across all plots, resulting in an overall frequency of each species of 20/40 plots, and an overall frequency of the joint-presence of two species of 10/40 plots. This design will allow making a proper distinction between selection and complementarity effects (cf. Hector et al. 2009), exploring the nature of the interactions within and among particular species along the diversity gradient and estimating the variation within treatment combinations.

The total number of plots in the experiment is, however, 127 and not 120. At the Hechtel-Eksel site, there is one extra plot left for spontaneous succession. At the Zedelgem and Gedinne sites an extra subtreatment was added, using one versus three provenances of oak (Zedelgem) and beech (Gedinne) in the first and second replicate of twenty plots, respectively (table 3). Therefore, two additional monocultures with the extra provenances were added in Zedelgem and four in Gedinne (two in Gribelle and two in Gouverneurs). Experiments manipulating genetic diversity within tree species are rare (but see Vehviläinen & Koricheva 2006 and Castagnyrol et al. 2012). However, high levels of genetic diversity may become of vital importance for sustained forest ecosystem functioning, especially in a rapidly changing environment (Aerts & Honnay 2011). This subtreatment will allow testing whether the performance differs between plots with a lower versus higher intraspecific genetic diversity and whether this effect depends on the diversity and identity of the other species present in the plot.

At all three sites, trees are planted on a 1.5 m × 1.5 m grid. The plot size is 42 m × 42 m at the Zedelgem site, resulting in a density of 784 trees per plots. The plots at the Gedinne site also measure 42 m × 42 m (784 trees), except thirteen plots that have a size of 42 m × 37.5 m (700 trees). The Hechtel-Eksel plots measure 36 m × 36 m and contain 576 trees. The total number of planted trees at the three sites is 32 810 (Zedelgem), 33 404 (Gedinne) and 23 040 (Hechtel-Eksel), making FORBIO one of the larger tree diversity experiments (see: www.treedivnet.ugent.be). To balance the

Table 3 – Overview of the provenances, the age and size of the planted trees at the three FORBIO sites and the overall tree survival rates (% surv) after the first (GS1) and second growing season (GS2).

* x+y; z+ means x years grown in nursery as seedling, y years grown in nursery after replanting, shoot height more than z cm.

Zedelgem (planted winter 2009-2010)			Hechtel-Eksel (late autumn 2012)			Gedinne (early spring 2010)			
Species	Provenance	Code* no trees planted	% surv GS1	% surv GS2	Species	Provenance	Code no trees planted	% surv GS1	% surv GS2
<i>Betula pendula</i>	Urkenbos	1+1; 100+	94.8	97.6	<i>Betula pendula</i>	Agnissart	1+1	4608	
<i>Fagus sylvatica</i>	Zoniënwood	3+0; 100+	77.9	88.9	<i>Larix kaempferi</i>	Cornichay	1+1	4608	
<i>Pinus sylvestris</i>	Groenendaal	1+2; 30+	57.0	86.2	<i>Pinus sylvestris</i>	Groenendaal	1+2	4608	
<i>Quercus robur</i>	Vekedelle West	1+3; 100+	75.1	90.9	<i>Pseudotsuga menziesii</i>	Fenfle	1+1	4608	
	Warandeduinen	3+0; 100+	52.8	96.9	<i>Quercus petraea</i>	Buggenhout - Boskapel	1+2	4608	
	Kwekerijweg	3+0; 100+	98.6	98.1					
<i>Tilia cordata</i>	Süddeutsches Hügel- und Bergland	1+1; 100+	98.2	97.0					
		Total	32810	80.4	92.7		Total	23040	
							Total	33404	79.2
									85.9

desire to obtain interspecific interactions as soon as possible with the wish to sustain the mixtures also in the long run, trees were planted in small monospecific patches of 3 × 3 trees (fig. 2). Cells are arranged in a checkerboard pattern in the two-species mixtures, but were randomly attributed to the species in the three- and four-species mixtures.

Before attributing the treatments to the plots, we controlled for any possible covariation between the treatments and the measured soil characteristics (see above). This was done in two steps: (1) by blocking each replication at the sites where there was an obvious gradient in environmental conditions (low-lying southwestern versus more elevated north-eastern part of the site in Zedelgem; Gribelle versus Gouverneurs subsites in Gedinne) and (2) by running ANOVA models to test whether there was any significant difference in the soil characteristics between the diversity levels and between plots with and without any of the five selected species. Treatments were randomly attributed to the (blocked) plots until no significant difference ($p > 0.1$) in any of the characteristics was present anymore. Analysis of data on ground-layer vegetation and soil biota collected after planting at the Zedelgem and Gedinne sites confirmed the lack of covariation (see electronic appendix 2).

Site preparation, (re-)planting and site management

At the Zedelgem site, the compacted layer at the bottom of the plough horizon (A_p) was loosened up in spring 2009 by means of a subsoiling treatment. In autumn 2009, prior to the planting, the entire site was worked with a rotary cultivator to remove the vegetation. In Gedinne, the entire site was mulched to fragment the slash and logging debris and to suppress the vegetation in winter 2010, prior to the planting.

Hechtel-Eksel experienced the most drastic site preparation. All the stumps of the former pine plantation were removed, and the site was disked in fall 2011 and 2012. In Zedelgem and Gedinne, a fence to prevent damage by hare/rabbit and deer, respectively, was established before planting. Plants (see table 3 for more details) were bought in commercial nurseries and were manually planted in winter 2009–2010 (Zedelgem), early spring 2010 (Gedinne) and late autumn 2012 (Hechtel-Eksel). Trees at the Zedelgem and Gedinne sites that were dead or exhibited uncomplete foliage ($> 1/3$ foliage loss) and/or a dead terminal shoot were replaced in winter-spring 2011 and 2012. The overall survival rate after the first growing season was c. 80% at both sites (table 3). However, the rates were quite variable between species and provenances. In Zedelgem, the survival rates of pine (c. 57%) and of one of the oak provenances (c. 53%) were remarkably low. In Gedinne, one beech provenance (c. 40% survival) stood out. For these three species, the low survival could be attributed to the poor quality of the planting material. Following replanting, the overall survival rate increased to c. 93% (Zedelgem) and c. 86% (Gedinne) after the second growing season, and the interspecific variability in survival strongly decreased. A second and last replanting at both sites has been done during winter – early spring 2012. After the planting, the vegetation has been mowed once a year in Zedelgem and twice a year in Gedinne. Vegetation management will stop as soon as the trees no longer risk to become overgrown by herbs, ferns or graminoids. Special attention was given to the elimination of spontaneously established trees. This will probably become an important issue at the Hechtel-Eksel site, where significant natural regeneration of pine and birch is expected. Decisions on possible future cleaning,

Distance	0.8	2.3	3.8	5.3	6.8	8.3	9.8	11	13	14	16	17	19	20	22	23	25	26	28	29	31	32	34	35	37	38	40	41	
Row	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
0.8	1	1	29	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	533	561	589	617	645	673	701	729	757
2.3	2	30	58	86	114	142	170	198	226	254	282	310	338	366	394	422	450	478	506	534	562	590	618	646	674	702	730	758	
3.8	3	31	59	87	115	143	171	199	227	255	283	311	339	367	395	423	451	479	507	535	563	591	619	647	675	703	731	759	
5.3	4	4	32	60	88	116	144	172	200	228	256	284	312	340	368	396	424	452	480	508	536	564	592	620	648	676	704	732	760
6.8	5	5	33	61	89	117	145	173	201	229	257	285	313	341	369	397	425	453	481	509	537	565	593	621	649	677	705	733	761
8.3	6	6	34	62	90	118	146	174	202	230	258	286	314	342	370	398	426	454	482	510	538	566	594	622	650	678	706	734	762
9.8	7	7	35	63	91	119	147	175	203	231	259	287	315	343	371	399	427	455	483	511	539	567	595	623	651	679	707	735	763
11	8	8	36	64	92	120	148	176	204	232	260	288	316	344	372	400	428	456	484	512	540	568	596	624	652	680	708	736	764
13	9	9	37	65	93	121	149	177	205	233	261	289	317	345	373	401	429	457	485	513	541	569	597	625	653	681	709	737	765
14	10	10	38	66	94	122	150	178	206	234	262	290	318	346	374	402	430	458	486	514	542	570	598	626	654	682	710	738	766
16	11	11	39	67	95	123	151	179	207	235	263	291	319	347	375	403	431	459	487	515	543	571	599	627	655	683	711	739	767
17	12	12	40	68	96	124	152	180	208	236	264	292	320	348	376	404	432	460	488	516	544	572	600	628	656	684	712	740	768
19	13	13	41	69	97	125	153	181	209	237	265	293	321	349	377	405	433	461	489	517	545	573	601	629	657	685	713	741	769
20	14	14	42	70	98	126	154	182	210	238	266	294	322	350	378	406	434	462	490	518	546	574	602	630	658	686	714	742	770
22	15	15	43	71	99	127	155	183	211	239	267	295	323	351	379	407	435	463	491	519	547	575	603	631	659	687	715	743	771
23	16	16	44	72	100	128	156	184	212	240	268	296	324	352	380	408	436	464	492	520	548	576	604	632	660	688	716	744	772
25	17	17	45	73	101	129	157	185	213	241	269	297	325	353	381	409	437	465	493	521	549	577	605	633	661	689	717	745	773
26	18	18	46	74	102	130	158	186	214	242	270	298	326	354	382	410	438	466	494	522	550	578	606	634	662	690	718	746	774
28	19	19	47	75	103	131	159	187	215	243	271	299	327	355	383	411	439	467	495	523	551	579	607	635	663	691	719	747	775
29	20	20	48	76	104	132	160	188	216	244	272	300	328	356	384	412	440	468	496	524	552	580	608	636	664	692	720	748	776
31	21	21	49	77	105	133	161	189	217	245	273	301	329	357	385	413	441	469	497	525	553	581	609	637	665	693	721	749	777
32	22	22	50	78	106	134	162	190	218	246	274	302	330	358	386	414	442	470	498	526	554	582	610	638	666	694	722	750	778
34	23	23	51	79	107	135	163	191	219	247	275	303	331	359	387	415	443	471	499	527	555	583	611	639	667	695	723	751	779
35	24	24	52	80	108	136	164	192	220	248	276	304	332	360	388	416	444	472	500	528	556	584	612	640	668	696	724	752	780
37	25	25	53	81	109	137	165	193	221	249	277	305	333	361	389	417	445	473	501	529	557	585	613	641	669	697	725	753	781
38	26	26	54	82	110	138	166	194	222	250	278	306	334	362	390	418	446	474	502	530	558	586	614	642	670	698	726	754	782
40	27	27	55	83	111	139	167	195	223	251	279	307	335	363	391	419	447	475	503	531	559	587	615	643	671	699	727	755	783
41	28	28	56	84	112	140	168	196	224	252	280	308	336	364	392	420	448	476	504	532	560	588	616	644	672	700	728	756	784

Figure 2 – Planting scheme of an exemplary four-species mixture (plot no 1, Zedelgem). Distances (m), row and tree numbers are given. Each tree species has a different colour: beech (white), birch (light grey), lime (darker grey) and pine (dark grey, white numbers).

Table 4 – Overview of the response variables that are currently being measured in the FORBIO experiment.

* tbd: to be determined.

#: Ze: Zedelgem; Ge: Gedinne; H-E: Hechtel-Eksel.

Ecosystem process	Process component	Measurements	Frequency	Status (autumn 2012)	
				no of trees (t)/ plots (p)/ bags (b) (Ze/Ge/H-E)#	no of sampling years (Ze/Ge/H-E)
Tree growth	tree vitality	ordinal vitality score	Yearly	32810t/33404t/23040t	3/3/0
	stem increment	diameter at stem base, tree height	Periodic	5502t/5656t/tbd*	1/1/0
	crown damage	loss of foliage mass, foliage discoloration, shoot dieback scores	Yearly	672t/706t/tbd	1/1/0
Element cycling	soil nutrient pool	topsoil nutrient concentration	Periodic	42p/44p/41p	1/1/1
	litter decomposition	litter decomposition rate (using in situ litterbags)	Periodic	600b/tbd/tbd	1/0/0
	soil carbon pool	topsoil carbon concentration	Periodic	42p/44p/41p	1/1/1
Biodiversity dynamics	understorey vegetation	species composition, % cover species	Periodic	168p/176p/tbd	1/1/0
	canopy arthropods	species composition (using emergence traps and vacuum sampling)	Periodic	40p/40p/tbd	1/1/0
	soil biota	earthworm species and biomass	Periodic	20p/20p/tbd	1/1/0
		microbial biomass and metabolic diversity	Periodic	20p/20p/tbd	1/1/0

pruning and thinning operations have not been taken yet and will depend on the future tree growth and survival.

OUTLOOK

Monitoring of ecosystem functioning started shortly after the planting in the Zedelgem and Gedinne sites and will start soon in Hechtel-Eksel. Multiple processes are being measured (table 4), ranging from tree growth over element cycling to associated biodiversity dynamics. As the trees grow older, we plan to add more processes, e.g. water cycling, to the monitoring scheme so that the picture of ecosystem functioning in the different treatments will be as complete as possible. Extra process components that will be added include, among others, tree quality development, stand structure development, tree phenology and litterfall dynamics. Yet, assuring that measurements are performed in all the plots, in order to include the full range of treatments, sites and processes in the analyses, is currently considered more important than adding extra ecosystem processes and components to the monitoring scheme (i.e. maximizing comprehensiveness).

The extensive and sustained data collection campaign will allow testing the hypotheses outlined in the introduction. Furthermore, the data will allow going beyond purely statistical assessments of the magnitude of complementarity effects, which is considered to be an important next step in BEF research (Cardinale et al. 2011). FORBIO's experimental design (e.g. having monocultures of all species in place, a similar tree density across treatments and sites, the possibility to measure the contribution of individual trees to eco-

system functioning) allows applying a wide range of mechanistic statistical models with parameters that refer to actual biological processes (cf. Hector et al. 2009). Nevertheless, these models will ideally be combined with experimental approaches that directly quantify the processes involved in species interactions. The repeated data collection will also allow testing the hypotheses at different points in time and in different stages of stand development. In grassland experiments, it has been shown that diversity effects become stronger over time (e.g. van Ruijven & Berendse 2005, Dybzinski et al. 2008). By contrast, Vilà et al. (2005) found that a positive diversity-productivity relationship was only evident before canopy closure in Mediterranean forests in Catalonia (Spain). Most likely, the impact of stand development stage on BEF relationships will be function-dependent. At our sites, it can be assumed that the diversity effect on stand growth will be stronger in the stem exclusion stage than in the stand initiation stage (sensu Oliver & Larson 1996). The opposite may be true for understorey vegetation dynamics, due to the often uniformly low light availability and, consequently, vegetation cover on the forest floor in the stem exclusion stage.

Not only basic science, but also forest management will benefit from the results coming from the FORBIO experiment. A recent survey among forest practitioners in Belgium demonstrated that there was surprisingly little common ground between current scientific knowledge and the practitioners' perception on ecosystem services of mixed species forest stands compared to monocultures (Carnol & Verheyen 2010). Communication of the outcomes of FORBIO to this non-scientific audience is therefore as important as the com-

munication to scientists. This can be done in a classical way via publications or a website, but, for instance, also by means of discussions at the experimental sites themselves. Finally, FORBIO is also a test case for uncommon, not well-known tree species mixtures. Traditionally, forest management in Belgium (and elsewhere) has focused on a rather limited number of stand types, and these stand types now dominate the forest area. In an era of global changes, in which the future performance of tree species is unsure (e.g. Campioli et al. 2012), these uncommon mixtures may become important learning objects for practitioners.

To conclude, we are convinced that FORBIO is an important ecosystem experiment that has the potential to deliver badly needed insights into the multiple relationships between biodiversity and ecosystem functioning, which will be valuable for both science and practice. Furthermore, we consider FORBIO as an open platform, meaning that any junior or senior scientist with an interest in studying BEF relationships at the FORBIO sites is welcome.

SUPPLEMENTARY MATERIAL

Supplementary data are available in pdf format at *Plant Ecology and Evolution*, Supplementary Data Site (<http://www.ingentaconnect.com/content/botbel/plecevo/supp-data>), and consists of the following: (1) soil survey protocols used to characterize the three FORBIO sites; (2) characterization of the initial understorey vegetation and soil biota community at the Zedelgem and Gedinne sites of the FORBIO experiment.

ACKNOWLEDGEMENTS

The authors wish to thank BELSPO, and Marc Van Heuckelom and Aline van der Werf in particular, for the financial and logistical support. It would not have been possible to establish the experiments without the continuous support of the 'Agentschap voor Natuur en Bos' in Flanders and the 'Département de la Nature et des Forêts' in Wallonia. We are also very grateful to the many volunteers who helped planting the trees. Elmar Robbrecht and two anonymous reviewers provided useful comments on an earlier version of this paper.

REFERENCES

Aerts R., Honnay O. (2011) Forest restoration, biodiversity and ecosystem functioning. *BMC Ecology* 11, 29. <http://dx.doi.org/10.1186/1472-6785-11-29>

Balvanera P., Pfisterer A.B., Buchmann N., He J.S., Nakashizuka T., Raffaelli D., Schmid B. (2006) Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters* 9: 1146–1156. <http://dx.doi.org/10.1111/j.1461-0248.2006.00963.x>

Bertness M.D., Callaway R. (1994) The role of positive forces in natural communities. *Trends in Ecology and Evolution* 9: 191–193.

Bock L. (coord.) (2007) Légende de la Carte Numérique des Sols de Wallonie – version 2. Gembloux, Faculté universitaire des Sciences agronomiques de Gembloux, Laboratoire de Géopédo-

logie, en relation avec le PCNSW (convention pour le compte de la Région Wallonne – DGA).

Campioli M., Vincke, C., Jonard, M., Kint, V., Demarée, G., Ponnelle Q. (2012) Current status and predicted impact of climate change on forest production and biogeochemistry in the temperate oceanic European zone : review and prospects for Belgium as a case study. *Journal of Forest Research* 17: 1–18. <http://dx.doi.org/10.1007/s10310-011-0255-8>

Cardinale B.J., Matulich K.L., Hooper D.U., Byrnes J.E., Duffy E., Gamfeldt L., Balvanera P., O'Connor M.I., Gonzalez A. (2011) The functional role of producer diversity in ecosystems. *American Journal of Botany* 98: 572–592. <http://dx.doi.org/10.3732/ajb.1000364>

Carnol M., Verheyen K. (2010) Les services écosystémiques dans les forêts mélangées et pures: perception des utilisateurs et connaissances scientifiques. *Forêt Wallone* 106: 49–59.

Castagneyrol B., Lagache L., Giffard B., Kremer K., Jactel H. (2012) Genetic diversity increases insect herbivory on oak saplings. *PLoS One* 7(8): e44247. <http://dx.doi.org/10.1371/journal.pone.0044247>

de Ferraris J.J.F. (1771–1778) Kabinetskaart van de Oostenrijkse Nederlanden.

Dybzinski R., Fargione J.E., Zak D.R., Fornara D., Tilman D. (2008) Soil fertility increases with plant species diversity in a long-term biodiversity experiment. *Oecologia* 158: 85–93. <http://dx.doi.org/10.1007/s00442-008-1123-x>

FAO (2010) Global Forest Resources Assessment 2010 – Main report. Rome, FAO.

Gamfeldt L., Hillebrand H., Jonsson P.R. (2008) Multiple functions increase the importance of biodiversity for overall ecosystem functioning. *Ecology* 89: 1223–1231. <http://dx.doi.org/10.1890/06-2091.1>

Gessner M.O., Swan C.M., Dang C.K., McKie B.G., Bardgett R.D., Wall D.H., Hättenschwiler S. (2010) Diversity meets decomposition. *Trends in Ecology and Evolution* 25: 372–380. <http://dx.doi.org/10.1016/j.tree.2010.01.010>

Hector A., Schmid B., Beierkuhnlein C., Caldeira M.C., Diemer M., Dimitrakopoulos P.G., Finn J.A., Freitas H., Giller P.S., Good J., et al. (1999) Plant diversity and productivity experiments in European grassland. *Science* 286: 1123–1127. <http://dx.doi.org/10.1126/science.286.5442.1123>

Hector A., Bagchi R. (2007) Biodiversity and ecosystem multifunctionality. *Nature* 448: 188–190. <http://dx.doi.org/10.1038/nature05947>

Hector A., Bell T., Connolly J., Finn J., Fox J., Kirwan L., Loreau M., McLaren J., Schmid B., Weigelt A. (2009) The analysis of biodiversity experiments: from pattern toward mechanism. In: Naeem S., Bunker D.E., Hector A., Loreau M., Perrings C. (eds) *Biodiversity, ecosystem functioning, and human wellbeing: an ecological and economic perspective*: 94–104. Oxford, Oxford University Press.

Hermly M., Honnay O., Firbank L., Grashof-Bokdam C.J., Lawesson J.E. (1999) An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. *Biological Conservation* 91: 9–22. [http://dx.doi.org/10.1016/S0006-3207\(99\)00045-2](http://dx.doi.org/10.1016/S0006-3207(99)00045-2)

Hobbie S.E., Reich P.B., Oleksyn J., Ogdahl M., Zytkowski R., Hale C.M., Karolewski P. (2006) Tree species effects on decomposition and forest floor dynamics in a common garden. *Ecology* 87: 2288–2297. [http://dx.doi.org/10.1890/0012-9658\(2006\)87%5B2288:TSEODA%5D2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2006)87%5B2288:TSEODA%5D2.0.CO;2)

Hooper D.U., Chapin F.S., Ewel J.J., Hector A., Inchausti P., Lavorel S., Lawton J.H., Lodge D.M., Loreau M., Naeem S., et al.

- (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75: 3–35. <http://dx.doi.org/10.1890/04-0922>
- Hooper D.U., Adair E.C., Cardinale B.J., Byrnes J.E.K., Hungate B.A., Matulich K.L., Gonzalez A., Duffy J.E., Gamfeldt L., O'Connor M.I. (2012) A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature* 486: 105–108. <http://dx.doi.org/10.1038/nature11118>
- IUSS Working Group WRB (2006) World reference base for soil resources 2006. World Soil Resources Reports No. 103. Rome, FAO.
- Leuschner C., Jungkunst H.F., Fleck S. (2009) Functional role of forest diversity: pros and cons of synthetic stands and across-site comparisons in established forests. *Basic and Applied Ecology* 10: 1–9. <http://dx.doi.org/10.1016/j.baae.2008.06.001>
- Loreau M., Hector A. (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature* 412: 72–76. <http://dx.doi.org/10.1038/35083573>
- Maron J. L., Marler M., Klironomos J. N., Cleveland C.C. (2011) Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters* 14: 36–41. <http://dx.doi.org/10.1111/j.1461-0248.2010.01547.x>
- Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: synthesis. Washington DC, Island Press.
- Morin X., Fahse L., Scherer-Lorenzen M., Bugmann H. (2011) Tree species richness promotes productivity in temperate forests through strong complementarity between species. *Ecology Letters* 14: 1211–1219. <http://dx.doi.org/10.1111/j.1461-0248.2011.01691.x>
- Nadrowski K., Wirth C., Scherer-Lorenzen M. (2010) Is forest diversity driving ecosystem function and service? *Current Opinion in Environmental Sustainability* 2: 75–79. <http://dx.doi.org/10.1016/j.cosust.2010.02.003>
- Oliver C.D., Larson B.C. (1996) Forest stand dynamics. Update edition. New York, John Wiley & Sons.
- Pacala S.W., Kinzig A.P. (2002) Introduction to theory and the common ecosystem model. In: Kinzig A.P., Pacala S.W., Tilman D. (eds) Functional consequences of biodiversity: empirical progress and theoretical extensions: 169–174. Princeton, Princeton University Press.
- Paquette A., Messier C. (2011) The effect of biodiversity on tree productivity: from temperate to boreal forests. *Global Ecology & Biogeography* 20: 170–180. <http://dx.doi.org/10.1111/j.1466-8238.2010.00592.x>
- Rockström J., Steffen W., Noone K., Persson Å, Chapin F.S., Lambin E.F., Lenton T.M., Scheffer M., Folke C., Schellnhuber H.J., et al. (2009) A safe operating space for humanity. *Nature* 461: 472–475. <http://dx.doi.org/10.1038/461472a>
- Scherber C., Eisenhauer N., Weisser W.W., Schmid B., Voigt W., Fischer M., Schulze E.-D., Roscher C., Weigelt A., Allan E., Beßler H., et al. (2010) Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature* 468: 553–556. <http://dx.doi.org/10.1038/nature09492>
- Scherer-Lorenzen M., Potvin C., Koricheva J., Schmid B., Hector A., Bornik Z., Reynolds G., Schulze E.-D. (2005) The design of experimental tree plantations for functional biodiversity research. In: Scherer-Lorenzen M., Körner C., Schulze E.D. (eds) The functional significance of forest diversity: a synthesis: 347–376. Heidelberg, Springer.
- Scherer-Lorenzen M., Schulze E.-D., Schumacher J., Weller E. (2007) Exploring the functional significance of forest diversity: a new long-term experiment with temperate tree species (BIO-TREE). *Perspectives in Plant Ecology, Evolution and Systematics* 9: 53–70. <http://dx.doi.org/10.1016/j.ppees.2007.08.002>
- Schnitzer S.A., Klironomos J.N., Lambers J.H.R., Kinkel L.L., Reich P.B., Xiao K., Rillig M.C., Sikes B.A., Callaway R.M., Mangan S.A., van Nes E.H., Scheffer M. (2011) Soil microbes drive the classic plant diversity–productivity pattern. *Ecology* 92: 296–303. <http://dx.doi.org/10.1890/10-0773.1>
- Sevenant M., Menschaert J., Couvreur M., Ronse A., Antrop M., Geypens M., Hermy M., De Blust G. (2002) Ecodistricten: ruimtelijke eenheden voor gebiedsgericht milieubeleid in Vlaanderen. Studieopdracht in het kader van actie 134 van het Vlaams Milieubeleidsplan 1997-2001. Brussels, Ministerie van de Vlaamse Gemeenschap, Administratie Milieu, Natuur, Land- en Waterbeheer.
- Studel B., Hector A., Friedl T., Löffke C., Lorenz M., Wesche M., Kessler M. (2012) Biodiversity effects on ecosystem functioning change along environmental stress gradients. *Ecology Letters* 15: 1397–1405. <http://dx.doi.org/10.1111/j.1461-0248.2012.01863.x>
- Suding K.N., Lavorel S., Chapin F.S., Cornelissen J.H.C., Diaz S., Garnier E., Goldberg D., Hooper D.U., Jackson S.T., Navas M.L. (2008) Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. *Global Change Biology* 14: 1125–1140. <http://dx.doi.org/10.1111/j.1365-2486.2008.01557.x>
- Tilman D., Reich P.B., Knops J., Wedin D., Mielke T., Lehman C. (2001) Diversity and productivity in a long-term grassland experiment. *Science* 294: 843–845. <http://dx.doi.org/10.1126/science.1060391>
- Van Ranst E., Sys C. (2000) Eenduidige legende voor de digitale bodemkaart van Vlaanderen (schaal 1: 20 000). Gent, Laboratorium voor Bodemkunde, Universiteit Gent.
- van Ruijven J., Berendse F. (2005) Diversity-productivity relationships: Initial effects, long-term patterns, and underlying mechanisms. *Proceedings of the National Academy of Sciences* 102: 695–700. <http://dx.doi.org/10.1073/pnas.0407524102>
- Vehviläinen H., Koricheva J. (2006) Moose and vole browsing patterns in experimentally assembled pure and mixed forest stands. *Ecography* 29: 497–506. <http://dx.doi.org/10.1111/j.0906-7590.2006.04457.x>
- Vilà M., Inchausti P., Vayreda J., Barrantes O., Gracia C., Ibáñez J.J., Mata T. (2005) Confounding factors in the observational productivity diversity relationship in forests. In: Scherer-Lorenzen M., Körner C., Schulze E.D. (eds) Forest diversity and function: temperate and boreal systems: 65–86. Heidelberg, Springer.
- Vilà M., Vayreda J., Comas L., Ibáñez J.J., Mata T., Obón B. (2007) Species richness and wood production: a positive association in Mediterranean forests. *Ecology Letters* 10: 241–250. <http://dx.doi.org/10.1111/j.1461-0248.2007.01016.x>
- Wilsey B.J., Teaschner T.B., Danesghar P.P., Isbell F.I., Polley H.W. (2009) Biodiversity maintenance mechanisms differ between native and novel exotic-dominated communities. *Ecology Letters* 12: 432–442. <http://dx.doi.org/10.1111/j.1461-0248.2009.01298.x>

Manuscript received 5 Dec. 2012; accepted in revised version 8 Jan. 2013.

Communicating Editor: Elmar Robbrecht.