

## ECOSYSTEM EFFECTS OF BIODIVERSITY MANIPULATIONS IN EUROPEAN GRASSLANDS

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**Abstract.** We present a multisite analysis of the relationship between plant diversity and ecosystem functioning within the European BIODEPTH network of plant-diversity manipulation experiments. We report results of the analysis of 11 variables addressing several aspects of key ecosystem processes like biomass production, resource use (space, light, and nitrogen), and decomposition, measured across three years in plots of varying plant species richness at eight different European grassland field sites. Differences among sites explained substantial and significant amounts of the variation of most of the ecosystem processes examined. However, against this background of geographic variation, all the aspects of plant diversity and composition we examined (i.e., both numbers and types of species and functional groups) produced significant, mostly positive impacts on ecosystem processes.

Analyses using the additive partitioning method revealed that complementarity effects (greater net yields than predicted from monocultures due to resource partitioning, positive interactions, etc.) were stronger and more consistent than selection effects (the covariance between monoculture yield and change in yield in mixtures) caused by dominance of species with particular traits. In general, communities with a higher diversity of species and functional groups were more productive and utilized resources more completely by intercepting

Manuscript received 20 October 2003; revised 29 June 2004; accepted 12 July 2004. Corresponding Editor: N. C. Kenkel.

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more light, taking up more nitrogen, and occupying more of the available space. Diversity had significant effects through both increased vegetation cover and greater nitrogen retention by plants when this resource was more abundant through  $N_2$  fixation by legumes. However, additional positive diversity effects remained even after controlling for differences in vegetation cover and for the presence of legumes in communities. Diversity effects were stronger on above- than belowground processes. In particular, clear diversity effects on decomposition were only observed at one of the eight sites.

The ecosystem effects of plant diversity also varied between sites and years. In general, diversity effects were lowest in the first year and stronger later in the experiment, indicating that they were not transitional due to community establishment. These analyses of our complete ecosystem process data set largely reinforce our previous results, and those from comparable biodiversity experiments, and extend the generality of diversity–ecosystem functioning relationships to multiple sites, years, and processes.

*Key words:* BIODEPTH, European plant-experiment network; biomass production; canopy structure; complementarity effect; decomposition; legumes; light use; multisite grasslands field study; nitrogen retention; plant species richness; resource use; selection effect.

## INTRODUCTION

The last decade has seen the intensified collaboration of population and community ecologists with physiological and ecosystem ecologists in the study of the relationship between biodiversity and ecosystem functioning (Schulze and Mooney 1993, Loreau et al. 2001, 2002, Kinzig et al. 2002). One of the main motivations for this research is the change in and loss of diversity seen as a result of human activities and climate change. Identifying general patterns in a research area such as this can be a major obstacle because even well-replicated ecological studies are often conducted at single points in space and time, and often focus on one or a small number of variables. This paper reports the results of a large-scale pan-European project (BIODEPTH—BIODiversity and Ecological Processes in Terrestrial Herbaceous ecosystems) that examined the relationship between plant diversity (species richness and functional-group richness of vascular plants) and ecosystem functioning in experimental grassland communities based on locally and naturally occurring species, established from seed under field conditions. A major aim of BIODEPTH was to explicitly address whether biodiversity effects on ecosystem functioning are consistent in space and time, over a range of climate and soil conditions, and with different local species pools. It is well known that natural variation has a major influence on ecosystem functioning (Schulze et al. 1995, Grime 1998, Huston and McBride 2002). In this experiment, we addressed the extent to which diversity affects ecosystem functioning at individual sites and the extent to which diversity interacts with variation in ecosystem functioning across sites (Hector et al. 1999, Fridley 2002).

Biodiversity effects on ecosystem processes could vary with environmental conditions if individual species performances and interactions are differentially affected by environmental variation such as changes in soil fertility and light availability (Tilman 1988, Bazzaz 1996, Fargione and Tilman 2002). However, if differential responses to environmental variation are aver-

aged out in multi-species communities this may increase the consistency of the biodiversity–ecosystem-functioning relationships across environmental gradients (Cropp and Gabric 2002). Only a few multisite biodiversity experiments have been carried out so far (BIODEPTH; CLUE [Changing land usage, enhancement of biodiversity and ecosystem development], Van der Putten et al. 2000; Emmerson et al. 2001), which try to identify the role of extrinsic factors (Tilman 1994, Fridley 2003) that vary and act at larger scales than the commonly investigated intrinsic factors (including community processes) that act at a local scale within systems.

Our own multisite analyses so far have focused mainly on aboveground biomass production (Hector et al. 1999, 2002a) from the second year of the experiment. We reported that the overall relationship between diversity and biomass production was positive and log linear, but that slopes from individual field sites ranged from positive to zero (Hector et al. 1999). Reviews of comparable experiments with plants (Schmid et al. 2002a) report relationships that all fall within this range from positive and linear (e.g., Tilman et al. 2001, He et al. 2002), to approximately log linear (e.g., Tilman et al. 1996, 1997a, Niklaus et al. 2001, Reich et al. 2001, van Ruijven and Berendse 2003) to slopes not significantly different from zero (e.g., Hooper and Vitousek 1997).

One major mechanism that could allow production to increase with diversity is complementary use of resources, such as through light partitioning, in which differences in morphology between species enhance the structural complexity of the vegetation and allow for more complete utilization of PAR (photosynthetic active radiation; Hirose and Werger 1994, Naeem et al. 1994, 1995, Spehn et al. 2000b, Fridley 2003). Diversity is also likely to increase complementarity in nutrient uptake, either by different species acquiring nutrients from different portions of the available pool in space, time, or chemical form, thereby increasing, e.g., total nitrogen retention (Spehn et al. 2002) and de-

creasing losses to leaching (Scherer-Lorenzen et al. 2003).

Biodiversity is not a one-dimensional ecological variable that acts in a simple way on ecosystem processes but is instead multifaceted, with complex effects. This makes the statistical analysis and interpretation of biodiversity experiments complex and even the proximate mechanisms difficult to isolate. Several major issues have already been identified that require special attention in analyses:

1) Effects of biodiversity in experimental communities can be separated into two major categories of explanation: niche complementarity (e.g., resource partitioning), plus positive (facilitative) interactions (Hector 1998, Loreau and Hector 2001) vs. "sampling effects" resulting from the greater probability of including a species or combinations of species with particular traits that dominate randomly assembled high-diversity communities (Huston 1997, Loreau et al. 2001). We employ the additive partitioning method of Loreau and Hector (2001) because it allows us to quantitatively define an overall net biodiversity effect and then separate this into a selection effect and a complementarity effect (see *Methods: Additive partitioning . . .*, below), whereas previous discussions have often treated the two mechanisms as mutually exclusive alternatives (Huston 1997, Huston et al. 2000).

2) One of the major functional traits of plants that affects many ecosystem processes is the ability to symbiotically fix atmospheric nitrogen. Hence, one of the major interactions likely to be of functional importance is the one between N fixers and non-fixing species. Whilst our experiment was not designed to isolate the role of legumes in generating biodiversity effects, we conducted analyses to test whether legumes formed a significant component of compositional diversity and whether additional species-richness effects existed beyond the effect of the presence of legume species.

3) Biodiversity effects on ecosystem functioning may be synergistic and simultaneously affect several interrelated ecosystem processes. For example, diverse communities may establish faster, as it is more likely that other species can compensate an eventual failure of single species due to poor growth or survival (e.g., caused by interspecific competition, disease, or herbivore attack). In communities with only one or a few species, phenological development of the stand is relatively synchronous, potentially leading to vegetation gaps during the growing season (McNaughton 1993). This lack of species redundancy in low-diversity communities (Levin 1997) will have subsequent effects on several different ecosystem processes; e.g., it can cause "open canopies," which differ in canopy structure and light climate from fully covered stands (Wenger and Hirose 1988). We therefore performed analyses of covariance to control for diversity-induced differences in plant cover and to examine the strength of additional effects of diversity on cover-related aboveground eco-

system processes (three-dimensional space, light and nutrient use, and biomass production).

#### *Caveats*

A few caveats should be kept in mind when interpreting our results. (1) The implications of our work for the consequences of species loss depend on the implied order of species loss which in our case is determined by our constrained random selection of species (see *Methods: Experimental design*, below). (2) We simulate species loss by assembling different diversity communities. An alternative approach to evaluate biodiversity–ecosystem functioning relationships is species removal from existing communities to create a diversity gradient (Wardle et al. 1999, Diaz et al. 2003). (3) The results of biodiversity experiments are not directly comparable with observational studies comparing productivity of natural or semi-natural ecosystems differing in species richness. This is because biodiversity experiments look at within-site effects of diversity on ecosystem processes, where environmental factors are relatively constant. Observational studies compare across sites that might vary in environmental factors that could overwhelm the effect of biodiversity. For the most recent literature surveys on observational studies see Grace (1999) and Mittelbach et al. (2001). For further discussion see Hector et al. (2000b, 2001a, b) and Schmid et al. (2002a).

One of the main aims of the present study is to present a comprehensive analysis of biodiversity–ecosystem functioning relationships that allows us to extend and, where possible, generalize previous findings to multiple processes across different sites in temperate and Mediterranean grassland ecosystems and to see how these ecosystem effects of biodiversity develop over time. The inclusion of sites with contrasting soils and climate, and with different regional sets of taxa, should add robustness to our test. We report multisite analyses of diversity effects on 11 ecosystem process variables: (1) above- and (2) belowground biomass over the full three years of the BIODEPTH experiment, (3) community cover, (4) stand height, (5) canopy biomass density, (6) center of gravity, (7) light use, (8) nitrogen pools in aboveground vegetation, (9) available inorganic nitrogen in soil, and (10, 11) decomposition of two different standard materials in the third year of the experiment. We ask the following specific questions: (1) How do different ecosystem processes vary in their response to altered plant diversity? (2) How do the effects of the manipulation of plant diversity on these processes develop over time? (3) Does this extended analysis of the ecosystem process variables over three years present a picture of consistent biodiversity effects across our eight sites or of location-specific differences?

#### METHODS

##### *Study sites*

This study was carried out at eight experimental sites along north–south and east–west transects across Eu-

TABLE 1. Comparison of ecosystem properties, by site, in the experimental (Exp.) plots at the highest diversity level with unmanipulated reference (Ref.) plots established in adjacent grasslands.

Plot†		Ecosystem property			
Type	No.	Species richness‡ (no./plot)	Vegetation cover (%)	Aboveground biomass (g/m <sup>2</sup> )	Canopy height (cm)
Germany					
Exp.	6	16.0	93.2 ± 1.9	1,051.8 ± 84.7	72.1 ± 2.9
Ref.	5	16.0 ± 0.0	100.0 ± 0.0	615.5 ± 135.8	58.0 ± 2.8
Portugal					
Exp.	4	14.0	78.8 ± 10.8	423.3 ± 102.5	ND
Ref.	4	14.5 ± 1.7	75.0 ± 13.2	400.0 ± 142.6	ND
Greece					
Exp.	8	18.0	99.4 ± 0.6	232.7 ± 50.7	53.4 ± 4.4
Ref.	2	21.0 ± 6.0¶	97.5 ± 2.5	271.6 ± 153.1	43.3 ± 0.0
Ireland					
Exp.	10	8.0	98.1 ± 1.0	1065.5 ± 53.2	37.0 ± 1.9
Ref.	4	11.0 ± 0.7	96.5 ± 0.9	951.0 ± 88.7	38.0 ± 2.3
UK: Silwood					
Exp.	10	11.0	99.0 ± 0.7	559.2 ± 53.8	29.5 ± 1.1
Ref.	5	14.8 ± 1.0¶	ND	680.5 ± 119.5	27.5 ± 1.9

Notes: Data are means ± 1 SE; ND = not determined. Data are from year 3 of the experiment, except those in italics are from year 2.

† Reference plots were only available at five sites.

‡ Species richness of experimental plots is number of species sown.

§ PAR: photosynthetically active radiation.

|| Only two reference plots were sampled.

¶ Species richness was not determined in the entire plot, but only in the biomass harvest quadrats.

rope that span several thousands of kilometers, in Germany, Greece, Ireland, Portugal, Sweden, Switzerland, and two sites in the United Kingdom (UK; Silwood Park near London, and Sheffield). Sites differed widely in climate, soil conditions, and other major environmental factors (Hector et al. 1999, Joshi et al. 2001). For more detailed information on single sites, for Switzerland see Diemer et al. (1997), Joshi et al. (2000), Koricheva et al. (2000), Spehn et al. (2000a, b), Stephan et al. (2000), Diemer and Schmid (2001), Pfisterer and Schmid (2002), and Pfisterer et al. (2004); for Sweden see Mulder et al. (1999, 2002), Koricheva et al. (2000), and Jumpponen et al. (2002); for Germany see Gastine et al. (2003) and Scherer-Lorenzen et al. (2003); for Greece see Troumbis et al. (2000, 2002); for Portugal see Caldeira et al. (2001); and for Silwood see Hector et al. (2000a, 2001a).

#### Species selection

At each study site, we compiled a list of species locally present in the grasslands. The communities therefore consisted of species that were typical of our sites and that frequently co-occur on the plot scale. We did not use atypical species. At each site the top level of diversity aimed to approximate average levels of diversity found in quadrats of plot size in the local grasslands (see Table 1).

#### Establishment of the experimental communities

The field experiments were established in spring 1995 in Switzerland, autumn 1996 in Portugal, and

spring 1996 at all other sites. Plots of 2 × 2 m (2 × 5 m in Sweden) were seeded with 2000 viable seeds/m<sup>2</sup> divided equally between the number of species in each experimental plant community (Hector et al. 1999). Plant species were representative of local grassland communities and seeds were locally collected as far as possible, or otherwise purchased from national commercial sources providing regional seeds. Reciprocal transplant tests with three common species revealed strong patterns of adaptations of local varieties to their "home" sites (Joshi et al. 2001). Prior to sowing, the existing vegetation of the study sites was removed and the soil seed bank was eliminated by continuous weeding (Switzerland, Sweden), steam sterilization (Germany), heat (soil was covered with black plastic for 2.5 months, Portugal), methyl bromide application (Ireland, Greece, UK: Silwood) or by establishing plots on calcareous sterilized sand with added nutrients (UK: Sheffield). To reduce post-application effects of methyl bromide on legumes or to ensure that even a rare legume would be inoculated (Sweden), *Rhizobium* was applied. Plots were weeded to remove unwanted species emerging from the remaining seed bank or invading from outside the experimental site. The plots were not subsequently fertilized during the experimental period. Former land use of the sites is listed in the Supplement (Table S1).

#### Soil conditions

Initial soil conditions (after the preparation of the field sites) were measured either in each plot or, where



TABLE 1. Extended.

PAR transmittance§ (%)	Belowground biomass (g/m <sup>2</sup> )	Soil inorganic N (mg/kg)	Cotton decomposition (% per day)	Wood decomposition (% per day)
$4.33 \pm 1.55$ $19.91 \pm 1.40$	$363.1 \pm 114.0$ $869.0 \pm 320.7$	$1.88 \pm 0.50$ $3.61 \pm 0.46$	$0.72 \pm 0.08$ $0.66 \pm 0.23$	ND ND
ND ND	$77.5 \pm 16.1$ ND	$3.00 \pm 0.11$ ND	$0.02 \pm 0.00$ ND	$0.02 \pm 0.00$ ND
$38.94 \pm 2.05$ $49.62 \pm 18.27$	$554.1 \pm 103.7$ $451.8 \pm 205.4$	$1.80 \pm 0.11$ ND	$1.32 \pm 0.01$ $1.52 \pm 0.00$	$0.07 \pm 0.02$ $0.08 \pm 0.05$
$3.06 \pm 0.32$ $5.56 \pm 1.60$	$689.4 \pm 46.8$ $808.6 \pm 76.1$	$8.69 \pm 1.52$ ND	$0.59 \pm 0.02$ $0.58 \pm 0.05$	ND ND
$5.78 \pm 0.97$ $1.47 \pm 0.4$	$1522.3 \pm 311.4$ $1905.0 \pm 673.4$	$1.36 \pm 0.16$ ND	$0.36 \pm 0.01$ $0.30 \pm 0.02$	$0.10 \pm 0.01$ $0.04 \pm 0.02$

plots had not yet been established, by using a stratified random-sampling scheme in which the experimental area was divided into a grid of at least 10 cells per block. Soil cores (4-cm diameter  $\times$  20 cm deep) were taken at random within each plot or cell, mixed, and a subsample taken to assess soil pH, available phosphorous, total carbon, total nitrogen, ammonium, and nitrate. At some sites, information on other nutrients was also collected, depending on the methods of local laboratories. To allow comparison between sites, one composite sample from each block at each site was analyzed for total C, total N, available P, and pH (in CaCl<sub>2</sub>) at the Institute of Environmental Sciences, University of Zurich (Zurich, Switzerland), following standard methods (Anonymous 1995; see Supplement: Table S1).

#### Experimental design

Our experiment has a multilevel, hierarchical design that tests the effects of diversity in the context of differences between sites and due to species composition. The experiment focuses on species number by establishing a gradient of species richness at each site, with five levels of diversity ranging from monocultures to higher diversity mixtures approximately matching background levels of diversity in comparable semi-natural grasslands at each site (Table 1; Supplement: Table 1). Species mixtures were assembled by random draws from the local pool of typical co-occurring species (see *Species selection*, above). Species selection at each site was constrained to vary the number of functional groups—grasses, N-fixing legumes, and other herbaceous dicots (forbs)—within the different levels of species richness and so that all communities included grasses (with the exception of a few polycultures deliberately established at the Swedish sites to enable some test of the effects of the grass functional group).

At low levels (one or two species), only one or two functional groups could be included, whereas at high levels it was unrealistic that all species belonged to only one or two functional groups. As a result the design unavoidably contains empty cells within the species richness and functional-group richness design matrix, and these two variables are therefore partially positively correlated (Schmid et al. 2002a). Each particular combination of species richness and number of functional groups (diversity level) was replicated with different species compositions (called “assemblages” in Hector et al. [1999] and in the Supplement to indicate inclusion of both monocultures and mixtures; here we use “composition” to be explicit about what this treatment replicates) at each site to separate the effects of species richness from the effects of species composition (Givnish 1994, Tilman et al. 1997b, Allison 1999, Schmid et al. 2002a, b). The number of replicates within diversity levels was reduced with increasing species richness because it was anticipated that the variability of ecosystem processes would be lower at high diversity as the species overlap among replicates inevitably increases if the total species pool is fixed (Schmid et al. 2002a, b). Unlike most other biodiversity experiments, BIODEPTH was designed to also quantify the variation due to species composition (variation left in the residual error of other analyses). Every monoculture and polyculture was repeated (with random positioning) in two replicated blocks per site (except Portugal, with a fully randomized design without blocks). There were two replicates of all compositions, except there were four replicates for one composition in Portugal, two in Ireland, one in Sweden, and one in UK: Sheffield. In total, the experiment comprised 480 plots, which replicated 200 different species compositions spread across eight sites. Some compositions (16

monocultures and one two-species mixture) occurred at more than one site, mainly at two to four sites, with two monocultures (*Plantago lanceolata*, *Trifolium repens*) occurring at six of the eight sites. This overall hierarchical design contains multiple error terms, which are explained under *Statistical analysis*, below.

#### *Experimental vs. "natural" reference grasslands*

In addition, at some sites (Germany [G], Portugal [P], Greece [GR], Ireland [IR], and UK Silwood) we established unmanipulated reference plots in neighboring grasslands to provide a natural comparison for the variables measured in our experimental plots. In general, all ecosystem properties measured in the unmanipulated reference plots of adjacent grasslands at 5 of the 8 sites (G, P, GR, IR, and UK Silwood) were very similar to the values obtained in the highest diversity level of the experimental plots (Table 1). Species richness matched the sown species numbers of the highest diversity levels of the experimental plots very closely, thus confirming the experimental design that adjusted the highest diversities to the background level of comparable grasslands. Some differences were observed at the German site for measures related to or influencing productivity (aboveground and belowground biomass, canopy height, photosynthetically active radiation transmittance, soil mineral N—but not cover) with higher values for the experimental plots, due to higher nutrient availability at the experimental site (Scherer-Lorenzen 1999). In Greece the experimental communities had a taller canopy than the reference plots, whereas in UK Silwood the experimental plots had higher PAR transmittance and somewhat lower biomass values than the reference.

#### *Realized species richness*

Realized plant-species richness was assessed from the biomass samples taken at harvest and the visual cover estimates at the whole plot level taken over the entire growing season. From all the surveys, we compiled a list of all the plant species present in each plot and year.

#### *Plant biomass*

Aboveground biomass of plants was determined by harvesting standing crop above 5 cm in one or two permanent quadrats of 20 × 50 cm per plot once (Greece, Portugal, Sweden, UK) or twice (Switzerland, Germany, Ireland) a year. Afterwards, the entire plots were mown to 5 cm, following the traditional hay-meadow managements. Plant samples were oven dried to a constant dry mass (constancy) and weighed.

#### *Canopy structure and light use*

The total cover of vegetation, i.e., the percentage of ground area covered by live plants, and the ground area covered by dead plants and litter, was estimated visually before each main harvest. The average canopy

height was measured with a sward stick (Hill Farming Research Organisation [HFRO], Edinburgh, UK) at the time of the biomass harvests. A clear window of 2 × 1 cm was lowered vertically down the stick towards the canopy (Barthram 1986) and the height of first contact with the canopy recorded, and the plot average of at least 10 repeat measurements was taken. At the time of peak biomass each year, aboveground biomass was harvested in canopy layers in a 20 × 50 cm permanent quadrat. Plants were cut at least at 5 cm, 20 cm, 35 cm, and 50 cm above the ground level (with a few exceptions; Ireland, no strata at 35 cm; Switzerland, additional strata at 15 cm). The number of strata depended on total canopy height (for Greece, Portugal, Sweden, UK Silwood, and Switzerland, last strata >50 cm; for UK Sheffield, last strata >35 cm; for Germany, strata every 15 cm to the top of each individual canopy). Samples were dried to constancy and weighed. We then calculated biomass density per layer as a measure of 3-dimensional space filling (see Naeem et al. 1994, 1995) by dividing the height of each layer by biomass (biomass/volume of layer, in grams per cubic meter). To get a measure of vertical biomass-density distribution in the canopy, we calculated the height of the center of gravity by multiplying the biomass of each layer with the mean height of the layer (=z) and dividing the sum of z by the total biomass (Spehn et al. 2000b).

Photosynthetically active photon-flux density (PPFD) was measured with a ceptometer (Delta-T Sunscan ceptometer; Delta-T Devices, Cambridge, UK) or LI-COR Line Quantum Sensor (Lincoln, Nebraska, USA) at the base of the canopy (3–5 cm above soil) in each plot before the main biomass harvest was taken. We then calculated the mean of at least three individual measurements per plot.

#### *Nitrogen in aboveground biomass*

Dried aboveground biomass samples were ground and analyzed for nitrogen content. Nitrogen in percentage of dry mass was measured by dry combustion using an automated C-H-N analyzer at three sites (Switzerland, LECO CHN-900 [LECO Corporation, Saint Joseph, Michigan, USA] Germany, C/N analyzer, Carlo Erba NA 1500, [Carlo Erba, Mailand, Italy]; Sweden, Europa Scientific ANCA-NT). In Ireland, Greece, Portugal, and UK Silwood the samples were digested and analyzed with a semi-automatic Kjeldahl procedure (Tecator Herndon, Virginia, USA).

#### *Root biomass and length*

We measured total community root biomass by taking two soil cores from each plot, avoiding the central area containing the permanent sampling area of the aboveground biomass harvests. Soil cores (4-cm diameter) were divided into two or four strata (0–10 cm and 10–20 cm or every 5 cm) and roots were extracted by washing and sieving (1-mm mesh size). Samples

were dried until constancy and weighted. At two sites (Switzerland, Germany), fine roots (<1 mm) were separated and length was determined by an image analysis system (Delta-T area meter [Delta-T Devices, Cambridge, UK]).

#### *Decomposition*

We measured the dry mass loss of cotton per day in the topsoil as an indicator for short-term decomposition. The cotton was a standard organic substrate containing ~95% cellulose, with an initial N concentration of 0.09%. Four repeat strips of cotton ( $12.2 \times 12.5$  cm—identical source used at all sites) were buried vertically (0–10 cm) in each plot over several months during the main growing period. Cotton strips were protected against rodents and other physical damage with a 1-mm nylon mesh (Germany, Greece, Ireland, UK Silwood, Sweden, Switzerland). Long-term decomposition was measured by dry mass loss of wooden birch sticks. Standardized sets of three flat birch sticks ( $11.5 \times 0.9 \times 0.2$  cm) were bundled using polyester thread, and buried vertically 1 cm below the surface, once or twice per growing season. Four bundles of sticks were used per plot and mean estimates of yearly decomposition were obtained from differences in dry mass. Only the central stick in the bundle was monitored. Initial N concentration of the sticks was 0.08%.

#### *Soil nitrogen*

Soil-soluble or exchangeable N concentrations were determined according to a generally agreed sampling protocol, but with different numbers of samples per year and different methods of chemical analysis across sites. Samples were taken with soil cores (diameter range: 2–5 cm) within the main rooting depth (10–20 cm, depending on site conditions). Two to 10 samples per plot were taken and pooled for the analysis. From the sieved samples (2-mm mesh size), aliquots were extracted either with KCl solution (Switzerland, Germany, Greece, and UK Silwood), or  $H_2SO_4$  (Kjeldahl-procedure, in Portugal and Ireland), and ammonia and nitrate concentrations were determined using standard soil-laboratory procedures. A second subsample was dried and the water content was used to calculate total plant-available mineral nitrogen ( $N_{min}$ ) as  $NH_4^+$ -N plus  $NO_3^-$ -N milligrams per kilogram of dry mass. Here, we present the results of the third year of the experiment. The soil was not sampled at the same time across the sites, so in our analysis we had to combine measurements dating early in the season (Ireland and Portugal), dating mid-season (Switzerland, Germany, Greece), and from the end of the growing season (UK Silwood). Soil N was not measured in Sweden and UK Sheffield during the third year.

#### *Statistical analysis*

The data were analyzed using the general-linear-modeling approach to multiple regression (e.g., Neter

and Wasserman 1974), implemented in GENSTAT (Payne et al. 1993), and the results of the model-fitting sequences were summarized in ANOVA tables (Green and Tukey 1960, Schmid et al. 2002b). The sequential analysis determined by our design and a priori hypotheses included main effects of site, block, species richness, functional group richness, and species composition, as well as interactions of diversity and species composition with sites. The effects of blocks (nested within sites) and species composition (nested within diversity) were considered random factors. Due to the hierarchical structure of the design, as indicated by the nesting described above, the experiment had multiple error terms (Schmid et al. 2002b). The error structure and hypothesis testing are briefly explained here with the expected mean squares and error term assignment given in full in Table 2. To account for spatial variation, sites were tested against blocks (within sites) and blocks against plots (within blocks; i.e., the overall residual). The most important aspect is that our design explicitly tests species and functional-group richness effects against species composition effects (and similarly the diversity-by-site interactions are tested against the composition-by-site interaction). Our design contains terms for the main effects of sites and species composition plus their interaction. However, it is important to note that to perfectly partition the two main effects and the site-by-composition interaction would require a design that repeated the same species composition at all eight sites. This was of course impossible due to the different plant communities present at the different locations. Instead, most species compositions are unique within a single site, with a smaller number (16 monocultures plus a single two-species mixture) occurring at more than one site. Consequently, it was impossible to avoid the site and composition terms being partly confounded and we cannot perfectly partition the variation due to site, species composition, and their interaction, and the values presented should be treated accordingly.

To test the effects related to time, we included year as a main factor in the analyses of realized species richness, biomass, and cover (Table 2), using the approach of orthogonal contrasts to repeated-measures analysis (Elashoff 1986). In this approach, polynomial contrasts are formed and tested against their own error terms, avoiding the problem of serial correlation and therefore the need to adjust the degrees of freedom (see Rosenthal and Rosnow 1985:65). Studies that conduct a large number of different tests sometimes employ a correction for multiple comparisons—often the sequential Bonferroni (Rice 1989)—because when inspecting summaries of a large number of tests, some significant results will be expected by chance alone. We do not use such a correction here for several reasons. First, most response variables showed significant effects, demonstrating that the experiment-wide level of significance is therefore far higher than would be

TABLE 2. Summary of repeated-measures ANOVA of realized species richness per plot from years 1–3.

Term	Source of variation	df	SS	MS	F
1	Site	7	937.0	133.9	446.3
2	Block (within site)	7	2.1	0.3	1.7
3	Species richness ( $\log_2$ )	1	16 749.3	16 749.3	41 873.3
4	Species richness (deviation from log-linearity)	9	4126.7	458.5	1146.3
5	Functional richness (linear)	1	4.8	4.8	12.0
6	Functional richness (deviation)	1	1.0	1.0	2.5
7	Site $\times$ Species richness ( $\log_2$ )	7	111.6	15.9	...
8	Site $\times$ Functional richness (linear)	7	6	0.9	...
9	Composition	173	67.0	0.4	...
10	Site $\times$ Composition	28	0.0	0.0	0.0
11	Plot	238	41.0	0.2	0.6
12	Year	1	0.5	0.5	1.7
13	Year $\times$ Site	7	109.7	15.7	157.0
14	Year $\times$ Block	7	0.5	0.1	0.3
15	Year $\times$ Species richness ( $\log_2$ )	1	0.6	0.6	1.5
16	Year $\times$ Functional richness (linear)	1	0.9	0.9	2.3
17	Year $\times$ Site $\times$ Species richness ( $\log_2$ )	7	222.4	31.8	1 370 689.7
28	Year $\times$ Site $\times$ Functional richness (linear)	7	28.9	4.1	176 724.1
19	Year $\times$ Composition	183	66.0	0.4	17 241.4
20	Year $\times$ Composition $\times$ Site	28	0.0	0.0	0.0
21	Residual	700	191.6	0.3	
22	Total	1421	22 668.0		

† Expected mean squares (without coefficients) show variance components for random and fixed effects and multiple error terms. Unique variance components of each term are in boldface;  $\sigma$  refers to random-effect components,  $S$  to fixed-effect components. Subscript definitions: P = Plot, B = Block, L = Site location, LC = Site  $\times$  Composition, S = Species richness ( $\log_2$ ), S' = Species richness (deviation from log-linearity), F = functional richness (linear), F' = Functional richness (deviation from linearity), C = Composition, Y = Year; YB = Year  $\times$  Block, YL = Year  $\times$  Site, YLC = Year  $\times$  Site  $\times$  Composition, etc.

‡ The appropriate error for each term is that which contains all other variance components except the one being examined so that the variance ratio (in this case  $F$ ) test removes these shared components, revealing the unique effect.

expected by chance alone. Second, our results are based on strong a priori logical arguments and theory, as well as supported by other recent studies (see *Introduction*, above, and *Discussion: Linking diversity effects . . .*, below). This also allowed us to set our main statistical model a priori. Third, such techniques increase the rate of type-II errors (failing to reject the null hypothesis when there is a significant result). The mathematical, logical, and practical arguments against applying multiple comparison methods, particularly in situations like our own, can be found in more detail in Moran (2003) and references therein.

The role of legumes in generating biodiversity effects observed in our experiment has been addressed in several previous publications (Scherer-Lorenzen 1999, Jumpponen et al. 2002, Mulder et al. 2002, Spehn et al. 2002, Scherer-Lorenzen et al. 2003). So here we restrict ourselves to two main questions. First, does the presence of legumes explain a significant amount of the species composition effect? Second, does controlling for the effects of legumes (entering legumes (presence vs. absence) first into the model as covariate) eliminate the effects of species richness?

#### *Additive partitioning of biodiversity effects*

The additive-partitioning method is a generalization of the relative yield (Harper 1977) and proportional deviation from expected value ( $D$ ) approaches (Loreau 1998). Using the method, the effect of biodiversity on aboveground biomass production can be partitioned

into a selection effect and a complementarity effect (which sum to the net biodiversity effect). The *net biodiversity effect*,  $\Delta Y$ , is measured by the difference between the observed yield of a mixture and its expected yield based on the average of the monoculture yields of the component species. The *selection effect* is the standard statistical covariance effect, in our case between the monoculture yield of species and their change in relative yield in the mixture. Positive selection occurs if species with higher-than-average monoculture yields dominate mixtures and negative selection when species with lower-than-average biomass dominate mixtures. Note that calculation of the selection effect uses  $N$  (number of species) rather than  $N - 1$  in the calculation of the covariance term since it is performed on the entire population of species in each mixture rather than a sample of species. The *complementarity effect* measures the net change in the average relative yield of species. A positive complementarity effect occurs when increases in some species are not exactly compensated by decreases in others, which can indicate resource-partitioning and related niche-differentiation processes. As the approach requires a comparison between performances of species in mixture and in monoculture, it can only be applied to the subset of experimental mixtures that contained species for which all monoculture yields were available. These various effects are related in the additive partitioning as follows:



TABLE 2. Extended.

<i>P</i>	ss %	Expected ms†	Error term‡
<0.001	4.1	$\sigma^2 + \sigma_p^2 + \sigma_B^2 + S_L^2$	2
0.1	<0.1	$\sigma^2 + \sigma_p^2 + \sigma_B^2$	11
<0.001	73.9	$(\sigma^2 + \sigma_p^2 + \sigma_{LC}^2 + S_S^2)$	9
<0.001	18.2	$(\sigma^2 + \sigma_p^2 + \sigma_{LC}^2 + S_S^2)$	9
<0.001	<0.1	$(\sigma^2 + \sigma_p^2 + \sigma_{LC}^2 + S_F^2)$	9
0.116	<0.1	$(\sigma^2 + \sigma_p^2 + \sigma_{LC}^2 + S_F^2)$	9
...	0.5	$\sigma^2 + \sigma_p^2 + \sigma_{LC}^2 + S_{LS}^2$	10
...	<0.1	$\sigma^2 + \sigma_p^2 + \sigma_{LC}^2 + S_{LF}^2$	10
...	0.3	$(\sigma^2 + \sigma_p^2 + \sigma_{LC}^2 + \sigma_C^2)$	10
1.000	<0.1	$(\sigma^2 + \sigma_p^2 + \sigma_{LC}^2)$	11
1.000	0.2	$\sigma^2 + \sigma_p^2$	21
0.197	<0.1	$\sigma^2 + S_C^2$	21
<0.001	0.5	$\sigma^2 + \sigma_{VB}^2 + S_{YL}^2$	14
0.934	<0.1	$\sigma^2 + \sigma_{VB}^2$	21
0.222	<0.1	$\sigma^2 + \sigma_{YLC}^2 + S_{YS}^2$	19
0.135	<0.1	$\sigma^2 + \sigma_{YLC}^2 + S_{YF}^2$	19
<0.001	1.0	$\sigma^2 + \sigma_{YLC}^2 + S_{YLS}^2$	20
<0.001	0.1	$\sigma^2 + \sigma_{YLC}^2 + S_{YLF}^2$	20
<0.001	0.3	$(\sigma^2 + \sigma_{YLC}^2 + \sigma_{YC}^2)$	20
1.000	<0.1	$(\sigma^2 + \sigma_{YLC}^2)$	21
	0.8	$\sigma^2$	

$$\begin{aligned} \Delta Y &= Y_O - Y_E = \sum_i RY_{O,i}M_i - \sum_i RY_{E,i}M_i \\ &= \sum_i \Delta RY_i M_i = N \cdot \overline{\Delta RY} \cdot \overline{M} + N \text{cov}(\Delta RY, M) \end{aligned}$$

where  $N$  = number of species in the mixture;  $M_i$  = yield of species  $i$  in monoculture;  $Y_{O,i}$  = observed yield of species  $i$  in mixture, and  $Y_O = \sum_i Y_{O,i}$  = total observed yield of the mixture;  $RY_{E,i}$  = expected relative yield of species  $i$  in the mixture, which is simply its proportion seeded or planted ( $1/N$ ), and  $RY_{O,i} = Y_{O,i}/M_i$  = observed relative yield of species  $i$  in the mixture;  $Y_{E,i} = RY_{E,i}M_i$  = expected yield of species  $i$  in the mixture, and  $Y_E = \sum_i Y_{E,i}$  = total expected yield of the mixture;  $\Delta Y = Y_O - Y_E$  = deviation from total expected yield in the mixture;  $\Delta RY_i = RY_{O,i} - RY_{E,i}$  = deviation from expected relative yield of species  $i$  in the mixture;  $N \cdot \overline{\Delta RY} \cdot \overline{M}$  = the complementarity effect, and  $N \text{cov}(\Delta RY, M)$  = the selection effect.

The additive-partitioning calculations follow Loreau and Hector (2001) with a few modifications for dealing with missing species (species were sometimes entirely absent—see observed species richness—but sometimes just absent from biomass samples). Species that were entirely missing occurred particularly in Portugal and in the third year of the experiment following a period late in the second year and early in the third year that was dry and extremely frosty. Species could have zero harvested biomass in monoculture, polyculture, or both. Where a species was missing in monoculture, expected values could not be calculated. These species therefore had to be ignored entirely by relative-yield-based methods (species richness was taken as the original richness minus the number of missing species). Where a species was present in monoculture but missing from a mixture in which it was established once,

it contributed the negative value of its expected contribution (i.e., a species expected to contribute 10 g that was missing contributed  $-10$  g). We standardized the data used here to make them as comparable across sites and across years within sites as possible. Details of the calculations and individual species biomass data used differ slightly from the single-year data used in Loreau and Hector (2001), although the results and conclusions of the analysis of the biodiversity effects over three years remain similar.

Note that the complementarity effect, and the relative yields it is based on, cannot distinguish facilitation from resource partitioning (and related niche-differentiation effects through natural enemies, etc.). Therefore, our complementarity effect covers all of these effects. More detailed mechanistic approaches are required to identify the underlying biology; we refer to relevant individual site papers where appropriate.

### Presentation of results

Some core ecosystem processes were monitored over three years and for these we present tables of repeated-measures ANOVA. The analyses often produced significant interactions of diversity with site or year or both. In the figures, we therefore present sites separately, showing observed means for each diversity level in each year. For some variables, we did not have a comprehensive data set across all sites and years and we therefore analyzed only the third year. The figures for single-year analyses also show sites separately but provide greater detail: we show the overall regression slope with means and standard errors of the replicate pairs of each species composition. We distinguish compositions with legumes from those without (since many

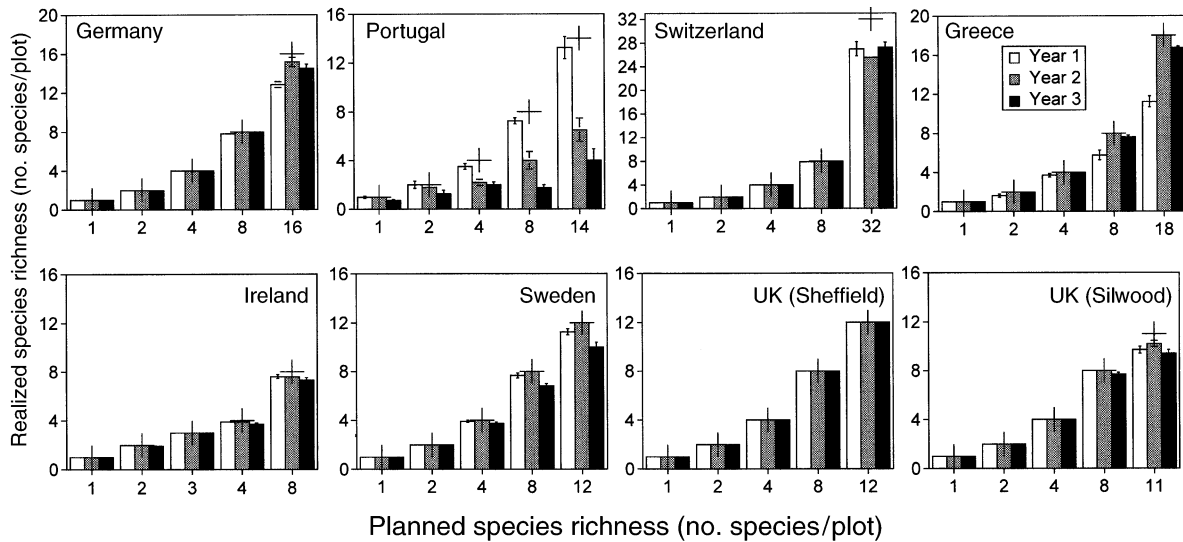


FIG. 1. Realized species richness of all sites in the first three years of the experiment. (Realized species richness is the number of species present in each plot and year.) Large crosses indicate planned species richness (the number of species sown in the plots at the start of the experiment). Data are means  $\pm$  1 SE of all compositions within species richness levels.

of the single-year variables were strongly influenced by this aspect of diversity).

## RESULTS

### *Realized species richness*

There was a strong correlation between the planned and realized diversity gradients over the three years of the experiment ( $R^2 = 0.93$ ; Table 2). However, realized numbers of species deviated sometimes from the numbers sown due to two causes: some species failed to germinate or successfully establish in the first year of the experiment; and, less commonly, some species went extinct from plots in the second or third year (Fig. 1). While many factors had significant effects on realized richness, they all explained  $<1\%$  of the variance apart from site (4.1%, Table 2). The greatest species loss occurred in Portugal, partly due to extreme climate conditions during the third year of the study. Therefore, because realized richness generally matched planned richness well, and because planned richness was the experimental treatment factor, all of the following analyses were calculated with planned richness as explanatory variable. Analyses that replace planned richness with realized richness show no difference from the general outcome and planned richness actually has significantly more explanatory power than realized richness (for a discussion of why, see Hector et al. [2000b, 2002b]).

### *Vegetation cover*

The effects of plant diversity (species and functional richness and species composition) on vegetation cover varied strongly with year and site (Fig. 2a, Table 3). Absolute cover increased over the three years of the experiment at five sites but not in Germany, Portugal,

and Sweden. In Ireland, there was an especially strong positive establishment effect of biodiversity, with low cover during the first year, but full cover in the subsequent years. Overall, vegetation cover was positively related to increasing numbers of plant species and functional groups (Table 3). The effect of diversity on cover was often stronger later in the experiment (except UK: Sheffield and Ireland). Interestingly, this was not due to higher increases in cover over time in high-diversity plots but the opposite: cover in low-diversity plots in Switzerland, Germany, Sweden, and UK: Silwood decreased from the first to the third year. The southernmost and northernmost European sites in Portugal and Sweden had lower vegetation cover than the other sites with less extreme climatic conditions. In these systems where productivity is limited, measurements in unmanipulated "reference" plots showed that cover was normally lower (Table 1). In Portugal, a dry season followed by uncharacteristic frosts killed many plants in the experimental and reference plots, reducing cover in both although more strongly in the experimental plots (average reduction in percent cover from year 2 to 3: experimental plot =  $-83\%$ , reference plots =  $-56\%$ ). In Sweden, ice cover created bare patches in one of the two blocks in the first and second winter and the most limiting factor for growth was low winter temperature, resulting in short growing seasons due to long snow cover or deep soil frost.

### *Aboveground biomass*

Overall diversity had a positive effect on aboveground biomass. Aboveground biomass increased log linearly with species richness and linearly with increasing number of functional groups (Table 3). However, across all three years the deviation around the species-

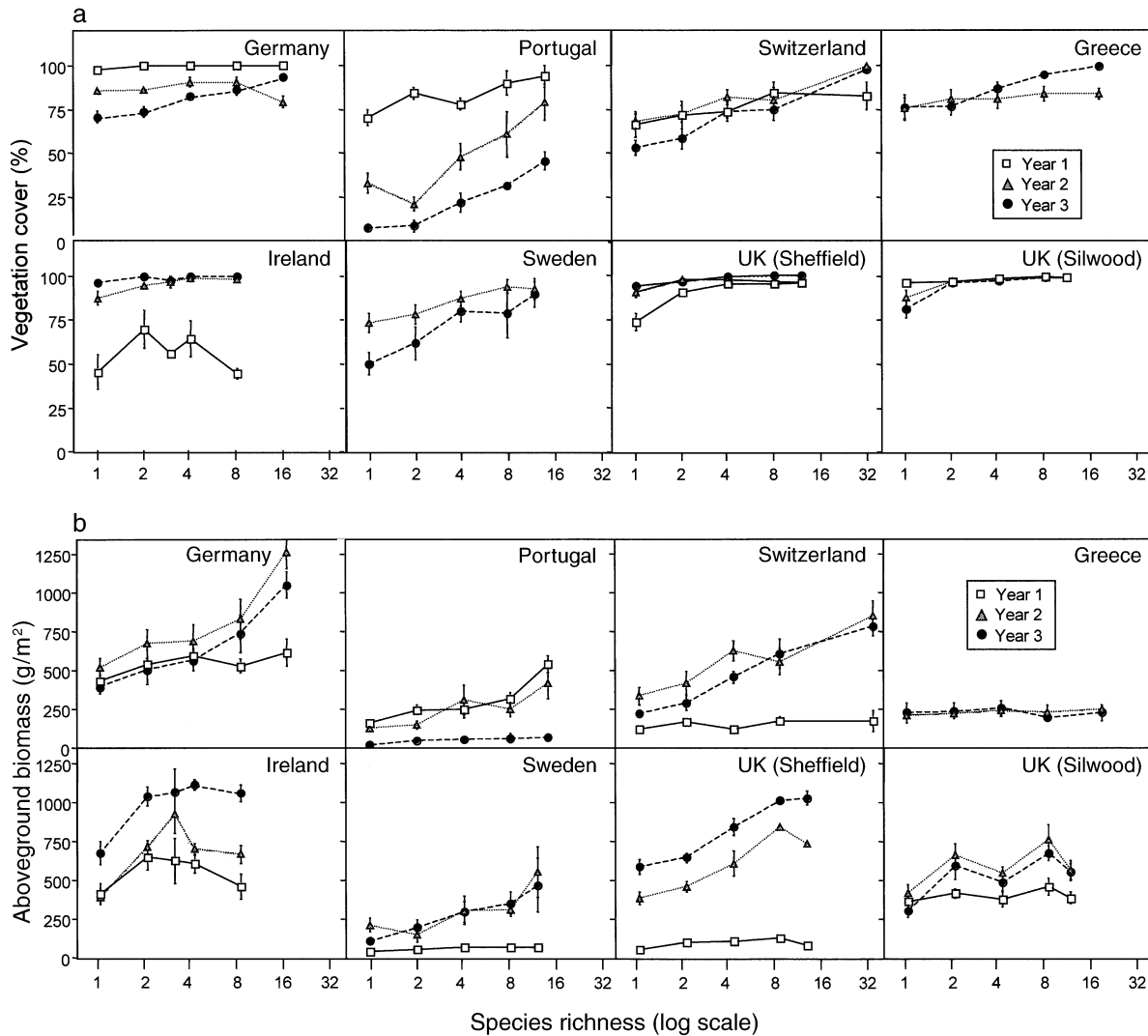


FIG. 2. Species richness effects (a) on vegetation cover (%) and (b) on aboveground biomass of all sites in the first three years of the experiment. Data are means  $\pm$  1 SE of all mixtures within species-richness levels.

richness effect became significant as well, and the effects of diversity varied with sites (Fig. 2b, Table 3). At most sites (except Portugal) the biodiversity–productivity relationship became more positive over time. However, in Greece the lack of a significant effect of diversity on productivity persisted. As reported in Hector et al. (1999), not surprisingly production was lower in the southern and northern sites where growing seasons are more restricted than in the mid-European sites. Biomass was usually lowest in the first year of the experiment—except in Portugal where it declined in year three due to extreme climatic events (see above)—and then increased with time (Ireland, UK: Sheffield) or settled at similar levels in years two and three (other sites; Fig. 2b).

#### Additive partitioning of biodiversity effects

There was a main effect of species richness on the complementarity effect: across all sites and years the

effect of species richness on aboveground biomass production was positive with a slope of  $2.5 \pm 0.5$ ;  $F_{1,69} = 10.1$ ,  $P < 0.01$ ; Table 4). The strength of the richness–complementarity effect relationship was weaker at the start of the experiment than in the second and third year (Fig. 3a), except for Portugal. The grand means for the net, complementarity, and selection effect were all highly significantly positive by the end of the experiment, and the net and complementarity effect increased on average over the three years (net effect years 1–3:  $37 \pm 6.4$  g/m<sup>2</sup>,  $80 \pm 9.5$  g/m<sup>2</sup>,  $99 \pm 9.4$  g/m<sup>2</sup>, respectively; complementarity effect:  $54 \pm 11.6$  g/m<sup>2</sup>,  $55 \pm 9.0$  g/m<sup>2</sup>,  $77 \pm 8.8$  g/m<sup>2</sup>, respectively; Fig. 3a), whereas the selection effect was more variable (selection effect:  $-17 \pm 9.9$  g/m<sup>2</sup>,  $25 \pm 6.1$  g/m<sup>2</sup>,  $21 \pm 4.8$  g/m<sup>2</sup>, respectively; Fig. 3b) (all data: means  $\pm$  1 SE). The selection effect was initially negative, meaning that communities were initially dominated by species with a lower-than-average monoculture biomass.

TABLE 3. Results of repeated-measures ANOVA for percent cover of vegetation and aboveground biomass (in grams per square meter) during the first three years of the experiment.

Source of variation	Cover					
	df	SS	MS	F	P	SS %
Site	7	288 869	41 267.0	12.6	0.002	34.1
Block (within site)	7	22 884	3269.0	27.6	0.000	2.7
Species richness ( $\log_2$ )	1	43 529	43 529.0	53.9	0.000	5.1
Species richness (deviation)	9	6225	691.7	0.9	0.565	0.7
Functional richness (linear)	1	6067	6066.6	7.5	0.007	0.7
Functional richness (deviation)	1	117	117.4	0.1	0.703	0.0
Site $\times$ Species richness ( $\log_2$ )	7	4697	671.0	1.0	0.466	0.6
Site $\times$ Functional richness (linear)	7	11 939	1705.6	2.5	0.040	1.4
Composition	173	139 690	807.5	1.2	0.313	16.5
Site $\times$ Composition	28	19 188	685.3	5.8	0.000	2.3
Plot	238	28 192	118.5	1.1	0.147	3.3
Year	1	18 505	18 504.9	174.9	0.000	2.2
Year $\times$ Site	7	131 094	18 727.7	24.2	0.000	15.5
Year $\times$ Block	7	5424	774.8	7.3	0.000	0.6
Year $\times$ Species richness ( $\log_2$ )	1	2550	2550.0	10.0	0.002	0.3
Year $\times$ Functional richness (linear)	1	0.2	0.2	0.0	0.978	0.0
Year $\times$ Site $\times$ Species richness ( $\log_2$ )	7	5051	721.6	2.5	0.039	0.6
Year $\times$ Site $\times$ Funct. richness (linear)	7	709	101.3	0.4	0.922	0.1
Year $\times$ Composition	183	46 711	255.3	0.9	0.691	5.5
Year $\times$ Composition $\times$ Site	28	8068	288.2	2.7	0.000	1.0
Residual	556	58 835	105.8			6.9
Total	1277	848 343				

### Canopy structure

Vertical distribution of biomass varied markedly between sites (Fig. 4, Table 5). This variation is partly due to differences in biomass yields, but also to strong differences in canopy heights and therefore in how dense the biomass is packed in the canopy. In some sites, canopies were tall, most notably in Germany, leading to a moderate biomass density ( $\sim 1000 \text{ g/m}^3$ ), while in other sites canopies were short and dense,

especially in Ireland ( $\sim 1500 \text{ g/m}^3$ ) and UK: Sheffield ( $\sim 2500\text{--}3000 \text{ g/m}^3$ ). Over the three years there was no significant overall diversity effect; instead, only individual layers at some sites differed significantly in density with changing diversity (Table 5). In contrast, during the third year, both canopy height and center of gravity of vertical biomass distribution significantly increased with diversity, although the effects varied with site (Fig. 5a, Table 6). Diversity-related changes in

TABLE 4. Net biodiversity effect (net effect), partitioned into complementarity (complementarity effect) and selection mechanisms (selection effect) on aboveground biomass for all sites in the third year of the experiment.

Source of variation	Net effect						Complementarity effect	
	df	SS	MS	F	P	SS %	SS	MS
Grand mean (constant)	1	33 552.1	33 552.1	187.0	0.0000	54.1	21 501.0	21 501.0
Site	7	5563.5	794.8	2.2	0.1756	9.0	4745.1	677.9
Block (within site)	6	2150.7	358.5	6.7	0.0000	3.5	1589.7	265.0
Species richness ( $\log_2$ )	1	2852.0	2852.0	15.9	0.0002	4.6	2142.9	2142.9
Species richness (deviation)	5	642.3	128.5	0.7	0.6136	1.0	1224.3	244.9
Functional richness (linear)	1	2612.4	2612.4	14.6	0.0003	4.2	1174.5	1174.5
Functional richness (deviation)	1	184.5	184.5	1.0	0.3141	0.3	177.6	177.6
Site $\times$ Species rich. ( $\log_2$ )	5	959.6	191.9	1.1	0.3847	1.5	2112.1	422.4
Site $\times$ Functional rich. (lin.)	7	1475.2	210.7	1.2	0.3287	2.4	1487.9	212.6
Composition	69	12 378.9	179.4	3.3	0.0000	19.9	14 703.8	213.1
Plot	101	5447.1	53.9	1.1	0.3398	8.8	6867.4	68.0
Year	1	3029.6	3029.6	59.9	0.0000	4.9	1474.0	1474.0
Year $\times$ Site	7	2568.9	367.0	3.0	0.0833	4.1	1962.7	280.4
Year $\times$ Block	7	847.4	121.1	2.4	0.0217	1.4	684.0	97.7
Year $\times$ Species richness ( $\log_2$ )	1	215.4	215.4	3.0	0.0866	0.3	0.8	0.8
Year $\times$ Functional richness (lin.)	1	235.2	235.2	3.3	0.0736	0.4	17.0	17.0
Year $\times$ Site $\times$ Species rich. ( $\log_2$ )	5	933.9	186.8	2.6	0.0311	1.5	499.8	100.0
Year $\times$ Site $\times$ Funct. rich. (lin.)	7	947.5	135.4	1.9	0.0822	1.5	1380.3	197.2
Year $\times$ Composition	75	5357.5	71.4	1.4	0.0251	8.6	6726.5	89.7
Residual	270	13 661.9	50.6			22.0	18 027.9	66.8
Total	577	62 063.7					66 998.2	



TABLE 3. Extended.

Biomass					
df	SS	MS	F	P	SS %
7	5 × 10 <sup>7</sup>	7 205 361	116.7	0.000	34.2
7	432 168	61 738.29	3.2	0.003	0.3
1	9 954 045	9 954 045	91.0	0.000	6.7
9	2 622 586	291 398.4	2.7	0.006	1.7
1	827 526	827 526	7.6	0.007	0.6
1	82 332	82 332	0.8	0.387	0.0
7	1 952 473	278 924.7	2.5	0.042	1.4
7	1 166 519	166 645.6	1.5	0.217	0.8
173	1.9 × 10 <sup>7</sup>	109 382.9	1.0	0.574	12.9
28	3 167 946	113 140.9	5.8	0.000	2.2
238	5 388 968	22 642.72	0.8	0.985	3.9
1	8 871 089	8 871 089	308.8	0.000	5.9
7	1.3 × 10 <sup>7</sup>	1 795 133	58.3	0.000	8.8
7	215 396	30 770.86	1.1	0.380	0.2
1	2 078 579	2 078 579	54.3	0.000	1.4
1	261 487	261 487	6.8	0.010	0.1
7	1 102 119	157 445.6	3.6	0.007	0.7
7	380 279	54 325.57	1.2	0.311	0.2
182	6 961 881	38 252.09	0.9	0.855	4.8
28	1 219 032	43 536.86	1.5	0.044	0.8
664	1.9 × 10 <sup>7</sup>	28 727.09			12.6
1418	1.5 × 10 <sup>8</sup>				

aboveground biomass and canopy structure also affected light interception and transmittance. Levels of transmitted PAR (photosynthetically active radiation) at the base of the canopies generally declined with increasing diversity (Fig. 5b, Table 6).

*Controlling for diversity effects on vegetation cover*

We repeated the analysis of aboveground biomass (Fig. 2a and b, Table 3) adding vegetation cover as a

covariate. Cover did indeed have a highly significant effect on biomass ( $F_{1,201} = 133.4, P < 0.001$ ) indicating that diversity effects on aboveground biomass were related in part to increased cover. Nonetheless, there was still a highly significant residual effect of species richness on biomass ( $F_{1,201} = 46.5, P < 0.001$ ), which was not explained simply by greater levels of vegetation cover. Effects of diversity (species and functional-group richness) on canopy-structure variables

TABLE 4. Extended.

Complementarity effect			Selection effect				
F	P	SS %	SS	MS	F	P	SS %
100.9	0.0000	32.1	3572.1	3572.1	22.8	0.0000	6.5
2.6	0.1364	7.1	9866.3	1409.5	24.1	0.0006	18.1
3.9	0.0015	2.4	351.1	58.5	1.8	0.0995	0.6
10.1	0.0023	3.2	416.4	416.4	2.7	0.1077	0.8
1.2	0.3432	1.8	167.0	33.4	0.2	0.9559	0.3
5.5	0.0218	1.8	52.1	52.1	0.3	0.5661	0.1
0.8	0.3645	0.3	0.1	0.1	0.0	0.9845	0.0
2.0	0.0921	3.2	586.2	117.2	0.7	0.5903	1.1
1.0	0.4407	2.2	2184.1	312.0	2.0	0.0687	4.0
3.1	0.0000	21.9	10 813.2	156.7	4.9	0.0000	19.8
1.0	0.4462	10.6	3218.9	31.9	0.6	0.9986	5.9
22.1	0.0000	2.2	1159.8	1159.8	21.6	0.0000	2.1
2.9	0.0938	2.9	1908.6	272.7	4.2	0.0395	3.5
1.5	0.1804	1.0	457.2	65.3	1.2	0.2930	0.8
0.0	0.9250	0.0	724.8	724.8	7.5	0.0075	1.3
0.2	0.6645	0.0	365.3	365.3	3.8	0.0549	0.7
1.1	0.3600	0.7	388.5	77.7	0.8	0.5469	0.7
2.2	0.0437	2.1	306.0	43.7	0.5	0.8636	0.6
1.3	0.0471	10.0	7203.7	96.1	1.8	0.0004	13.2
		26.9	14 481.9	53.6			26.5
			54 651.0				

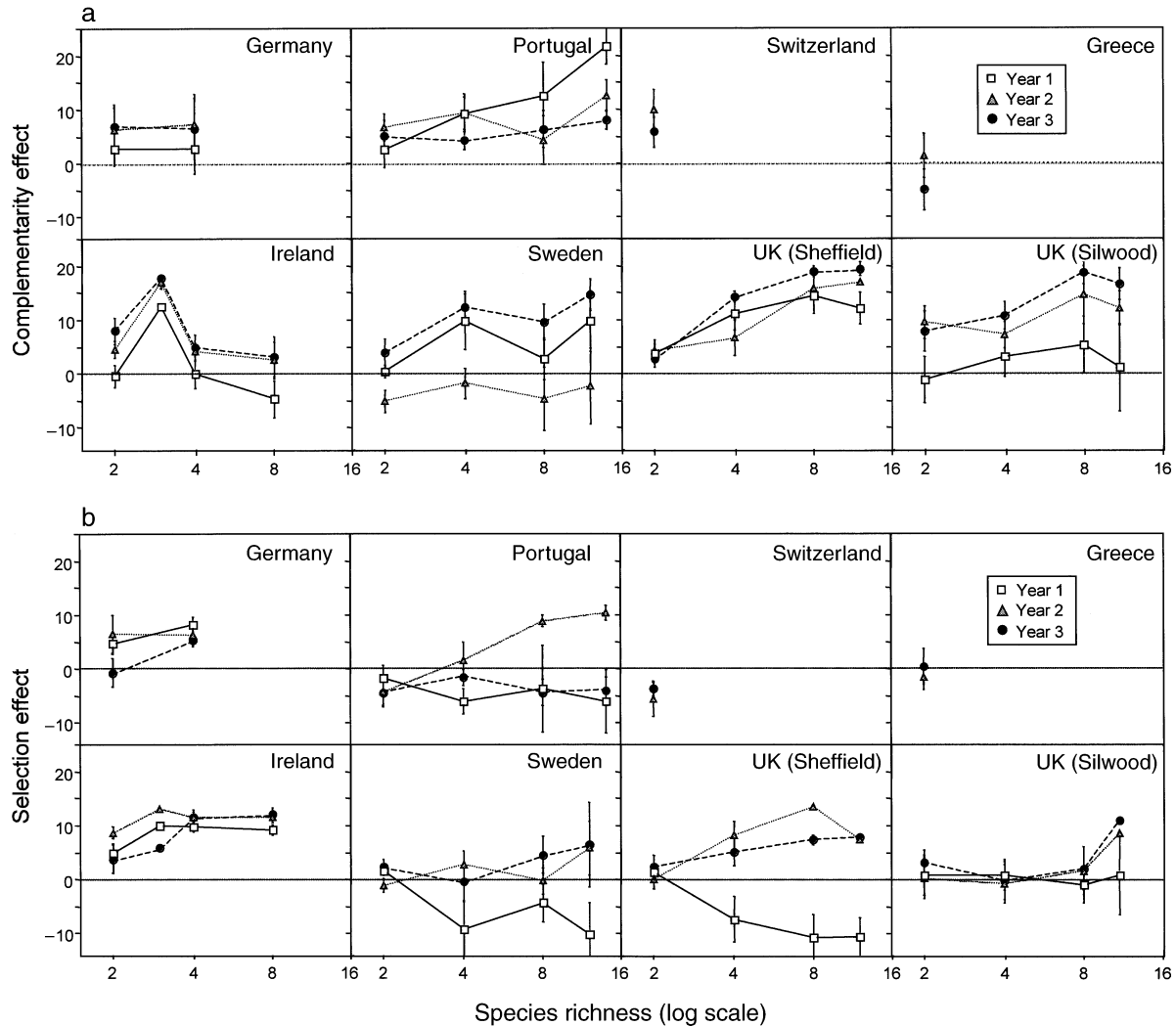


FIG. 3. Complementarity and selection effects as a function of species richness in the first three years of the experiment at all sites. (a) Contribution of complementarity to over-yielding of aboveground biomass (complementarity effect). (b) Selection effect on aboveground biomass. Data are means  $\pm$  1 SE of all mixtures within species richness levels. Values are square-root transformed to meet the assumptions of analyses but preserve the original positive and negative signs.

remained significant for light use, canopy height, and center of gravity of vertical biomass distribution,  $P < 0.05$ ).

#### Nitrogen in aboveground biomass

Total N content in aboveground biomass increased with diversity (Fig. 6, Table 7) in parallel with the increase in aboveground biomass (Table 3, Fig. 2a). Germany, Switzerland, and Sweden showed the strongest increase in biomass and N content, mainly driven by legume presence (see *The role of legumes . . .*, below), whereas at the other sites only a weak increase was measured. Results of the third year were similar to published results from the second year of the experiment (Spehn et al. 2002).

#### Root biomass

The effect of species richness on root biomass was consistent across sites and generally slightly positive (Fig. 7, Table 8). However, there were also large and highly significant differences in root biomass between sites explaining 53% of total variation, which might not only reflect differences in vegetation type, soil chemistry, and texture, but also differences in methodology (e.g., efficiency of root extraction from soils of differing types).

#### Soil nitrogen

Total inorganic (soluble) nitrogen concentrations differed strongly among sites (Fig. 8), with mean values ranging from 1.7 mg/kg (Greece) up to 29.0 mg/kg

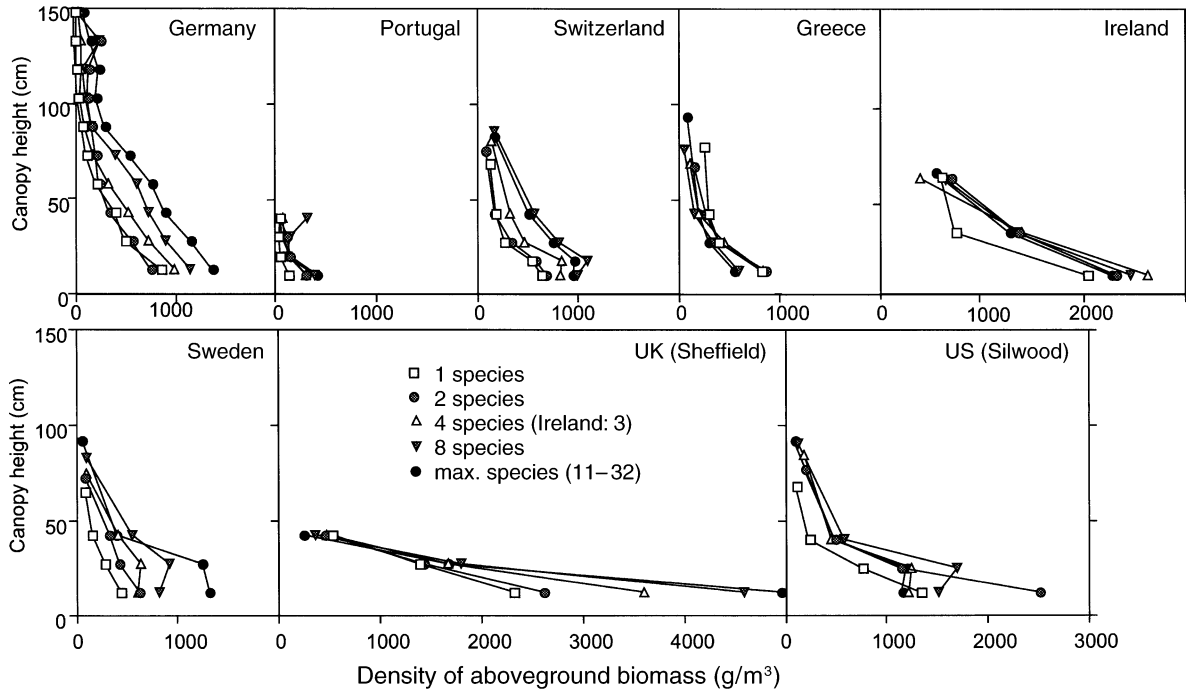


FIG. 4. Species richness effects on density of aboveground biomass in different canopy layers at all sites.

(Switzerland), reflecting different soil types, former land use, and soil fertility (see Supplement) and presumably to a much lesser extent also different soil-analysis methods (see *Methods: Soil nitrogen*, above). Consequently, up to 79% of the total variation in soil N in our full statistical model was attributed to the site term. There was no overall effect of species richness on N concentrations in the soil, but increasing the number of plant functional groups led to a decrease in soil

N (Fig. 8, Table 9). Species composition had the largest effect, explaining more than half of the sum of squares remaining after eliminating the large site effect.

#### Decomposition

The amount of standard material decomposed differed mainly between sites, with Portugal and UK (Sheffield and Silwood) showing a lower cotton decomposition rate than the other sites (less than 0.5%

TABLE 5. Results of repeated-measures ANOVA for different layers of canopy density in the third year.

Source of variation	df	SS	MS	F	P	SS %
Site	7	563 714 954	80 530 708	36.6	0.000	13.9
Block (within site)	7	15 414 174	2 202 025	1.5	0.167	0.4
Species richness ( $\log_2$ )	1	5 337 796	5 337 796	1.9	0.170	0.1
Species rich. (deviation from log-lin.)	9	15 227 455	1 691 939	0.6	0.794	0.4
Functional richness (linear)	1	12 339 538	12 339 538	4.4	0.038	0.3
Functional richness (deviation)	1	843 247	843 247	0.3	0.584	0.0
Site $\times$ Species richness ( $\log_2$ )	7	22 843 110	3 263 301	1.9	0.110	0.6
Site $\times$ Functional richness (lin.)	7	3 589 047	512 721	0.3	0.950	0.1
Composition	166	466 250 781	2 808 740	1.6	0.066	11.5
Site $\times$ Composition	28	48 433 417	1 729 765	1.1	0.392	1.2
Plot	229	335 619 338	1 465 587	3.0	0.000	8.3
Layers ( $\log_2$ )	1	1 047 493 706	1 047 493 706	2114.7	0.000	25.8
Layers $\times$ Site	7	292 270 197	41 752 885	12.7	0.002	7.2
Layers $\times$ Block	7	23 088 453	3 298 350	6.7	0.000	0.6
Layers $\times$ Species richness ( $\log_2$ )	1	665	665	0.0	0.988	0.0
Layers $\times$ Functional richness (lin.)	1	6 519 965	6 519 965	2.4	0.123	0.2
Layers $\times$ Location $\times$ Species richness ( $\log_2$ )	7	44 494 696	6 356 385	5.7	0.000	1.1
Layers $\times$ Site $\times$ F. richness (lin.)	7	2 411 164	344 452	0.3	0.943	0.1
Layers $\times$ Composition	176	478 809 893	2 720 511	2.5	0.004	11.8
Layers $\times$ Site $\times$ Composition	27	29 928 422	1 108 460	2.2	0.000	0.7
Residual	1304	645 909 009	495 329			15.9
Total	2001	4 060 539 028				

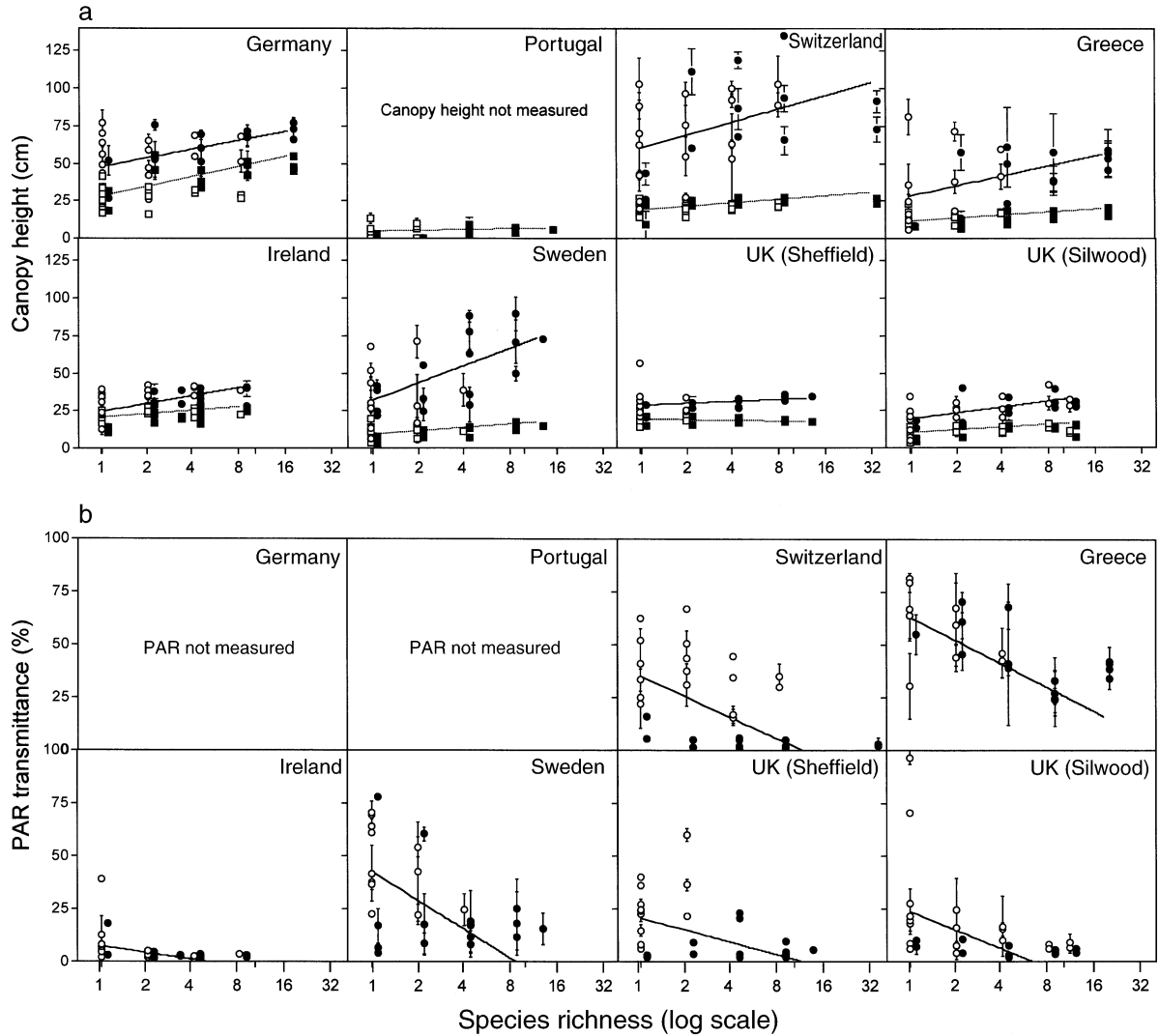


FIG. 5. Species richness effects on (a) total canopy height (circles) and center of gravity of vertical biomass distribution (squares) and on (b) PAR transmittance (percentage of photosynthetically active radiation measured at the bottom of the canopies) in year 3 of the experiment at all sites. Solid symbols represent compositions containing one or several legume species; open symbols represent compositions without legumes. Data are means  $\pm$  1 SE of each composition; the regression slope is from the overall statistical model (Table 6).

TABLE 6. Results of ANOVA of canopy height, center of gravity, and photosynthetically active radiation (PAR) at the base of the canopy in the third year.

Source of variation	Height						Center of gravity		
	df	SS	MS	F	P	SS %	df	SS	MS
Site	6	107 185	17 864	40.1	0.000	41.6	7	35 706	5101
Block (within site)	7	3122	446	5.3	0.000	1.2	7	104	15
Species richness ( $\log_2$ )	1	27 281	27 281	49.3	0.000	10.6	1	2063	2063
Species richness (deviation)	8	1805	226	0.4	0.914	0.7	9	958	106
Functional richness (linear)	1	23	23	0.0	0.839	0.0	1	13	13
Functional richness (deviation)	1	717	717	1.3	0.257	0.3	1	5	5
Site $\times$ Species richness ( $\log_2$ )	6	8871	1479	5.4	0.001	3.4	7	851	122
Site $\times$ Funct. richness (lin.)	6	645	107	0.4	0.876	0.3	7	409	58
Composition	152	84 011	553	2.0	0.018	32.6	173	8296	48
Site $\times$ Composition	26	7071	272	3.2	0.000	2.7	28	1570	56
Residual	202	17 000	84				238	1961	8
Total	416	257 731					479	51 936	



per day, Fig. 9). Again, this difference probably reflects a mixture of biological differences and differences in soil type, etc. (see *Methods: Soil conditions*, above). On average, species and functional diversity had a positive effect on cotton decomposition, and functional diversity also had a positive effect on wood decomposition (Table 10). However, the relationship was clearly driven mainly by the Greek site, which showed the strongest response to an increase in species richness (Fig. 9).

*The overall effect of species composition*

There were significant differences among the 200 different species compositions at the eight sites with regard to all ecosystem processes (significant Composition or Site  $\times$  Composition terms in Tables 1–9) except for root biomass and wood decomposition. Because we had 200 different compositions formed from over 100 different species, we concentrated mainly on the role of nitrogen-fixing species as a group. Individual species effects are being analyzed for publication in a later paper.

*The role of legumes in generating biodiversity effects*

In general, a significant part of the differences between species compositions (after having included the effect of species and functional-group richness in the models) was explained by the presence of legumes. However, there were several significant ( $P < 0.05$ ) site  $\times$  legume interactions for PAR ( $F_{6,26} = 3.6$ ), belowground biomass ( $F_{7,28} = 3.2$ ), and soil N ( $F_{6,12} = 18.91$ ). Similarly, the year  $\times$  site  $\times$  legume interaction was also sometimes significant in the repeated-measures analysis: aboveground biomass ( $F_{7,28} = 5.8$ ), complementarity effect ( $F_{7,75} = 3.5$ ). The effect of legumes clearly often varied with site, time, or both. However, sometimes the effects of legumes were simpler. For the selection effect, the influence of legumes interacted with time but not site (legume  $\times$  year interaction  $F_{1,75} = 11.6$ ,  $P < 0.01$ ). Second, the positive effect of legumes on cellulose (cotton-strip) decomposition did

not differ between sites, but showed a significant main effect ( $F_{1,184} = 22.45$ ,  $P < 0.001$ ). Finally, for canopy height, legumes did not contribute significantly to the effect of species composition ( $F_{1,152} = 0.83$ ,  $P > 0.3$ ).

A significant (all  $P < 0.05$ ) residual main effect of species richness remained, when the presence of legumes was added first as a covariate in the analyses: for aboveground biomass, ( $F_{1,173} = 39.2$  vs.  $F_{1,173} = 91.0$  in Table 3), for complementarity effect ( $F_{1,69} = 3.5$  vs.  $F_{1,69} = 10.1$  in Table 4), for canopy height ( $F_{1,152} = 38.0$  vs.  $F_{1,152} = 49.3$  in Table 6), for PAR ( $F_{1,134} = 11.3$  vs.  $F_{1,134} = 51.6$  in Table 6), for belowground biomass ( $F_{1,173} = 6.8$  vs.  $F_{1,173} = 8.8$  in Table 8), and for soil N ( $F_{1,139} = 11.30$  vs.  $F_{1,139} = 0.9$  in Table 9). For the selection effect in this modified analysis, species richness interacted significantly with site ( $F_{5,69} = 1.1$ ,  $P = 0.0068$  vs.  $F_{5,69} = 0.7$  in Table 4).

DISCUSSION

Most biodiversity manipulation experiments to date have been conducted at single sites (but see Emmerson et al. 2001 and Van der Putten 2000), usually over relatively short time periods (but see Tilman et al. 2001) and often focusing on a small number of ecosystem processes (but see Hooper and Vitousek 1997). Here, we analyze data (see Supplement) on a wide range of ecosystem processes collected with standardized experimental protocols at eight different European grassland sites for a minimum of three years. The general conclusion of our study is that ecosystem functioning at our European grassland sites was influenced by all three of our experimental variables. Not surprisingly, differences between locations and differences in species composition both explained substantial and significant amounts of the variation of most of the ecosystem processes examined. Nevertheless, against this background of geographic variation, and when tested against the variation due to species composition, all ecosystem processes examined were significantly affected by changes in one or more aspects of diversity (species and/or functional-

TABLE 6. Extended.

Center of gravity			PAR					
<i>F</i>	<i>P</i>	ss %	df	SS	MS	<i>F</i>	<i>P</i>	ss %
343.3	0.000	68.8	5	72 847	14 569	21.4	0.001	40.0
1.8	0.087	0.2	6	4089	682	9.6	0.000	2.2
43.0	0.000	4.0	1	21 542	21 542	51.6	0.000	11.8
2.2	0.023	1.8	7	969	138	0.3	0.938	0.5
0.3	0.603	0.0	1	943	943	2.3	0.135	0.5
0.1	0.747	0.0	1	22	22	0.1	0.819	0.0
2.2	0.069	1.6	5	3676	735	1.6	0.223	2.0
1.0	0.425	0.8	5	2245	449	0.9	0.474	1.2
0.9	0.733	16.0	134	55 906	417	0.9	0.673	30.7
6.8	0.000	3.0	18	8511	473	6.7	0.000	4.7
			158	11 213	71			
			341	181 963				

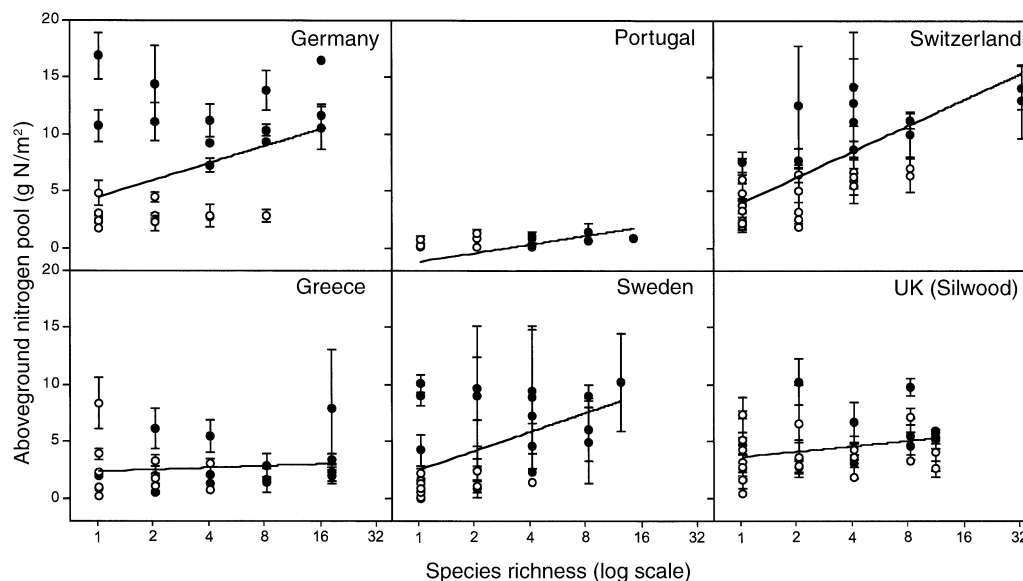


FIG. 6. Species richness effects on aboveground biomass nitrogen in year 3 of the experiment. Solid symbols represent compositions containing one or several legume species; open symbols represent compositions without legumes. Data are means  $\pm$  1 SE of each composition; the regression slope is from the overall statistical model (Table 7).

group richness). Communities with higher levels of diversity reached higher cover, were often more productive, and utilized resources more completely including light, soil nitrogen and space. In short, our extended analysis reveals that the effects of diversity are more widespread than just the previously analyzed variables and years (Hector et al. 1999, Spehn et al. 2002).

However, the effects of diversity varied with both site and time. Some processes, such as short-term (cotton) decomposition and canopy spacefilling (biomass density) were more strongly affected at some sites than at others, and long-term (wood) decomposition was not significantly affected by changes in species richness at any site, but only by the number of functional groups. In general, the effects of diversity were stronger and more consistent aboveground than below (see the following section and Joshi et al. 2004).

#### *Linking diversity effects to different ecosystem processes*

Aboveground, higher-diversity communities showed a consistent pattern of higher complementarity-effect values, greater resource use in terms of light and space, higher productivity, and therefore larger pools of N in biomass. This linkage of greater complementarity, resource use, and productivity is consistent with theories based on niche differentiation and resource partitioning (Tilman et al. 1997b, Loreau 1998, 2000), but is partly also based on facilitation (e.g., through fixed N as well as possible influence of nurse-plant effects, reduction of physical stress, mechanical support, etc. [Callaway 1995, Mulder et al. 2001, Bruno et al. 2003]). While interactions between legumes and non-legumes have been addressed in some ways by theory (Schwinning and Parsons 1996), the increase in the resource supply

TABLE 7. Results of ANOVA for aboveground biomass nitrogen pool in the third year.

Source of variation	df	SS	MS	F	P	SS %
Site	5	1609.7	321.9	14.1	0.006	23.2
Block (within site)	5	114.5	22.9	3.9	0.002	1.7
Species richness ( $\log_2$ )	1	736.6	736.6	42.5	0.000	10.6
Species rich. (deviation from log-linear)	8	227.3	28.4	1.6	0.122	3.3
Functional richness (linear)	1	233.0	233.0	13.4	0.000	3.4
Functional richness (deviation)	1	2.6	2.6	0.1	0.698	0.0
Site $\times$ Species richness ( $\log_2$ )	5	442.2	88.4	5.3	0.005	6.4
Site $\times$ Functional richness (linear)	5	146.2	29.2	1.7	0.181	2.1
Composition	126	2191.5	17.4	1.0	0.498	31.6
Site $\times$ Composition	16	267.8	16.7	2.9	0.000	3.9
Residual	166	963.0	5.8			
Total	339	6934.6				

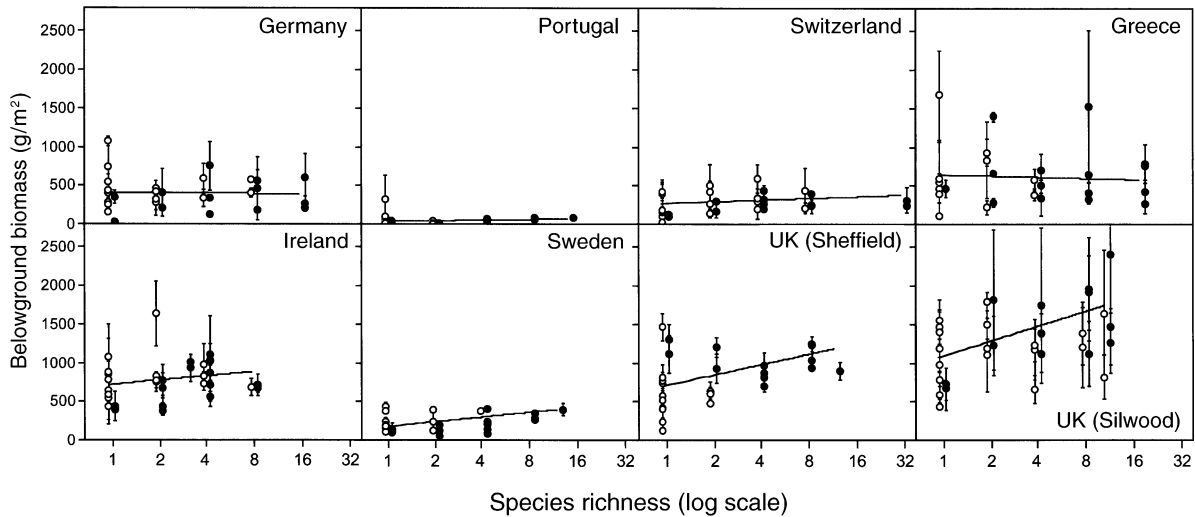


FIG. 7. Species richness effects on root biomass of the top 20-cm soil layer in year 3 of the experiment. Solid symbols represent compositions containing one or several legume species; open symbols represent compositions without legumes. Data are means  $\pm$  1 SE of each composition; the regression slope is from the overall statistical model (Table 8).

driven by some species through  $N_2$  fixation and how this affects the relationship between biodiversity and ecosystem functioning is something that has yet to be properly addressed by theory.

The picture belowground was not so clear. Existing theory predicts (Tilman et al. 1997b, Loreau 1998) that with increasing diversity and therefore higher uptake, levels of unconsumed soil N should fall due to interspecific differences in exploiting different portions of the available N pool either in space or in time. This type of effect has been observed in some other biodiversity experiments (Ewel et al. 1991, Tilman et al. 1996, 1997a, Hooper and Vitousek 1997, Niklaus et al. 2001; but see Naeem et al. 1995, Hooper and Vitousek 1998, Symstad et al. 1998, Kenkel et al. 2000). Apart from differences in methods that partly account for the observed variability between sites, differences in soil N pools may also be a result of regionally different contributions of biological  $N_2$  fixation to overall N availability. Using isotope techniques at three sites (Germany, Sweden, and Portugal) we have shown that

legumes were actively fixing atmospheric N, which was also transferred to neighboring non-legume species (Mulder et al. 2002, Spehn et al. 2002). At all other sites, except Greece, both biomass and tissue-N data also support this observation. Depending on the presence or absence of actively  $N_2$ -fixing legumes in mixtures, we saw an altered pattern of diversity on soil N pools. Communities without legumes showed little overall pattern in available soil N. With legumes, however, there was generally a decline in available soil N with increasing diversity. More detailed analyses revealed that there was no correlation between the abundance of legumes and soil mineral N across all sites, except UK Silwood where higher legume biomass was associated with higher soil N pools by the third year. Lower levels of unconsumed soil N are often associated with greater root biomass (Tilman et al. 2002, Scherer-Lorenzen et al. 2003). We also observed in communities containing legumes an increase in fine-root biomass with greater diversity, which may imply a higher uptake capacity for soil nutrients. These results support

TABLE 8. Summary of ANOVA for root biomass (0 to -20 cm) in the third year.

Source of variation	df	SS	MS	F	P	SS %
Site	7	68 695 652	9 813 664	57.7	0.000	53.0
Block (within site)	7	1 190 714	170 102	1.6	0.132	0.9
Species richness ( $\log_2$ )	1	1 232 544	1 232 544	8.8	0.003	1.0
Species rich. (deviation from log-linear)	9	1 210 921	134 546	1.0	0.477	0.9
Functional richness (linear)	1	400 968	400 968	2.9	0.093	0.3
Functional richness (deviation)	1	13 811	13 811	0.1	0.754	0.0
Site $\times$ Species richness ( $\log_2$ )	7	1 307 461	186 780	1.4	0.243	1.0
Site $\times$ Functional richness (linear)	7	2 550 668	364 381	2.7	0.027	2.0
Composition	173	24 300 029	140 462	1.1	0.455	18.7
Site $\times$ Composition	28	3 729 102	133 182	1.3	0.176	2.9
Residual	238	25 054 230	105 270			
Total	479	129 686 100				

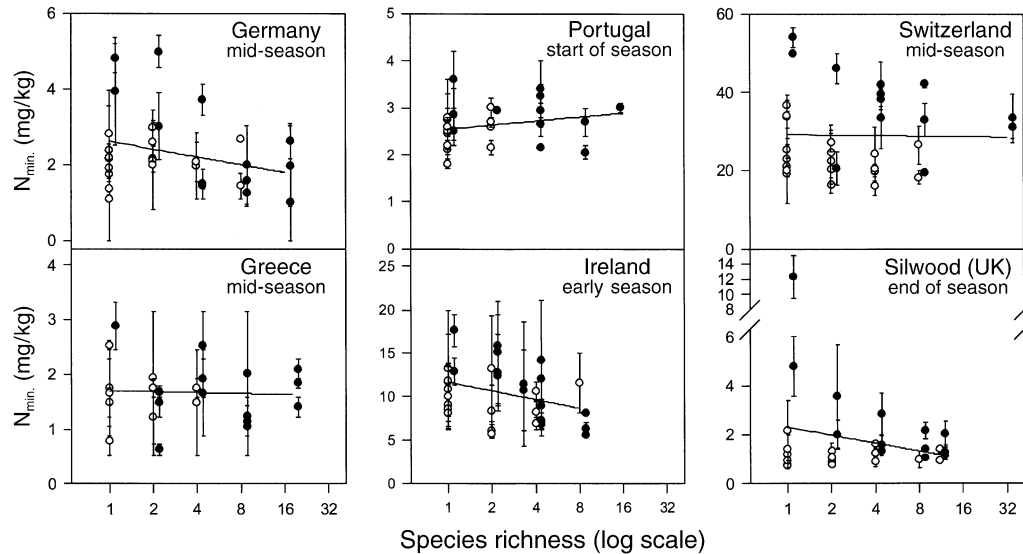


FIG. 8. Species richness effects on soil inorganic nitrogen concentrations within the rooting zone (top 20-cm soil layer) in year 3 of the experiment. Note the different scales for the y-axes. Solid symbols represent compositions containing one or several legume species; open symbols represent compositions without legumes. Data are means  $\pm$  1 SE of each composition; the regression slope is from the overall statistical model (Table 9).

the view that more diverse systems are more efficient in nitrogen uptake when additional N input occurs through fixation or when soils are not N limited (Scherer-Lorenzen et al. 2003).

The belowground process that showed the least influence of diversity was decomposition. There was a significant effect only of functional-group richness and not of species richness on wood decomposition. The increase in cotton decomposition with greater diversity was clearly mainly driven by a strong relationship only at the Greek site. This is a good example of different processes responding differently to changes in diversity, since in Greece, where plants grew on an unusual soil high in  $Mg^{2+}$ ,  $Ca^{2+}$  and very low in  $PO_4^{3-}$  (see Supplement), aboveground production and many other processes were largely unaffected (Troumbis et al. 2000). In general, direct plant-diversity effects on processes such as wood decomposition would imply niche

differentiation between plant species influencing this particular process (Lawton 2000). More plausible are indirectly driven diversity effects through changes in biotic (e.g., fauna) and abiotic (e.g., moisture) soil conditions, which are more likely to be idiosyncratically linked to species identity than to species richness, as shown by earlier work on diversity effects on litter decomposition (Wardle et al. 1997).

Why do such differences in above- and belowground responses occur? First, belowground responses may be delayed due to long-term effects of previous land use—and in our case site preparation (see *Methods: Establishment of experimental . . .*, above)—on soil chemical, physical, and biological properties. This could mask weak diversity–function relations for some time until they manifest over the long-term (Nilsson et al. 1999, Compton and Boone 2000, Hooper et al. 2000). Second, indirect effects of plant diversity on microbial

TABLE 9. Results for ANOVA for soil mineral nitrogen pools in the third year.

Source	df	SS	MS	F	P	SS %	SS % <sup>†</sup>
Site	5	36 329	7265.8	145.9	0.000	79.0	
Block (within site)	5	249	50.0	4.9	0.000	0.5	2.6
Species richness ( $\log_2$ )	1	35	35.0	0.9	0.349	0.1	0.4
Species rich. (deviation from log-linear)	8	130	16.3	0.4	0.913	0.3	1.3
Functional richness (linear)	1	289	289.0	7.3	0.008	0.6	3.0
Functional richness (deviation)	1	10	10.0	0.3	0.616	0.0	0.1
Site $\times$ Species richness ( $\log_2$ )	5	42	8.4	0.1	0.983	0.1	3.0
Site $\times$ Functional richness (linear)	5	614	122.8	1.9	0.151	1.3	8.3
Composition	139	5501	39.6	0.6	0.924	12.0	52.4
Site $\times$ Composition	15	960	64.0	6.3	0.000	2.1	9.9
Residual	181	1845	10.2			4.0	19.1
Total	366	46 004				100.0	100.0

<sup>†</sup> After eliminating the large site effect.



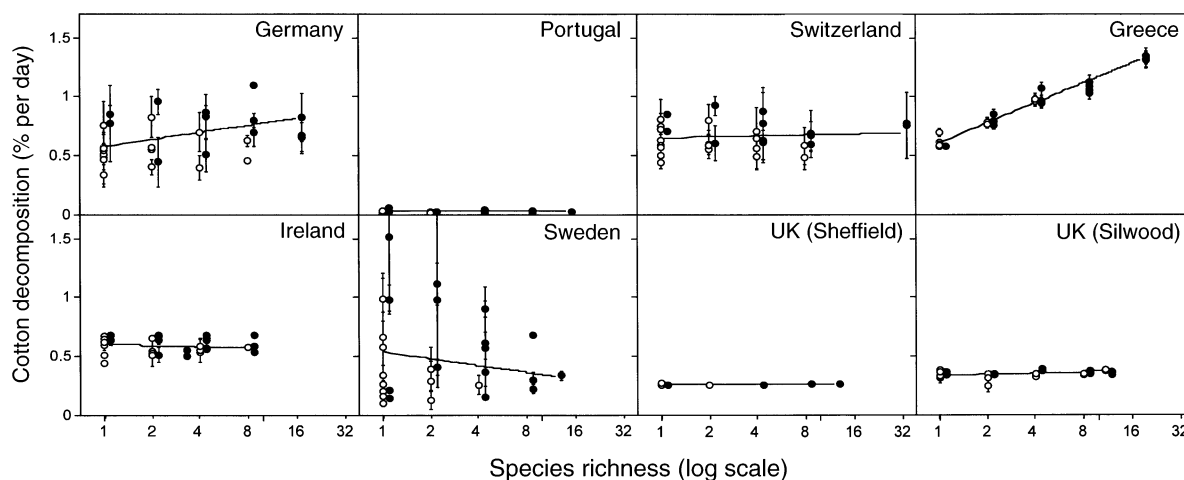


FIG. 9. Species richness effects on decomposition of cotton-strip standard material (percentage dry-mass loss per day) in year 3 of the experiment. Solid symbols represent compositions containing one or several legume species; open symbols represent compositions without legumes. Data are means  $\pm$  1 SE of each composition; the regression slope is from the overall statistical model (Table 10).

processes (e.g., via altered soil microclimate or via changes in root exudates) such as mineralization, nitrification, or immobilization may equal or even exceed direct effects of plant uptake, as shown by Hooper and Vitousek (1998), Niklaus et al. (2001), and Zak et al. (2003). Third, soil food-web properties and related soil processes like decomposition and mineralization might be more tightly coupled to soil abiotic conditions and species composition than to plant species diversity (Wardle et al. 1999), which may explain why no consistent pattern was found over our large gradient in site characteristics. Fourth, apart from abiotic stress conditions at particular sites, functional redundancy of soil organisms (e.g., Brussaard et al. 1997, Bradford et al. 2002), and the fact that belowground processes are much slower than aboveground responses (Wardle et al. 1999, Wardle 2002) have been suggested as explanations for the lack of quick responses in biogeochem-

ical processes to changes in producer diversity (Joshi et al. 2004).

#### *Variation of diversity effects over time*

For the variables measured over multiple years, biodiversity effects were often lowest in the first year and increased in year two, or three, or both. For example, aboveground biomass increased in its mean value (Ireland and UK Silwood) or in diversity slope (Germany, Switzerland, Sweden, and UK Sheffield) over time. However, such trends were never universal: aboveground biomass remained unaffected by diversity in all years in Greece (but leaf-area index increased significantly with a log-linear increase in species richness in the third year at the Greek site) and mean biomass declined dramatically in Portugal (with a reduced slope). Similarly, looking at mean values the complementarity and net biodiversity effects also tended to

TABLE 10. Results of ANOVA for decomposition of standard material (cotton and wood) in the third year.

Source of variation	Cotton						Wood					
	df	SS	MS	F	P	SS %	df	SS	MS	F	P	SS %
Site	7	26.8	3.8	12.2	0.002	57.6	4	0.2039	0.0510	27.1	0.004	50.8
Block (within site)	7	2.2	0.3	16.8	0.000	4.7	4	0.0075	0.0019	2.5	0.045	1.9
Species richness ( $\log_2$ )	1	0.6	0.6	13.5	0.000	1.2	1	0.0001	0.0001	0.2	0.685	0.0
Species rich. (deviation from log-linear)	9	1.5	0.2	3.8	0.000	3.1	7	0.0026	0.0004	0.6	0.742	0.6
Functional richness (linear)	1	0.3	0.3	6.8	0.010	0.6	1	0.0047	0.0047	7.8	0.006	1.2
Functional richness (deviation)	1	0.0	0.0	0.0	1.000	0.1	1	0.0011	0.0011	1.8	0.180	0.3
Site $\times$ Species richness ( $\log_2$ )	7	1.5	0.2	2.1	0.089	3.2	4	0.0016	0.0004	0.9	0.492	0.4
Site $\times$ Functional richness (linear)	7	0.1	0.0	0.1	0.994	0.3	4	0.0029	0.0007	1.7	0.239	0.7
Composition	160	7.1	0.0	0.4	0.999	15.2	114	0.0688	0.0006	1.4	0.390	17.1
Site $\times$ Composition	24	2.5	0.1	5.6	0.000	5.4	9	0.0039	0.0004	0.6	0.812	1.0
Residual	219	4.1	0.0				140	0.1047	0.0007			
Total	443	46.6					289	0.4018				

increase with time, indicating that positive effects of diversity on biomass are not caused only by interspecific differences in maximal growth rate. In contrast, the selection effect was more variable between years and sites. These results thus partly support the conceptual model of a transition from dominance of sampling effects at the beginning of biodiversity experiments to dominance of complementarity effects at later stages, as outlined by Pacala and Tilman (2002). This transition occurs due to changes in competitive dynamics, with dominance of exponential-growth dynamics at very early stages and slower competitive dynamics later on. While we saw an increase in complementarity over time, the sampling effect was not strong at the beginning of our experiment. Similarly, cover showed surprisingly complex changes over time. Overall mean values became both more positive (e.g., Ireland) and negative (Germany, Portugal). The slope with diversity also became steeper at some sites (e.g., Switzerland). In some cases changes occurred only over part of the diversity gradient: e.g., UK Silwood, where cover of some monoculture plots declined with time, vs. UK Sheffield, where cover of low-diversity plots increased with time. We discuss this to point out that cover patterns were not a simple product of establishment effects but that combinations of mortality and developmental cycles in key species can also lead to canopy gaps, and these effects can vary with diversity. In some cases, mortality probably reflects expected population processes, e.g., thinning or natural decline of the initial cohorts (effects of life history), and in others the impact of extreme climatic events (e.g., Portugal).

#### *Variation of diversity effects with site*

At the design level our experiment is primarily focused on testing effects of diversity (the central question) and not differences due to site and species composition (which are already well known). A study focused on abiotic explanations for site differences would need a much larger number of sites. We are also limited in the interpretation of site effects on some ecosystem processes as they reflect differences in both biology and methodology (for example, soil N was not estimated at the same time within the growing season). Nevertheless, as would be expected, site had strong effects on all ecosystem processes. Gradients in temperature and rainfall between sites correlate with production, with reduced productivity in most northern and most southern sites (see Hector et al. 1999: Note 20). Site differences in the impact of legumes may be explained by levels of available phosphorous (Spehn et al. 2002). In some cases certain sites were unusual, e.g., Portugal experienced a strong external effect from a dry period and particularly frosty winter. Greece showed no or little effect of diversity on aboveground biomass production (see also Troumbis et al. 2000) and on most of the measured ecosystem processes, except for cotton decomposition where it showed the strongest

effect of species richness. The fact that diversity had no effect on biomass at our least productive site (Greece) and increased with aboveground production, is consistent with Fridley (2002, 2003), who found that effects of experimental diversity gradients on productivity significantly increased with soil fertility (but see Kenkel et al. 2000).

Our first publication on aboveground biomass patterns from the second year of the experiment (Hector et al. 1999) showed no significant interaction between diversity and site, implying a single positive slope with different intercepts for different sites (Hector et al. 1999: Fig. 1). Our multisite repeated-measures analyses of a larger range of ecosystem processes examined over three years present a picture of broader and stronger biodiversity effects, but also revealed significant interactions of diversity with site, time, or both on aboveground biomass. When drawing comparisons between these results with those of Hector et al. (1999), it needs to be considered that they include data from the first year when the plots were establishing and patterns appear to be different mainly due to lack of time to develop. Nevertheless, the changing patterns over time are qualitatively consistent with Tilman et al. (2001) who also found differences over time: an initially saturating relationship between diversity and biomass became linear in a longer-term analysis (our changes were less strong but also over a shorter period). More recently, Fukami and Morin (2003) have demonstrated that ecosystem functioning can be affected by history: even under highly controlled conditions the same set of species can achieve different productivities depending on the order of community assembly.

#### *Diversity effects in the context of composition and location*

In terms of the proportion of the total variation explained (i.e., the multiple  $R^2$  shown as the percentage sums of squares explained in our tables), site and composition (plus sometimes their interaction) explained the largest absolute portions. However, these variables also require a lot of degrees of freedom to do it—especially species composition with 173 degrees of freedom. Composition is therefore a complex variable that is hard to interpret or use for prediction (but see Hector et al. 1999: Fig. 3, Petchey et al. 2004). In contrast, while species richness explains less of the sums of squares for treatments its contribution is more parsimonious in only requiring a single degree of freedom for the regression. What is more, with diversity on a log scale we have a simple linear relationship, which is straightforward to use for prediction. A critical feature of our design is that it explicitly replicated species composition (variation that is pooled into the error term of most other biodiversity experiments because there are no replicates for specific compositions), and the species richness effect is tested in the context of the composition effect by dividing the mean square for

species richness by the mean square for composition in the  $F$  test.  $F$  values larger than 1 then indicate that the average effect of species richness (per degree of freedom) is greater than the average effect of composition. Thus, when degrees of freedom are taken into account together with sums of squares, the average species-richness effect (as assessed by the  $F$  value) is 90 times larger than the average species-composition effect (for biomass). Thus, while diversity explains less of the total variation than do site and composition it is important to acknowledge that this relatively simple and easy-to-obtain measure can make simple and repeatable predictions about levels of productivity and other ecosystem processes.

#### *Biodiversity effects and biological mechanisms*

The results of our extended analyses of the selection effect on aboveground biomass are broadly consistent with previously published results (Loreau and Hector 2001). While the selection effect became more positive later in the experiment it was both weaker (e.g., its percentage sums of squares for the (log) species richness effect is one quarter that for the complementarity effect) and more variable than the complementarity effect. Dominance through the selection effect was neither by the most productive species (the selection effect was sometimes even negative, that is by species with lower-than-average monoculture yields) nor was it the main driving force behind our results. In contrast, the complementarity effect produced one of the simplest and most consistent results in our experiment: there was a general positive relationship between diversity and the complementarity effect across all years and sites. This was evident even in the face of a wide variety of different soil and climate conditions across the range of sites in the experiment. Even though a variety of different soil and climate conditions were present due to the range of sites in the experiment, a positive complementarity effect seemed to be the rule. Analysis of different forms of transgressive overyielding produced qualitatively similar results to those already reported in Hector et al. (2002a).

What biological mechanisms underlie these effects of changes in diversity? Higher levels of vegetation cover could explain the diversity effects in some sites, notably in Portugal (Caldeira et al. 2001). This was due to better establishment of some species in higher-diversity mixtures relative to lower-diversity plots and monocultures (probably a “nurse crop” effect), or to higher mortality or reduced growth in low-diversity compositions after two or three years. However, significant effects of species richness remained after controlling for the influence of diversity via cover.

In many of our analyses additional diversity effects remain after controlling for the effects via increased cover, legume presence, and similar likely causes. Interactions of plants with mycorrhizas (e.g., van der Heijden et al. 1998), differentiation in rooting depth (Ber-

endse 1982), resource type (McKane et al. 2002), and hydrological niche (Silvertown et al. 1999) might be possible mechanisms for these “additional” biodiversity effects. The additive-partitioning analyses reveal that both selection and complementarity effects played a role, with the latter dominating. Niche complementarity may also occur in relation to other trophic levels through the effects of natural enemies (see Mulder et al. 1999, Koricheva et al. 2000, Spehn et al. 2000a, Joshi et al. 2004).

It appears that effects of biodiversity began almost as soon as our seeds germinated through “nurse crop”-type effects (in the first year, and at sites with many annuals also in later years) since establishment of some species was better in high-diversity communities. Sampling effects may then have acted through the inclusion of fast-growing species in higher diversity communities (Huston 1997, Pacala and Tilman 2002), although we did not see a strong influence of sampling on biomass production. The vegetation in higher diversity communities then exploited more available space both in two (cover) and three (canopy volume) dimensions as well as filling space more intensively through increased canopy density aboveground and greater root biomass and density belowground. These in turn led to greater light interception and greater availability of N in soil due to the additional  $N_2$  fixation in communities with legumes. The legume effect represents both resource partitioning through use of atmospheric vs. soil N and, later in the experiment, facilitation as fixed N was taken up by non-legumes. While we cannot tease all of these effects apart completely, our analyses suggest they develop at least partly in concert, rather than as a simple, linear, causal chain. Path analysis of the legume effect at the Swedish site revealed that initially the simple presence or absence of legumes explained most of the diversity effect, but later in the experiment numbers of both legume and non-legume species became important as well as significant additional effects of diversity per se (Mulder et al. 2002). However, path analysis may be of limited use since we already know the different effects of biodiversity are largely colinear (Petraitis et al. 1996, Allison 1999, Naeem 2002). Species richness is colinear with the presence and number of legumes in our design, therefore effects will be attributed to the single strongest effect, as we suspect often happens when we give priority to the legumes in analyses given that complementarity effects also occur in experiments where legumes are not included (van Ruijven and Berendse 2003).

The influence of diversity in communities with and without legumes could also be examined with appropriate design constraints, namely, by having equal numbers of mixtures with and without legumes across the gradient of species richness. However, this would be hard or impossible to achieve at low levels of diversity, where there may be a limited number of legume vs. non-legume species, and at high levels of diversity,

where species from all functional groups must be included to achieve the desired high level of diversity. We still know relatively little about the details of competition, complementarity, and coexistence in grassland communities. While the first generation of biodiversity experiments has generated a lot of new information about the relationship between diversity and ecosystem processes, and developments in analytical methods can tell us something about the causes, only a new generation of more mechanistic experiments will reveal more about the underlying biology.

### Conclusions

Altering biodiversity through changes in the numbers and types of plant species and functional groups in our experimental communities significantly affected all 11 ecosystem processes examined over the three-year period. In general, high-diversity communities were more productive, had stronger complementarity effects, and exploited more resources by intercepting more light, taking up more nitrogen, and utilizing more 2- and 3-dimensional space. These longer-term results give greater support to niche-differentiation models as an explanatory mechanism of the results, as complementarity was the stronger underlying biodiversity effect. Diversity effects were stronger aboveground than belowground. In particular, clear diversity effects on decomposition were only observed at one site. Our results therefore reinforce our previous findings and reveal stronger and more extensive effects of altered plant diversity. They also extend the generality of diversity–ecosystem functioning relationships to multiple sites, years, and processes. However, because the effects of biodiversity vary with time and site, understanding this variation will help integrate the results of biodiversity–manipulation experiments with studies of the control of ecosystem functioning at the larger scale.

### ACKNOWLEDGMENTS

Many colleagues too numerous to list have assisted with the project; in particular we thank Phil Heads for project coordination and John Nelder for advice on statistical analyses. Christian Körner, Pascal Niklaus, and three anonymous reviewers provided valuable comments on the manuscript. The European Science Foundation (LINKECOL) funded an exchange with the Centre of Population biology at Silwood Park (UK), where parts of this manuscript were prepared. Funds of the Institute of Environmental Sciences of the University of Zurich and the Botanical Institute of the University of Basel contributed to the preparation of the manuscript. The BIODEPTH project was funded by the European Commission within the Framework IV Environment and Climate program (ENV-CT95-0008) and by the Swiss Federal Office for Education and Science (Project EU-1311 to B. Schmid).

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#### SUPPLEMENT

A supplement containing the data from the BIODEPTH project (15 ecosystem-process variables measured at eight different European grassland field sites over three years) together with metadata and a table with site information is available in ESA's Electronic Data Archive: *Ecological Archives* M075-001-S1.