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Acta Oecologica 29 (2006) 85–96

ACTA OECOLOGICA
INTERNATIONAL JOURNAL OF ECOLOGY

www.elsevier.com/locate/actoec

Original article

Effect of functional group richness and species richness in manipulated productivity–diversity studies: a glasshouse pot experiment

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Received 7 March 2005; accepted 9 August 2005

Abstract

Species and functional group (grasses, legumes, creeping nonlegume forbs, rosette nonlegume forbs) richness of species assemblages composed of 16 species from four functional plant groups were manipulated to evaluate the productivity-diversity relationships in a greenhouse pot experiment. Pots were filled with sand, and supplied at two levels of nutrients. The plants were grown in monocultures, two, four, eight and 16 species mixtures. Individual two, four, and eight species mixtures differed in the richness of functional groups. Although the two characteristics of biodiversity, i.e. species and functional group richness, were necessarily correlated, it was shown that it is possible to separate their effect statistically, and also test for their common effect without pronounced loss of test power. There was a pronounced increase of average aboveground biomass and a mild increase in belowground biomass with biodiversity. The effect of functional group richness was more pronounced than the effect of the number of species. By using the method of Loreau and Hector (Nature 411 (2001) 72), selection and complementarity effects were statistically separated, and the overyielding index was calculated as a ratio of the productivity of a mixture to the productivity of its most productive component (to demonstrate transgressive overyielding). Positive values of complementarity and transgressive overyielding were both found, particularly in some rich communities and under high nutrient levels. Complementarity significantly increased only with functional group richness and mainly under high nutrients in the belowground biomass. Some species, when grown in monocultures, had decreased productivity under higher nutrients, and thus were more productive in mixtures than in monocultures. It seems that those species suffered from too high nutrient levels when grown in monocultures, but not in the presence of other species, which were able to use the nutrients in high concentrations and effectively decrease the nutrient levels. As a consequence, mixtures of high diversity were always more productive under high nutrients. The difference in species proportions between high and low nutrients, characterized by chord distance, increased with species richness. The relative change in productivity decreased with the number of functional groups. This suggests that species richness might lead to stabilization of aggregate characteristics (like total productivity) under changing environmental conditions by changing the proportions of individual species.

Keywords: Complementarity; Overyielding; Selection effect; Species richness; Plant functional groups

1. Introduction

The critical role of species composition and richness in the dynamics and functioning of ecosystems has been discussed many times over the past two decades. The dependence of productivity on plant species diversity is a hotly debated topic (Naeem et al., 1994; Tilman et al., 1996; Tilman, 1997a; Grime, 2001). Diversity experiments, where the number of species is manipulated and ecosystem functioning (most often productivity) is measured as a response variable, became a standard part of experimental ecology (Naeem and Li, 1997; Hector et al., 1999; Tilman, 1999; Van der Putten et al., 2000; Tilman et al., 2001). However, their proper design and interpretation of their results is still an open question (Johnson et al., 1996; Bengtsson et al., 1997; Grime, 1997; Huston, 1997; Tilman, 1997a, 1997b; Hodgson et al., 1998; Lawton et al., 1998; Naeem et al., 1999; Sankaran and McNaughton, 1999; Hector et al., 2000; Huston et al., 2000; Kaiser, 2000; Naeem et al., 2000; Wardle et al., 2000; Lepš et al., 2001; Cameron, 2002). The observed response of ecosystem processes to species or functional group diversity can be

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doi:10.1016/j.actao.2005.08.003

generated by a combination of different effects (Tilman, 1997a; Loreau, 1998a, 2000; Loreau and Hector, 2001). The complementarity effect arises from niche differentiation: When two species use resources in different ways, their mixture should be able to use the resources more effectively than corresponding monocultures, and consequently, achieve higher productivity. Unless there is intimate knowledge of the mechanisms of species interaction, we are not able to distinguish direct facilitation from niche differentiation, and consequently, both mechanisms are often subsumed under the name complementarity (Loreau and Hector, 2001). Second, the sampling effect model (Aarsen, 1997; Huston, 1997; Tilman et al., 1997) hypothesizes that species differ in their competitive abilities, and that better competitors are also more productive. Under this assumption, communities that have greater diversity should, on average, be more productive because they are more likely to contain one or more highly productive species (selection or chance effect is sometimes used in the same or very similar meaning). Whereas complementarity is generally accepted as a genuine biodiversity effect, it is often questioned whether the sampling effect is no more than an artifact of the design of biodiversity experiments (e.g. Huston, 1997).

The most convincing evidence that biodiversity is beneficial would come from a demonstration of overyielding, i.e. the productivity of a species mixture being higher than the productivity of any of its constituent species grown in isolation (trasnsgressive overyielding sensu Hector et al., 2002). Transgressive overyielding can not be achieved through the sampling effect, and is, consequently, proof that other mechanisms ("complementarity") played a role. Similarly, only overyielding demonstrates that the mixture's function (productivity) can not be replaced by any of the monocultures. Obviously, monocultures of all the species are required to demonstrate overyielding (Garnier et al., 1997). Recently, Loreau and Hector (2001) suggested a new method that enables numerical separation of the sampling and complementarity effects. Their method is based on the relative yield total (RYT, De Witt, 1960) philosophy, and does not require transgressive overyielding to demonstrate complementarity. Their method is based on non-transgressive overyielding (i.e. productivity of a mixture being higher than the weighted average of the corresponding monocultures).

Many researchers consider ecosystem processes to be more consistently associated with functional composition (presence of certain plant functional types or traits) and/or functional richness (number of different plant functional types) than with species richness itself (Lepš et al., 1982; Grime et al., 1997; Hooper, 1998; Hooper and Vitousek, 1998; for a comprehensive review see Díaz and Cabido, 2001). Symstad et al. (1998); Spehn et al. (2002) found that, whereas total plant biomass increases with diversity, most of the diversity effects are attributable to the presence of the functional group of N-fixers. According to Symstad (2000), higher functional group richness increases ecosystem stability, particularly the resistance to invasion.

The belowground environment is more heterogeneous and, consequently, provides more opportunities for complementarity than the aboveground part of the ecosystem. Also, because the importance of competition for light increases with increasing nutrients, it was hypothesized that complementarity should be expected mainly in low nutrient environments.

Lehman and Tilman (2000) predicted that species rich communities should be more stable in aggregated characteristics, like total biomass, but that this stability can be at the expense of the stability of individual populations. Using similar reasoning, the difference between total biomass of assemblages with the same species composition, but grown under a different nutrient regime, should decrease with diversity, but their compositional dissimilarity should increase.

To test these hypotheses, a glasshouse pot experiment was performed, where species richness and functional richness of plant assemblages, and fertility of their environment were manipulated. We used 16 perennial grassland species grouped in four functional types, planted in 10 possible combinations of species and functional group richness, and at two nutrient levels. The pot experiments lack the variability of natural habitats, and consequently restrict (in comparison with field conditions) the possibility of niche differentiation. All of those restrictions have to be taken into account in interpreting the results. However, the pot experiments can be much more replicated than field experiments (which is very important, particularly as we need the monocultures of all of the constituent species), and enable better control of environmental conditions.

The aim of the study was to evaluate diversity effects on productivity in our experimental mixtures, and to separate the effects of species number, functional group number and soil nutrients on these effects. Special attention was paid to differences in the responses of above and belowground biomass.

2. Materials and methods

2.1. Selected experimental species

We selected 16 species which are common and often grow together in mesotrophic meadows in Central Europe. All of them are polycarpic perennials, and can be classified into four functional groups: four grass species — the narrow leaved *Festuca rubra*; the broad-leaved *Trisetum flavescens*, *Alopecurus pratensis* and *Holcus lanatus*; four rosette hemicryptophytes — *Lychnis flos-cuculi*, *Hypochaeris radicata*, *Plantago media* and *Leontodon autumnalis*; four herbs with creeping aboveground stolons — *Veronica officinalis*, *Glechoma hederacea*, *Fragaria vesca* and *Prunella vulgaris*; four nitrogen-fixers (legumes) — *Lotus corniculatus*, *Anthyllis vulneraria*, *Trifolium pratense* and *Lathyrus pratensis*.

2.2. Experimental design

The glasshouse experiment was set up in early May 2002. Round pots (diameter 19 cm, height 18 cm) were filled with

Table 1
Treatments, number of species combinations and number of replications used in each, high and low nutrients pots

Treatment	Number of species in mixture	Number of species combinations	Number of replicates
1	Monoculture of each species	16	2x
2	Two species within one functional group	8	1x
3	Two species from two groups	8	1x
4	Four species (each species is from one group)	4	1x
5	Four species (each two species are from different groups)	4	2x
6	Four species that composed one functional group	4	1x
7	Eight species from two groups	6	1x
8	Eight species from three groups	8	1x
9	Eight species from four groups	6	1x
10	Sixteen species (i.e. four functional groups)	1	8x

sand. Seeds of 1–16 species were sown at the same density, so that each pot contained 160 seeds, and were grown under two nutrient levels. The low nutrient treatment was fertilized each week with a commercial formula (Kristalon start: N 19%, P_2O_5 6%, K_2O 20% and MgO 3%) in concentration of 10 g per 10 l of water, while the concentration in the high nutrient treatment was 20 g per 10 l of water. The experiment was set up in a completely randomized design. Ten combinations of species and functional group richness were used, with different species combinations within each combination (treatments, Table 1), yielding 184 pots at the beginning of the experiment. All species were equally represented at each species richness and functional group richness. An equal number of seeds of each species was sown in each combination. As far as possible, all of the functional groups were equally represented in mixtures containing more functional groups (in mixtures with three functional groups and eight species, the ratio of species belonging to individual groups was 3:3:2). During the experiment, plants were grown under natural daylight conditions in a glasshouse and watered when needed. Six pots were lost for various reasons during the experiment. After 4 months, soil from all 178 pots was rinsed thoroughly, plants were sorted into species, dried and weighed (shoots, i.e. aboveground biomass and roots, i.e. belowground biomass separately).

2.3. Data analysis

Productivity was characterized by above and belowground biomass. For each mixture, the Overyielding index and the complementarity and selection effects were calculated and estimated. The overyielding index (*OI*) was calculated by

$$OI = Y/MAX\left(M_i\right),$$

Where Y is biomass of a mixture and M_i is the biomass of ith species grown in monoculture. *OI* is equivalent to the I_1 index of Garnier et al., (1997) and to $D_{\max}$ (Loreau, 1998a). For statistical analyses, log (*OI*) was used because it is centered around zero in the case that the productivity of the mixture is the same as the productivity of the most productive monoculture (Spaekova and Lepš, 2001). The average value of two replicates of each monoculture was used as M_i.

The method of Loreau and Hector (2001) was used for partitioning selection and complementarity effects. According to them, the net biodiversity effect, ΔY, is characterized by the difference between the observed yield of a mixture and its expected yield under the null hypothesis that there is no selection and complementarity effect:

$$\Delta Y = Y_O - Y_E = N\overline{\Delta RYM} + N\,\mathrm{cov}(\Delta RY, M),$$

where ΔRY_i is the deviation from expected relative yield of species i in mixture, calculated as the difference between expected and observed relative yields. The observed relative yield of a species in mixture is the ratio of its yield in mixture and its yield in monoculture. The expected relative yield is the proportion of the species sown. The complementarity effect is proportional to the average of ΔRY over all species in the mixture, whereas selection is proportional to the covariance of ΔRY and yield of the species in monoculture. N is the number of species in a community.

The dependence of above and belowground biomass and log (*OI*), complementarity and selection effects on the number of species, number of functional groups and nutrient level was analyzed by general linear models (GLM). Species richness and functional group richness were continuous predictors, and nutrient level the categorial predictor. The number of species and number of functional groups are inevitably correlated predictors: the number of functional groups can not exceed the number of species. Also, because there was a closed species pool, the 16 species treatment means inevitably the highest number of functional groups. In GLM, the test of partial effects statistically separates the unique effects of functional group richness and species richness, and consequently, enables their comparison. However, because of correlation between the number of species and number of functional groups, there can be a large shared effect between these two. Consequently, both partial effects can be non-significant, whereas their common effect and their marginal (i.e. effects in absence of the correlated predictor) effects are highly significant. Consequently, we tested also for the common effect (called biodiversity) and for the marginal effects. As the aim of the study was whether biodiversity effects are the same under low and high nutrients, parallelisms (interactions by covariates) were also tested for. Significant result of this test means that the lines are nor parallel, and so the effects are different under low and high nutrients. To stress this, the effect will be called ″Interaction″ in further text. In analyzes of dependence of differences between high and low nutrients on the diversity measures, there is no categorial predictor, and, consequently, multiple regression is used (which is a special case of GLM). The common effect of the two diversity indices is then characterized by the significance of ANOVA of the entire model. Consequently, in this case, the significance of the model corresponds to the significance of ″Biodiversity″ in GLM.

This approach was used to comply with the methodology used in similar experiments (Hector et al., 1999). However, one should be aware that the approach is statistically correct for the biomass values only. In the derived characteristics (*OI*, complementarity, selection), the same monoculture values are used in various pots (i.e. replications), and, consequently, the values are not independent, thus the degrees of freedom are inflated (Spaekova and Lepš, 2001).

Differences between both productivity and relative species composition of pots with the same species composition but different nutrient regime were characterized in the following way: First, pairs were formed between low and high nutrient pots with the same species composition; where there were more replicate with the same species composition, the pairing was random. (Thanks to this, all of the resulting values are independent, and can be correctly used in statistical analyses). For each pair, the following characteristics were calculated.

Plain difference between biomass in high and low nutrient level treatments

$$D = W_h - W_l$$

i.e. the positive value mean increase of biomass in high nutrients. Because the productivity of some combinations was higher in low nutrients, and we were interested in the stability with respect to change of nutrient level, the absolute value of the difference was calculated, i.e. $AD = |D|$, as a characteristic of resistance.

Because it could be expected that the magnitude of the difference is proportional to the productivity of the species combination, the standardized difference (D_{st}) and standardized absolute value of difference (AD_{st}) were calculated by dividing the difference by the average productivity in high and low nutrient treatments:

$$D_{st} = D/((W_h + W_l)/2) \qquad AD_{st} = AD/((W_h + W_l)/2)$$

Compositional similarity was characterized by the standardized Euclidean distance (chord distance, Orloci, 1978)

$$CD = \sqrt{\sum_{i=1}^{s} (X_{h,i} - X_{l,i})^2}$$

where *S* is the number of species in the combination, $X_{h,i}$ and $X_{l,i}$ are the biomass values of *i*th species in high and low nutrient pots, respectively, after standardization by sample norm, so that the length of the sample vector is unity, i.e.

$$\sum_{i=1}^{s} X_i^2 = 1$$

This value was naturally not calculated for the monocultures, where no change in species composition was possible.

3. Results

Aboveground biomass increased with both the number of species and number of functional groups (Fig. 1). The GLM analysis (Table 2) showed that the explanatory power (measured by sum of squares) of the number of functional groups was roughly three times higher than that of the number of species. Of the partial effects, only that of functional group numbers on aboveground biomass was significant. The effect was much more pronounced under high nutrients (significant interaction). Similarly, the effect of biodiversity on belowground biomass was positive, although none of the partial effects was significant (but the total effect of biodiversity was still highly significant). Although the interaction was not sig-

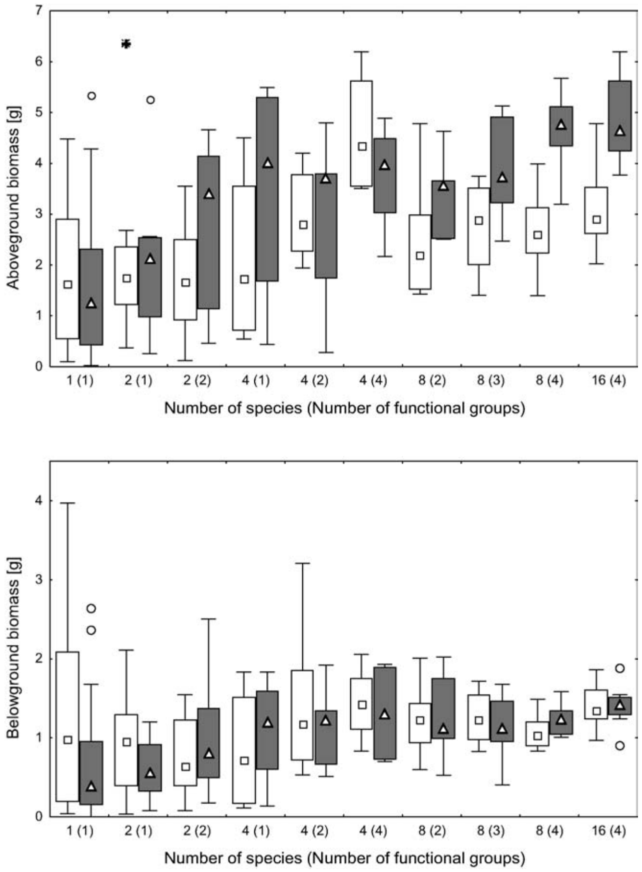

Fig. 1. Above and belowground biomass in pots. Biomass for low nutrient treatment is shown by empty boxes, for high nutrient treatment by filled boxes. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e. range of data within {lower quartile – 1.5 × interquartile range; upper quartile + 1.5 × interquartile range}), (o) – outliers, (*) - extremes.

Table 2
GLM results for the number of species, functional group richness, biodiversity, fertilization in (A) aboveground biomass and (B) belowground biomass. The test of parallelism is included

A) Aboveground biomass					
	SS	df	MS	*F*	*P*
Number of species	5.31	1	5.31	2.83	0.094
Functional group richness	13.53	1	13.54	7.21	**0.008**
Biodiversity	115.15	2	57.58	31.62	**0.000**
Fertilization	12.22	1	12.22	6.51	**0.012**
Interaction	17.11	2	8.56	4.81	**0.009**
Error	326.58	174	1.88		
B) Belowground biomass					
	SS	df	MS	*F*	*P*
Number of species	0.26	1	0.26	0.55	0.459
Functional group richness	0.74	1	0.74	1.58	0.209
Biodiversity	5.16	2	2.58	5.29	**0.006**
Fertilization	2.21	1	2.21	4.72	**0.031**
Interaction	1.68	2	0.83	1.99	0.139
Error	81.39	174	0.47		

nificant, there was still a tendency for stronger dependence under high nutrients. The effect of nutrient level was significant for both above and belowground biomass.

The relationship between biomass production in monoculture and dominance in mixtures was significantly positive under the low nutrient level for both above and belowground biomass. However, species of intermediate or low-to-intermediate monoculture biomass were the most successful ones in mixtures under high nutrient levels, both for above and belowground (Fig. 2). Biomass in monoculture was thus a poor predictor of the success of a species in mixture. Some species were even more productive in the 16 species mixture than in monoculture.

The analysis of log (*OI*) for aboveground biomass showed that its value increased slightly with the number of species; the increase was found under low nutrients only (significant interaction), but on average, the value was higher under high nutrients (Table 3). Values of log (*OI*) for belowground biomass increased similarly with biodiversity under both low and high nutrient levels.

The selection effect value (calculated according to Loreau and Hector, 2001) for aboveground biomass increased with the number of species, but slightly decreases in belowground biomass (Table 3). For aboveground biomass, the relationship was similarly positive under both high and low nutrient levels, with higher values in the low nutrient treatment. The selection effect was negative and decreased with number of species for belowground biomass under both nutrient levels, the decrease being more pronounced in the low nutrient treatment. The complementarity effect did not seem to be affected by any biodiversity characteristics, but increased (mainly with

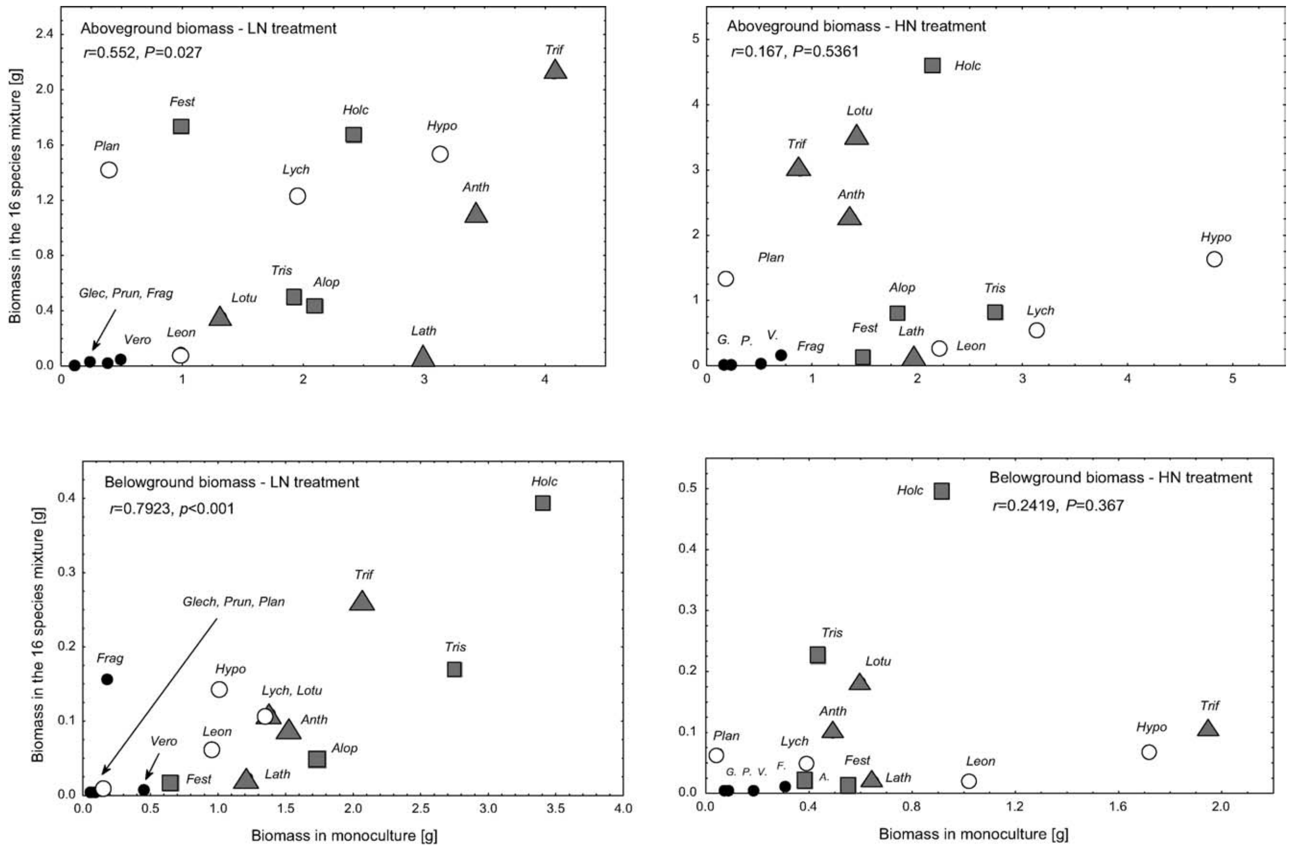

Fig. 2. The relationship between species biomass in monoculture and in mixture of all 16 species. Above and belowground biomass for each of the low (LN) and high nutrient (HN) treatments are shown. *Alop – Alopecurus, Fest – Festuca, Holc – Holcus, Tris – Trisetum, Hypo – Hypochaeris, Leon – Leontodon, Lych – Lychnis, Plan – Plantago, Frag – Fragaria, Anth – Anthyllis, Lath – Lathyrus, Lotu – Lotus, Trif – Trifolium, G. – Glechoma, P. – Prunella, V. – Veronica.* Symbols for functional groups: triangle – legumes, square – grasses, empty circle – rosettes, full circles – creeping forbs.

Table 3

Summary of GLM analyses for log (*OI*), net effect (Net), selection (Select) and complementarity effects (Compl) in (A) aboveground and (B) belowground biomass

A) Aboveground biomass

	log(*OI*)			Net			Select			Compl		
	F	*P*		*F*	*P*		*F*	*P*		*F*	*P*	
Number of species	4.03	**0.047**	▲	1.14	0.287		12.59	**0.000**	▲	0.51	0.477	
Functional group richness	0.60	0.440		1.09	0.297		0.000	0.977		0.24	0.625	
Fertilization	13.46	**0.000**	▲	2.36	0.127		9.35	**0.003**	▼	12.02	**0.000**	▲
Biodiversity	0.42	0.659		3.94	**0.022**		0.83	0.439		1.25	0.289	
Interaction	4.66	**0.012**		0.84	0.434		2.19	0.117		0.84	0.434	

B) Belowground biomass

	log(*OI*)			Net			Select			Compl		
	F	*P*		*F*	*P*		*F*	*P*		*F*	*P*	
Number of species	1.07	0.301		5.04	**0.027**	▲	7.03	**0.009**	▼	3.19	0.077	
Functional group richness	1.06	0.304		0.12	0.725		0.12	0.724		4.92	**0.028**	▲
Fertilization	39.77	**0.000**	▲	25.24	**0.000**	▲	0.6	0.437		10.68	**0.001**	▲
Biodiversity	3.54	**0.032**		0.81	0.447		11.68	**0.000**		8.52	**0.000**	
Interaction	0.14	0.986		0.09	0.918		3.97	**0.022**		2.12	0.124	

DF error for each GLM analysis has the same value of 172. ▲ indicates significant increase in the values of biodiversity indices with the number of species and functional groups; ▼ indicates significant decrease in biodiversity values.

functional group richness) belowground. Its value was higher under high nutrients (Fig. 3). The net effect is the combination of the selection and complementarity effects. Its value increased with diversity in aboveground biomass (only common effect significant), while for belowground biomass, it increased with species richness (Table 3).

The plain non-standardized difference (D) for aboveground biomass increased with biodiversity (the partial effects were not significant), but no dependence was found in belowground biomass (Fig. 4 and Table 4). For some monocultures and low diversity mixtures, the difference was negative, i.e. their biomass was higher in low nutrients. On the contrary, in species rich mixtures, aboveground productivity was always higher under high nutrient levels. The plain standardized difference (D_{st}) and absolute non-standardized difference (AD) did not change with diversity in aboveground biomass, but the total biodiversity effect on AD was significantly negative for belowground biomass. Standardized absolute value of the difference ($\mathrm{AD}_{\mathrm{st}}$) significantly decreased with the number of functional groups in both above and belowground biomass. Chord distance increased with the number of species in the aboveground biomass (Fig. 4 and Table 4).

4. Discussion

4.1. Increase of productivity with diversity

This study demonstrated a pronounced increase in average aboveground biomass and a mild increase in average belowground biomass with biodiversity characteristics. In both, the effect of functional group richness was more pronounced than the effect of number of species. This supports the conclusions of Díaz and Cabido (2001) about the importance of functional differentiation. For aboveground biomass, the increase was much more pronounced under high nutrients. Surprisingly, some low diversity mixtures and monocultures produced more aboveground biomass under low nutrients, and some productive species were more productive in mixtures than when grown in monocultures, usually under high nutrient levels. This might be partially caused by high sowing densities of the species. Typically, *P. media*, *H. lanatus* (and some other species) suffered from high seedling density in monocultures, resulting in low final yield. This effect was more pronounced in the high nutrient treatment. It has been known for a long time in agronomy that increasing the sowing density over some threshold might lead to decreased final yield (see Silvertown and Doust, 1993, pp. 50–53 for discussion). One should be aware, however, that, if this is the case for the most productive species, decreasing its sowing density in mixture might be enough to increase the final yield of the mixture (and getting high values of all "biodiversity effect" indices, including *OI*).

There is, however, an alternative explanation for this effect. This phenomenon was observed mainly under high nutrients. Some of the species might suffer from too high nutrient levels when in monoculture. The presence of other species that are not negatively affected by high nutrients might help to decrease the nutrient level to a level acceptable to other species. If this explanation is correct, then this effect would be a real case of complementarity, i.e. biomass increase caused by the differential ability of species to take up nutrients, or could be even interpreted as facilitation (nutrient uptake of one species improved the conditions for another species). The fact that the increase of aboveground biomass with diversity was much more pronounced under high nutrients, and, also, that the value of the complementarity effect was generally higher under high nutrients, supports this explanation.

4.2. Complementarity and sampling effects

In pot biodiversity experiments, a positive effect of species richness on primary production was usually caused by

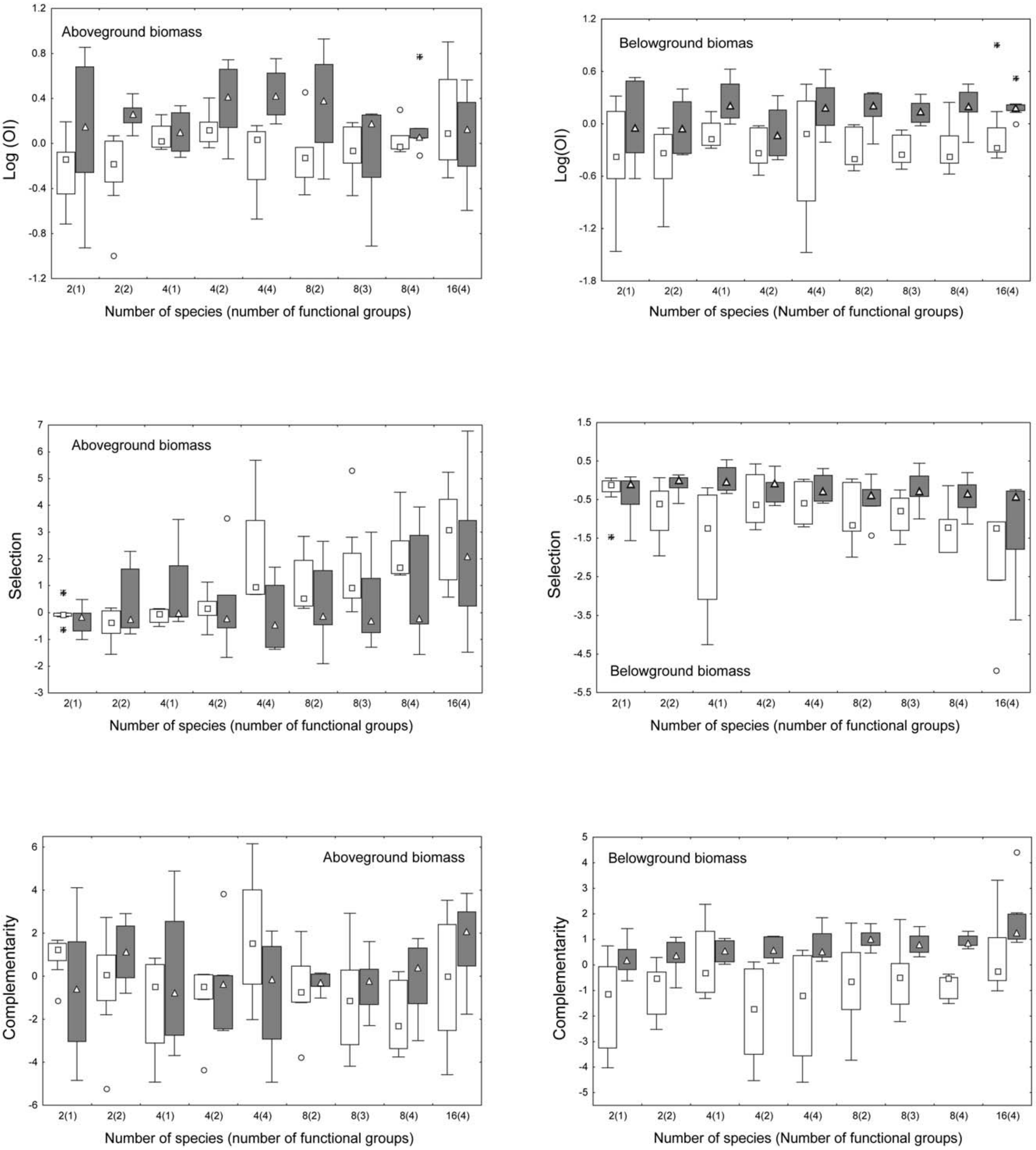

Fig. 3. Dependence of log(*OI*), selection effect and complementarity effect on species richness for above and belowground biomass. Biomass for low nutrient treatment is shown by empty boxes, for high nutrient treatment by filled boxes. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e. range of data within {lower quartile – 1.5 × interquartile range; upper quartile + 1.5 × interquartile range}), (o) – outliers, (*) - extremes.

the sampling effect (positive selection effect) rather than complementarity (Spaekova and Lepš, 2001; Fridley, 2002; Mikola et al., 2002). However, in those studies, the number of individuals was kept constant by thinning the germinated individuals or planting constant numbers of individuals, and was lower than in our case. In this study, the species were sown at high densities and underwent selfthinning during the experiment. This probably resulted in a much lower correlation between monoculture productivity and success in competition (compare this study with Spaekova and Lepš, 2001). The difference in the time of germination is another factor decreasing the correlation between productivity and competitive ability.

Our analyses have shown that the complementarity and selection effects, as calculated by the methods of Loreau and Hector (2001), behave differently from each other. This is

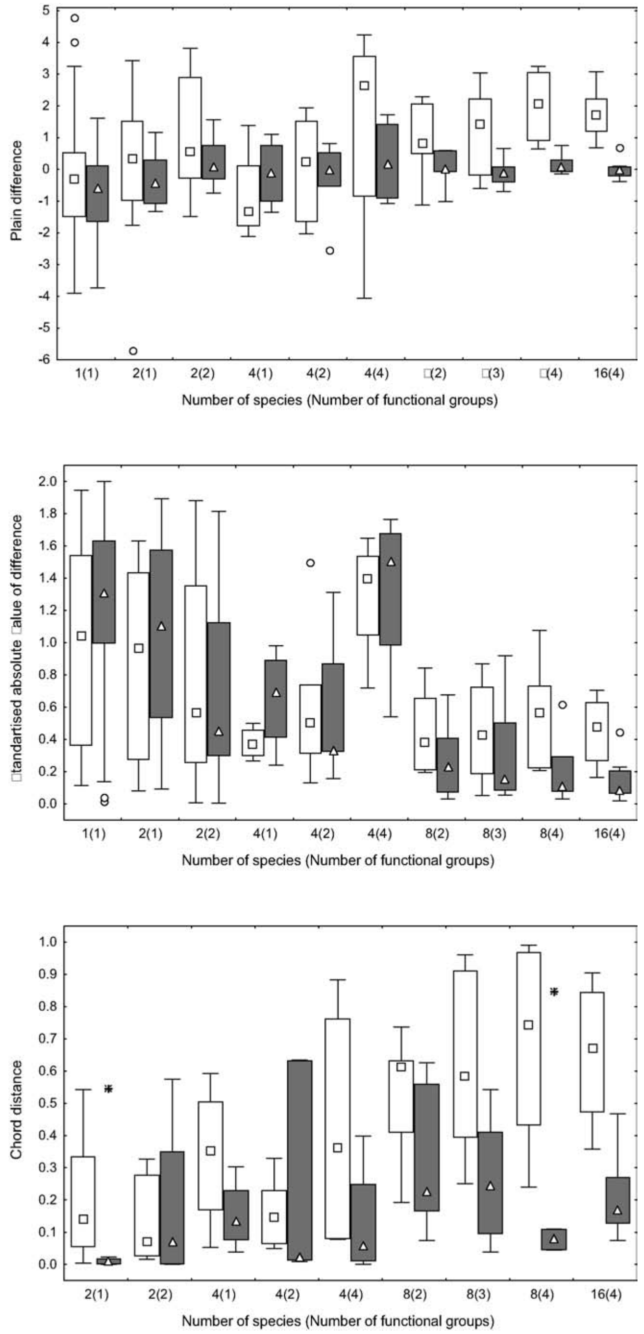

Fig. 4. Plain difference, standardized difference of absolute value and euclidean distance between biomass of high and low nutrient levels. Aboveground biomass is shown by empty boxes, belowground biomass by filled boxes. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e. range of data within {lower quartile – 1.5 × interquartile range; upper quartile + 1.5 × interquartile range}), (o) – outliers, (*) - extremes.

not surprising, because each of them is based on different mechanisms. The matter is probably even more complicated. Although the meaning of those two parts of the biodiversity effect is intuitively clear from the formula, the biological mechanisms affecting their values probably vary from case to case. In particular, it is probably not always complementarity in resource use that leads to positive numerical values. It is based on the RYT methodology, the limitations of which have been nicely shown by Loreau (1998b). This is particularly true when the selection effect became negative.

The calculated complementarity value could also reflect the effect of facilitation (Loreau, 1998b). Legumes, the nitrogen fixers, were one of our functional groups. Of them, *T. pratense* is renown as being a very efficient N_2-fixer (Spehn et al., 2002). Furthermore, agricultural intercropping experiments have shown that nitrogen-fixers in low diversity systems commonly increase nitrogen availability for their neighbors (Vandermeer, 1989). Facilitation by legumes through nitrogen fixation leading to higher biomass production is probably common in similar experiments (Hector et al., 1999; Tilman et al., 2001). However, nitrogen facilitation by legumes should be effective mainly under low nutrient conditions. The opposite is true: when individual pots were compared, *OI* values for aboveground biomass indicate transgressive overyielding (i.e. $OI > 1$) in 40.7% and 77.2% of the low and high nutrient pots, respectively. Even bigger difference was found for belowground biomass: transgressive overyilding was found in 13.8% and 67.2% of low and high nutrient pots. Consequently, other causes were more likely to produce the high complementarity values than nitrogen facilitation by legumes.

4.3. Effect of individual species traits

The experimental mixtures were often dominated by *Holcus*, whereas some species (e.g. *Hypochaeris*), more productive in the monocultures, were subordinate (Fig. 2), mainly under high nutrient levels. Although *Holcus* had intermediate monoculture productivity in high nutrients, it was able to quickly elevate its canopy in mixtures. *Holcus* has the ability to overtop and shade its neighbors by allocation more resources to the stems. Therefore, to be a successful competitor for light in a mixture, a plant must sacrifice some of its potential production when growing in monoculture (Hector et al., 2002). In general, creeping forbs had very low productivity in monocultures, and were nearly eliminated in mixtures, contributing positively to the selection effect. On the contrary, rosette forbs were able to exhibit high productivity in monocultures, but were overtopped in mixtures (usually by grasses, which were not so productive in monocultures). This contributed negatively to the selection effect. It shows that, depending on the experimental setup and/or environmental conditions and species set, the high productivity in monoculture need not be a good predictor of success in competition. A combination of these effects often lead to a negative selection effect and positive complementarity. The resulting productivity depended on the relative strength of the contributing effects. Similar effects were discussed in Hooper and Vitousek (1997); Dukes (2001); Deutschman (2001).

4.4. Effect of nutrient level

The plain difference in productivity between the high and low nutrient treatments is positively correlated with diver-

Table 4
Results of multiple regression with number of species and number of functional groups as explanatory variables for D, AD, D_{st}, AD_{st} and ED in (A) aboveground and (B) belowground biomass

A) Aboveground biomass						
Regression: multiple					simple	
Difference between HN and LN	Explanatory variable	ANOVA	Beta	Sign.	*R*	Sign.
D	Model	$F(2,85) = 4.5452$		*		
	Number of species		0.2934	n.s.	0.3106	**
	Number of functional group		0.0212	n.s.	0.2589	*
AD	Model	$F(2,85) = 0.1473$		n.s.		
	Number of species		0.0942	n.s.	0.0159	n.s.
	Number of functional group		–0.0965	n.s.	–0.0202	n.s.
D_{st}	Model	$F(2,85) = 1.9386$		n.s.		
	Number of species		0.1952	n.s.	0.2086	n.s.
	Number of functional group		0.0165	n.s.	0.1747	n.s.
AD_{st}	Model	$F(2,85) = 8.3167$		***		
	Number of species		–0.0538	n.s.	–0.3453	***
	Number of functional group		–0.3597	*	–0.4033	***
ED	Model	$F(2,55) = 16.052$		***		
	Number of species		0.5012	**	0.5990	***
	Number of functional group		0.1389	n.s.	0.4920	***
B) Belowground biomass						
Regression: multiple					simple	
Difference between HN and LN	Explanatory variable	ANOVA	Beta	Sign.	*R*	Sign.
D	Model	$F(2,85) = 1.5443$		n.s.		
	Number of species		0.0437	n.s.	0.1653	n.s.
	Number of functional group		0.1501	n.s.	0.1855	n.s.
AD	Model	$F(2,85) = 9.2770$		***		
	Number of species		–0.2384	n.s.	–0.4056	***
	Number of functional group		–0.2064	n.s.	–0.3996	***
D_{st}	Model	$F(2,85) = 0.9575$		n.s.		
	Number of species		0.0452	n.s.	0.1339	n.s.
	Number of functional group		0.1095	n.s.	0.1461	n.s.
AD_{st}	Model	$F(2,85) = 24.551$		***		
	Number of species		–0.2569	n.s.	–0.5631	***
	Number of functional group		–0.3779	*	–0.5861	***
ED	Model	$F(2,55) = 0.1251$		n.s.		
	Number of species		0.0655	n.s.	0.0673	n.s.
	Number of functional group		0.0025	n.s.	0.0486	n.s.

ANOVA is the ANOVA of the entire model. Betas are the standardized partial regression coefficients. Simple r is correlation coefficient of the variable with the response (characterizing the marginal effect of the predictor). (***) $P < 0.001$, (**) $P < 0.01$, (*) $P < 0.05$, (n.s.) $P > 0.05$.

sity. In fact, some of the low diversity mixtures and monocultures had decreased productivity with increasing nutrients. Consequently, the average change came from negative values for monocultures to highly positive values for species rich mixtures. With increased species number, the probability rises that the mixture contains a species able to take advantage of the nutrient amendment. Moreover, it seems that the presence of such species might improve conditions for other species, e.g. those that were harmed by high nutrient levels. On the contrary, the absolute value of the standardized difference decreased with diversity, particularly with functional group richness, showing that productivity of more diverse mixtures is more resistant to change in nutrient. The change in species composition characterized by the chord distance increased with diversity. This corresponds well to the predictions of Tilman (1999): the stability of the aggregate characteristic, here productivity, is achieved through a change in species composition.

4.5. Limitations of pot experiments

We are aware that pot experiments are rather limited in their realism. In particular, the spatial and temporal variability of the environment, which could be important for the manifestation of complementarity, is highly restricted in pots. However, there are two advantages to pot experiments. First, we were able to separate the roots of individual species, when rinsing the substrate carefully; this is not feasible in normal soils in field experiments. Further, field experiments impose much greater restrictions on the number of replications. Monocultures are essential for proper analysis. In calculating the indices (*OI*, selection, complementarity), monoculture values are included. This means that a possible underestimation of monoculture biomass of highly productive species causes an overestimation of biodiversity effects in many samples. Consequently, the monocultures should be replicated (we used two replicates, but more would be desirable for statistical analyses).

Also, nutrient concentrations that are high to harm some species are not common in natural conditions. This effect was also amplified by using sand as the substrate. Nevertheless, the results showing that some species are harmed by high nutrient level when grown in monoculture, but when grown in mixture, the other species are able to deplete the nutrients to acceptable levels, suggest a mechanism by which diversity (either species or functional) could improve resource use by the whole community. The fact that several of the species did grow better in mixtures (a phenomenon not observed in a similar experiment by Špaekova and Lepš, 2001) suggests that pure sand is a rather stressful environment for some species (particularly pure sand in combination with a high nutrient level was detrimental for some species). As shown several times (e.g. Pugnaire et al., 2004), facilitative interactions are more common in stressed environments, and it can be reasonably expected that the presence of roots of other species could improve soil properties for some species. As a result, the complementarity and overyielding were both observed in many cases.

4.6. Species and functional group richness

Interestingly, in biomass analyses, functional group richness was a better predictor than the number of species. Four a priori defined functional groups were used and the statistical analysis showed that functional group richness had good explanatory power. In this study the functional groups behaved consistently. All the creeping herbs exhibited low productivity, both in low and high nutrients, and also in monocultures and mixtures. The rosette plants (with the exception of *P. media*) showed high productivity in monocultures, where they took the advantage of high nutrient levels, but were highly suppressed in mixtures, particularly under high nutrients. Because they did not produce any stems, they were overgrown by other species in mixtures and being shaded and outcompeted by taller plants. The legume monocultures had generally (much) higher productivity under low nutrients (with the exception of *Lotus,* where productivity was roughly the same). Under high nutrients, they mostly achieved higher biomass in mixture than in monocultures. The only exception was *L. pratensis*, a species with delayed germination, which is an important competitional disadvantage in short term experiments. The grasses did not show consistent behavior; on the contrary, the behavior of *Holcus* was somewhat similar to that of legumes. Nevertheless, in most cases, functional group membership did not only correspond to species morphology, but also reasonably predicted species behavior in the experiment.

As noted by Tilman et al. (2002), any a priori definition of functional groups is a problem, particularly as it might be uncertain which of the species traits will be important in competitive interactions. In manipulative experiments focused directly on the effect of functional groups, however, one could select groups of species that are very similar to each other and sufficiently distinct from the others. This was the case in our experiment. Nevertheless, some of the functional traits were not reflected in the classification of functional groups, and still affected considerably the final outcome of the experiment (e.g. delayed germination in *L. pratensis*). Whereas the importance of germination speed for competition was exaggerated due to the experimental setup, similar mechanisms probably play a role in nature. For example, Hooper and Vitousek (1997) used early and late seasonal annuals and showed how annuals were able to suppress the productivity of the bunchgrasses, partially because of their phenology.

Unlike Tilman et al. (2002) we do not see much of a problem in separation the effect of functional group and species richness. Whereas we agree with Tilman et al. (2002) that "classical" GLM, with a type III sums of squares, provides a very conservative test for partial effects (i.e. high Type II error rate), in our view, it is no problem to test within GLM also for their common effect; this test has usually (much) higher power that tests for partial effects. On the contrary, we see serious problems with analyses of derived characteristics (like complementarity, OI, selection), because their values are not independent observations. They are used here only to provide results comparable with other published papers, but we are aware that the significance can be seriously inflated.

4.7. Design of biodiversity experiments

To design a biodiversity experiment is generally a difficult task. For simple logistic reasons, the possible number of replicates is limited, and consequently, one can not examine all possible species combinations. As Allison (1999) demonstrated, the power of the test is then affected by the way in which the species mixtures are assembled (i.e. how species and functional group richness are combined). Our design corresponds to the Three-tier factorial design of Allison (1999). Under this design, the number of functional groups and total number of species are necessarily correlated. However, the analysis that includes both the partial effect of species number and their common effect is able to separate the effects and concurrently provides a test which is sufficiently strong for the general "biodiversity effect". The important fact (not reflected in Allison, 1999) is that there are two sources of "random" variation: the variability caused by species composition (various replications of the same "treatment", i.e. containing the same number of species and functional groups, have different species composition), and a random variability. In our experience, the first part (i.e. the species composition specific effect) of the variability is greater, particularly in strongly controlled conditions. Under those circumstances, we believe that the design varying species composition among replicates of each combination of species functional group richness is necessary whenever possible.

As has been shown by Fukami et al. (2001), the similarity between replicates increases with species richness in most designs with a limited species pool. This is also reflected by the fact that variability in biomass decreases with species richness. Fortunately, the trend of decreasing variability with species richness is not so strong that it would invalidate statistical analyses (any GLM analysis assumes homoscedascity, i.e. constant variability), because there is no simple remedy. The log transformation improves homoscedascity when mean and variability are positively correlated (here they were negatively correlated). Similarly, none of the distributions commonly used in generalized linear models is based on a distribution where mean and variance are negatively correlated. Using the open species pool, however, would require a very large number of monocultures. Alternatively, some species would not be grown as monocultures, but would be part of species mixtures. In this case, however, neither *OI*, nor the other diversity indices, can be calculated.

5. Conclusions

In a pot greenhouse experiment, average biomass increased with both number of species and number of functional groups. The effect of functional groups was more pronounced. The increase was more pronounced under high nutrients. Contrary to many previous studies, there was low correlation between species performance in monoculture and in mixture. We demonstrated that this can be caused by a series of factors in our experimental setup. As a result, the selection effect in our experiment was low, or even sometimes negative. The relative change in productivity decreased with richness, whereas the shift in species composition increased. This suggests that richness could stabilize the community aggregate characteristics through a shift in species proportions.

Acknowledgments

This study was funded by the TLinks project of V Framework Program of the European Community within the Energy, Environment and Sustainable Development Program (contract number EVK2-CT-2001-00123) and national grants MSM6007665801, GACR206/03/H034.

References

Aarsen, L.W., 1997. High productivity in grassland ecosystems: effected by species diversity or productive species? Oikos 80, 183–184.

Allison, G.W., 1999. The implications of experimental design for biodiversity manipulations. Am. Nat. 153, 26–45.

Bengtsson, J., Jones, H., Setälä, H., 1997. The value of biodiversity. Trends Ecol. Evol. 12, 334–336.

Cameron, T., 2002. 2002: the year of the "diversity-ecosystem function". Trends. Ecol. Evol. 17, 495–496.

Deutschman, D.H., 2001. Design and analysis of biodiversity field experiments. Ecol. Research 16, 833–843.

Díaz, S., Cabido, M., 2001. Vive la différence : plant functional diversity matters to ecosystem processes. Trends Ecol. Evol. 16, 646–655.

Dukes, J.S., 2001. Productivity and complementarity in grassland microcosms of varying diversity. Oikos 94, 468–480.

Fridley, J.D., 2002. Resource availability dominates and alters the relationship between species diversity and ecosystem productivity in experimental plant communities. Oecologia 132, 271–277.

Fukami, T., Naeem, S., Wardle, D.A., 2001. On similarity among local communities in biodiversity experiments. Oikos 95, 340–348.

Garnier, E., Navas, M.-L., Austin, M.P., Lilley, J.M., Gifford, R.M., 1997. A problem for biodiversity-productivity studies: how to compare the productivity of multispecific plant mixtures to that of monocultures? Acta Oecologica 18, 657–670.

Grime, J.P., 1997. Biodiversity and ecosystem function: the debate deepens. Science 277, 1260–1261.

Grime, J.P., 2001. Plant Strategies, Vegetation Processes and Ecosystem Properties. John Wiley & Sons, Chichester.

Grime, J.P., Thompson, K., Hunt, R., Hodgson, J.G., Cornelissen, J.H.C., Rorison, I.H., Hendry, G.A.F., Ashenden, T.W., Askew, A.P., Band, S.R., Booth, R.E., Bossard, C.C., Campbell, B.D., Cooper, J.E.L., Davison, A.W., Gupta, P.L., Hall, W., Hand, D.W., Hannah, M.A., Hillier, S.H., Hodkinson, D.J., Jalili, A., Liu, Z., Mackey, J.M.L., Matthews, N., Mowforth, M.A., Neal, A.M., Reader, R.J., Reiling, K., Ross-Fraser, W., et al., 1997. Integrated screening validates primary axes of specialisation in plants. Oikos 79, 259–281.

Hector, A., Bazeley-White, E., Loreau, M., Otway, S., Schmid, B., 2002. Overyielding in grassland communities: testing the sampling effect hypothesis with replicated biodiversity experiments. Ecol. Lett. 5, 502–511.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G., Finn, J.A., Freitas, H., Giller, P.S., Good, J., Harris, R., Högberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Körner, C., Leadley, P.W., Loreau, M., Minns, A., Mulder, C.H.P., O'Donovan, G., Otway, S.J., Pereira, S.J., Prinz, A., Read, D.J., Scherer-Lorenzen, M., Schulze, E.-D., Siamantziouras, A.-S.D., Spehn, E.M., Terry, A.C., Troumbis, A.Y., Woodward, F.I., Yachi, S., Lawton, J.H., 1999. Plant diversity and productivity experiments in European Grasslands. Science 286, 1123–1127.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G., Finn, J.A., Freitas, H., Giller, P.S., Good, J., Harris, R., Högberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Körner, C., Leadley, P.W., Loreau, M., Minns, A., Mulder, C.H.P., O'Donovan, G., Otway, S.J., Pereira, S.J., Prinz, A., Read, D.J., Scherer-Lorenzen, M., Schulze, E.-D., Siamantziouras, A.-S.D., Spehn, E.M., Terry, A.C., Troumbis, A.Y., Woodward, F.I., Yachi, S., Lawton, J.H., 2000. No consistent effect of plant diversity on productivity. Response. Science 289, 1255a.

Hodgson, J.G., Thompson, K., Wilson, P.J., Bogaard, A., 1998. Does biodiversity determine ecosystem function? The ecotron experiment reconsidered. Funct. Ecol. 12, 843–856.

Hooper, D.U., 1998. The role of complementarity and competition in ecosystem responses to variation in plant diversity. Ecology 79, 704–719.

Hooper, D.U., Vitousek, P.M., 1997. The effects of plant composition and diversity on ecosystem processes. Science 277, 1302–1305.

Hooper, D.U., Vitousek, P.M., 1998. Effects of plant composition and diversity on nutrient cycling. Ecol. Monogr. 68, 121–149.

Huston, M.A., 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia 110, 449–460.

Huston, M.A., Aarsen, L.W., Austin, M.P., Cade, B.S., Fridley, J.D., Garnier, E., Grime, J.P., Hodgson, J., Lauenroth, W.K., Thompson, K., Vandermeer, J.H., Wardle, D.A., 2000. No consistent effect of plant diversity on productivity. Science 289, 1255a.

Johnson, K.H., Vogt, K.A., Clark, H.J., Schmitz, O.J., Vogt, D.J., 1996. Biodiversity and the productivity and stability of ecosystems. Trends Ecol. Evol. 11, 372–377.

Kaiser, J., 2000. Rift over biodiversity divides ecologists. Science 289, 1282–1283.

Lawton, J.H., Naeem, S., Thompson, L.J., Hector, A., Crawley, M.J., 1998. Biodiversity and ecosystem functioning: getting the Ecotron experiment in its correct context. Funct. Ecol. 12, 848–852.

Lehman, C.L., Tilman, D., 2000. Biodiversity, stability, and productivity in competitive communities. Am. Nat. 156, 534–552.

Lepš, J., Brown, V.K., Diaz Len, T.A., Gormsen, D., Hedlund, K., Kailová, J., Korthals, G.W., Mortimer, S.R., Ridriguez-Barrueco, C., Roy, J., Santa Regina, I., van Dijk, C., van der Putten, W.H., 2001. Separating the chance effect from other diversity effects in the functioning of plant communities. Oikos 92, 123–134.

Lepš, J., Osbornová-Kosinová, J., Rejmánek, M., 1982. Community stability, complexity and species life-history strategies. Vegetatio 50, 53–63.

Loreau, M., Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 411, 72–76.

Loreau, M., 1998. Biodiversity and ecosystem functioning: a mechanistic model. Proc. Natl. Acad. Sci. USA 95, 5632–5636 (a).

Loreau, M., 1998. Separating sampling and other effects in biodiversity experiments. Oikos 83, 600–603 (b).

Mikola, J., Salonen, V., Setälä, H., 2002. Studying the effects of plant species richness on ecosystem functioning: does the choice of experimental design matter? Oecologia 133, 594–598.

Naeem, S., Li, S., 1997. Biodiversity enhances ecosystem reliability. Nature 390, 507–509.

Naeem, S., et al., 2000. Reply to Wardle et al. Bull. Ecol. Soc. Am. 81, 241–246.

Naeem, S., Chapin, F.S., Constanza, R., Ehrlich, P.R., Golley, F.R., Hooper, D.U., Lawton, J.H., O′Neil, R.V., Mooney, H.A., Osvaldo, E.S., Symstad, A.J., Tilman, D., 1999. Biodiversity and ecosystem functioning: maintaining natural life support processes. Issues in Ecology 4, 1–14.

Naeem, S., Thompson, L.J., Lawler, S.P., Lawton, J.H., Woodfin, R.M., 1994. Declining biodiversity can alter the performance of ecosystems. Nature 368, 734–737.

Orloci, L., 1978. Multivariate Analysis in Vegetation Research, W. Junk, the Hague.

Pugnaire, F.I., Armas, C., Valladares, F., 2004. Soil as a mediator in plant–plant interactions in a semi-arid community. J. Veg. Sci. 15, 85–92.

Sankaran, M., McNaughton, S.J., 1999. Determinants of biodiversity regulate compositional stability of communities. Nature 401, 691–693.

Silvertown, J.W., Doust, J.L., 1993. Introduction to Plant Population Biology. Blackwell Sci. Publ., Oxford.

Spaekova, I., Lepš, J., 2001. Procedure for separating the selection effect from other effects in diversity-productivity relationship. Ecol. Lett. 4, 585–594.

Spehn, E.M., Scherer-Lorenzen, M., Schmid, B., Hector, A., Caldeira, M.C., Dimitrakopoulos, P.G., Finn, J.A., Jumpponen, A., O'Donnovan, G., Pereira, J.S., Schulze, E.-D., Troumbis, A.Y., Körner, C., 2002. The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. Oikos 98, 205–218.

Symstad, A.J., 2000. A test of the effects of functional group richness and composition on grassland invasibility. Ecology 81, 99–109.

Symstad, A.J., Tilman, D., Willson, J., Knops, J., 1998. Species loss and ecosystem functioning: effects of species identity and community composition. Oikos 81, 389–397.

Tilman, D., 1997. Distinguishing between the effects of species diversity and species composition. Oikos 80, 185 (a).

Tilman, D., 1997. Community invasibility, recruitment limitation, and grassland biodiversity. Ecology 78, 81–92 (b).

Tilman, D., 1999. The ecological consequences of changes in biodiversity: a search for general principles. Ecology 80, 1455–1474.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., Siemann, E., 1997. The influence of functional diversity and composition on ecosystem processes. Science 277, 1300–1302.

Tilman, D., Knops, J., Wedin, D., Reich, P., 2002. Plant diversity and composition: effects on productivity and nutrient dynamics of experimental grasslands. In: Loreau, M., Naeem, S., Inchausti, P. (Eds.), Biodiversity and Ecosystem Functioning. Oxford University Press, Oxford, pp. 21–35.

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T., Lehman, C., 2001. Diversity and productivity in a long-term grassland experiment. Science 294, 843–845.

Tilman, D., Wedin, D., Knops, J., 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystem. Nature 379, 718–720.

Van der Putten, W.H., Mortimer, S.R., Hedlund, K., van Dijk, C., Brown, V.K., Lepš, J., Rodriguez-Barueco, C., Roy, J., Diaz Len, T.A., Gormsen, D., Korthals, G.W., Lavorel, S., Santa Regina, I., Šmilauer, P., 2000. Plant species diversity as a driver of early succession in abandoned fields: a multi-site approach. Oecologia 124, 91–99.

Vandermeer, J., 1989. The Ecology of Intercropping. Cambridge University press, Cambridge.

Wardle, D.A., Huston, M.A., Grime, J.P., Berendse, F., Garnier, E., Setälä, H., 2000. Biodiversity and ecosystem function: an issue in ecology. Bulletin of the Ecological Society of America 81, 235–239.

De Witt, C.T., 1960. On competition. Versl. Landbouk. Onderz. 66, 1–82.