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Effects of species and functional group richness on production in two fertility environments: an experiment with communities of perennial plants

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ABSTRACT

The effects of species and functional group richness of sown species and fertilization on productivity and invasion of colonizers (i.e. unsown species) were studied in a three year field experiment. Both species and functional group numbers positively affected the average yield of sown species, but the effect changed among the three years. The biomass of colonizers generally decreased with the biomass of sown species, and so was negatively affected by the diversity characteristics of sown species. All three characteristics of biodiversity (selection, complementarity and overyielding effects) varied greatly during the study period, depending on both species and functional group richness and nutrient amendment. Functional differences among species led to the detection of high values of complementarity (resource use complementarity and/or facilitation), however this effect was not sufficient to cause transgressive overyielding. The compositional difference between fertilized and non-fertilized control plots increased with both time and species richness, whereas the relative difference in productivity decreased. This shows that the changes in species composition toward the one best-suited to the given environment, can lead to optimal resource use among plant species, and, as a consequence, diminish variation in community functioning in a changing environment.

1. Introduction

Plant species diversity has been shown to enhance ecosystem productivity (Naeem et al., 1994; Tilman et al., 1996, 1997, 2001; Hector et al., 1999; Tilman, 1999; Loreau, 2000), but the mechanisms behind this relationship remain controversial (Huston, 1997; Loreau et al., 2001; Huston et al., 2000; Wardle et al., 2000). In experiments where species assemblages are randomly constructed, species-rich plots have a higher probability of including those species that have the greatest individual effect on production, a phenomenon called the “sampling effect” (Aarsen, 1997; Huston, 1997; Tilman et al., 1997). Determining whether sampling effect might apply in natural ecosystems has been difficult, because no

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experiments have examined the sensitivity of sampling effect in environmental context. Sampling effect may be particularly important in environments that promote species dominance, where one or a few species have a strong individual control over productivity (Fridley, 2001). Higher production than expected from the sampling effect may be caused by complementary resource use or facilitative interactions among species (Vandermeer, 1989; Tilman et al., 1997, 2001; Hooper, 1998; Hector et al., 1999). However, this phenomenon needs further investigation (Austin et al., 1988; Garnier et al., 1997).

The phenomenon that a species mixture produces more than expected from the monocultures yields of constituent species is usually called overyielding. However, there are various ways, how the ‘‘expectation’’ is calculated (Trenbath, 1974; Vandermeer, 1989). As a consequence, two categories of overyielding are distinguished (Hector et al., 2002). Non-transgressive overyielding is defined as situation when the yield of a mixture is greater than expected based on a weighted average of the monoculture yields of the component species, as indicated by values greater than one for relative yield totals (RYTs; Trenbath, 1974; Vandermeer, 1989). Transgressive overyielding (Trenbath, 1974; Vandermeer, 1989) occurs when a mixture yields more than any monoculture of the component species.

Several studies have reported significant positive effects of plant species richness on ecosystem functioning (Tilman et al., 1996, 2001; Hector et al., 1999), while some studies have found functioning to be mainly influenced by species and functional group composition, i.e. the identity of the species present (Hooper and Vitousek, 1997; Wardle et al., 1997). Diversity is often equated with species richness and other components of diversity have frequently been underestimated. In several studies, the rates and magnitude of ecosystem processes have been found to be more consistently associated with functional composition (presence of certain functional types or traits) and/or functional richness (number of functional groups) than with species richness (Lepš et al., 1982; Grime et al., 1997; Hooper and Vitousek, 1998; Reich et al., 2001; Symstad, 2000; Tilman et al., 1997). Broader issues concerning functional groups are reviewed in Mooney et al. (1996), Diaz and Cabido (2001), Diaz et al. (2003) and Hooper et al. (2002). Determining the relative contributions of functional and species richness to ecosystem functioning is important in management and conservation, because it provides information on ecological redundancy (Walker, 1992; Lawton and Brown, 1993; Naeem, 1998), where ‘‘ecological redundancy’’ refers to taxonomically different species that exhibit similar or related ecological functions.

Most of the above biodiveristy experiments were carried out for relatively short time and were accompanied by weeding. It has been shown that the diversity-productivity relationships disappeared quickly after cessation of weeding (Pfisterer et al., 2004). In communities of perennial plants, the development of mature individuals takes often several years, and so does the development of relationships between species. Whereas at the beginning, the success of individual species is more determined by their ability to germinate and grow fast (Silvertown and Doust, 1993), the importance of competitive ability increases with time. Very probably, the species establishing slowly become more and more the drivers of ecosystem functioning. With initial seed input, we would describe the process as directed succession. Our experience shows (Lepš et al., 2007) that in communities originating from sowing of grassland species on ex-arable land, the successional dynamics, although fastest during the first years, could be observed for nearly a decade. The classical successional theory (e.g. Odum, 1971) predicts that the ‘‘division of labour among species’’ – and so also the complementarity, should increase with successional age. Consequently, it is important to know how the results will change during the first years after sowing. Most studies assessing diversity/productivity relationships have been relatively short-term (<3 years) which may underestimate the strength of complementarity or facilitation (Hooper and Dukes, 2003). Any successional development is influenced by the weather conditions of individual years. Unless we start the same experiments in several subsequent years (which, to our knowledge have not been yet done, at least for experiments of this size), it is extremely difficult to disentangle the successional development from random effects, mostly weather, in individual years.

It has been demonstrated that nutrient addition brings about an increase in the average height of vegetation, which leads to a competitive advantage of tall growth forms over shorter ones (Tilman, 1988). Nutrient addition may promote light partitioning by accentuating growth form differences between species, thereby enhancing the structural complexity of vegetation. Furthermore, stratification of canopy layers promotes coexistence and increases production in some herbaceous and grassland communities (Mitchley, 1988; Liira and Zobel, 2000). If those mechanisms prevail, we can expect enhancing of biodiversity effects following fertilization. On the other hand, nutrient addition means release from nutrient limitation and increase in the importance of competition for light. As the competition for light is probably more asymmetric than competition for nutrients, this often leads to elimination of shorter species and decrease of species diversity (Lepš, 1999). This would suggest that the underground competition provides more opportunities for complementary use resources; this would predict higher complementarity in nutrient poor conditions, where the competition for nutrients is more important than competition for light.

The more species in the mixture, the higher is the probability that a species combination suits to particular conditions will be present. The response of individual species will be then amplified by the interspecific competition. Those premises led Tilman (1999) to predict that species richness will decrease the variability of aggregated characteristics (like total biomass); this will be achieved through increased variability in species composition. Following his logic, we can predict that the differences in production between control and fertilized plots sown with identical species mixtures will diminish with species richness, whereas differences in species composition will increase. Because in vegetation development, the initial species composition is more affected by diaspore availability, and the competitive equilibria develop with time, we can expect that those differences in species composition will increase with time.

To assess the relative degree to which fertilization, species and functional group diversity influence productivity in plant communities, a three year field experiment was performed in which species and functional group richness and fertilization were manipulated. Because weeds were establishing from the soil seed bank during the course of the experiment, and the experiment was run without weeding of the plots, it could also show how the diversity of resident sown species affects colonizer invasion. Specifically, our goals were to determine: (i) how is the productivity affected by species and functional group richness; (ii) how are those effect affected by soil nutrients; (iii) how those effect change during the first three years after establishment of the experiment; and (iv) how is the magnitude of difference between fertilized and control plots related to species richness.

2. Methods

The experiment was conducted in an abandoned agricultural field, last cultivated in 2001, at Benešov in South Bohemia, CZ (49° 92′ N, 15° 00′ E, altitude approximately 660 m). This region experiences a temperate climate, with mean annual temperature of 6.4 °C and mean annual precipitation of 680 mm.

A taxonomically diverse group of polycarpic perennial species with well-known growth characteristics was chosen for this experiment. These species represent major components of the surrounding grasslands. Species from four distinct functional groups were selected: 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*.

During March 2002, treatments were established in 2 × 2 m plots in a completely randomized design. Ten combinations of species and functional group richness were used, with different species combinations within treatments (Table 1), yielding 184 plots. As our previous experience from similar experiments show that the variability in mixture performance caused by differences in species composition is much greater than that caused by spatial variability in environmental conditions, no blocking was used. The treatments were assigned to plots in a restricted random manner, so that each level of diversity was spread evenly over the entire plot. Mixtures of 1–16 species were sown at the same density of 3000 seeds m^{-2}. This number was equally divided among all the species in the mixture. As far as possible, all of the functional groups were equally represented in mixtures with more than one functional group. Mixtures were grown under two nutrient treatments: non-fertilized Control and Fertilized. The plots assigned to the nutrient addition treatment were amended with 40 g NPK m^{-2} yr^{-1} (Total N: 12%, P_2O_5: 12%, K_2O: 12%; Synferta P-NPK, AGRO CS Česká Skalice, CZ), applied in April 2002 and March 2003, 2004 and 2005.

The 2 × 2 m^2 plots were harvested for aboveground biomass in June 2003, 2004 and 2005 by clipping at ground level in two 15 × 15 cm subplots and then completely mown. The two subsamples were then pooled, separated into individual sown species and the rest of aboveground biomass (associated with the summed biomass of all colonizing species, including those sown in other treatments), oven-dried and weighed.

The aboveground biomass of sown and colonizing species is a measure of the growth success of the sown and colonizer species respectively in different biodiversity treatments. Generalised linear models (GLM) were used to compare the influence of species number, functional group richness (continuous predictors) and fertilization (categorical predictor) on the biomass of sown and colonizer species (response). Species richness and functional group richness were continuous predictors, while nutrient level was a categorical one. In a design with a closed data set, the number of species and functional groups is inevitably correlated (in our case, the correlation was 0.813). Consequently, the strength of the test of partial effect of each of the two correlated predictors is decreased. (In fact, those *partial* effects test whether there is an effect of one variable, when the other is held constant – in this case, whether there is an effect of functional group richness when the species richness does not change, or whether there is an effect of species richness when the functional group richness does not change.) Consequently, we have also performed common test for the overall effect of diversity – in fact, we tested the null hypothesis that the common effect of species and functional diversity is null (for details, see Lanta and Lepš, 2006). This common effect is called hereafter referred to as ‘Biodiversity’.

Table 1 – Treatments for control and fertilized plots used in the manipulated experiment

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

Besides biomass, each plot was also characterized by biodiversity effect indices, namely the transgressive overyielding index (OI), and by the additive partitioning biodiversity effects technique (Loreau and Hector, 2001). Those indices are calculated on the basis of the sown species only; we are interested in the effect of sown mixture richness on productivity, and also in its ability to suppress the arable weeds. OI is defined as the total biomass of mixture/monoculture biomass of the component species with the largest monoculture value (Loreau, 1998). An OI value >1 for a mixture indicates transgressive overyielding (Trenbath, 1974; Vandermeer, 1989; Hector et al., 2002). At the second step, the role of sampling effects among diversity and fertility levels were measured by using the additive partitioning biodiversity effects technique (Loreau and Hector, 2001) to separate the productivity responses of mixtures into selection effect (SE) and complementarity (CE). The net difference in yield for a mixture, ΔY, is the observed yield (Y_o) minus expected (Y_e) and is equal to the summation of both complementarity and selection effect:

$$\Delta Y = Y_o - Y_e = SE + CE.$$

This procedure estimates SE for each plant mixture by calculating the covariance between species monoculture yields and their deviance from expected relative yields in mixture (based on their planted proportions), multiplied by the total number of species in mixture. CE for a mixture is calculated as the average deviance over all of the species in the mixture and over species average monocultural yields. If species of higher than average monocultural yields dominate a mixture, the associated SE is positive; dominance by less productive species leads to a negative SE. CE gives similar information as RYT (relative yield total). These indices were then used as response variables in GLM, similarly as the biomass data. This approach is generally used in biodiversity studies (e.g. Hector et al., 1999). It was used here so that the analysis is comparable with other studies. However, we are aware that each monoculture value enters into all of the mixtures where the species is present, and, consequently, the individual values are not independent. As a result, the significance of analyses could be inflated (Lanta and Lepš, 2006).

Differences between both the productivity of the sown species and the relative composition of sown species in plots with the same species composition, but different nutrient regime, were characterized in the following way: First, pairs were formed between control and fertilized plots with the same species composition; where there were more replicates with the same species composition, the pairing was random. The following characteristics were calculated for each pair:

Plain difference between biomass in control and fertilized plots

$$D = W_f - W_c$$

i.e., the positive value mean increase of biomass in high nutrient treatments. Because the productivity of some combinations was higher in low nutrient treatments, and we were interested in stability with respect to a 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

Table 2 – Summary of GLM for aboveground biomass of sown residents and colonizers

	df	Sown species 2003		Colonizers 2003		Sown species 2004		Colonizers 2004		Sown species 2005		Colonizers 2005	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Species richness	1, 180	18.04	0.000▲	0.58	0.446	7.49	0.007▲	4.36	0.038▼	5.72	0.018▲	0.51	0.477
Functional group richness	1, 180	0.06	0.809	0.44	0.506	0.74	0.391	3.99	0.047▼	2.36	0.126	4.77	0.030▼
Biodiversity	2, 180	24.79	0.000	2.77	0.065	17.99	0.000	2.31	0.102	20.99	0.000	4.09	0.018
Fertilization	1, 180	12.33	0.001▲	1.47	0.226	5.28	0.023▲	0.35	0.557	1.04	0.211	0.53	0.469

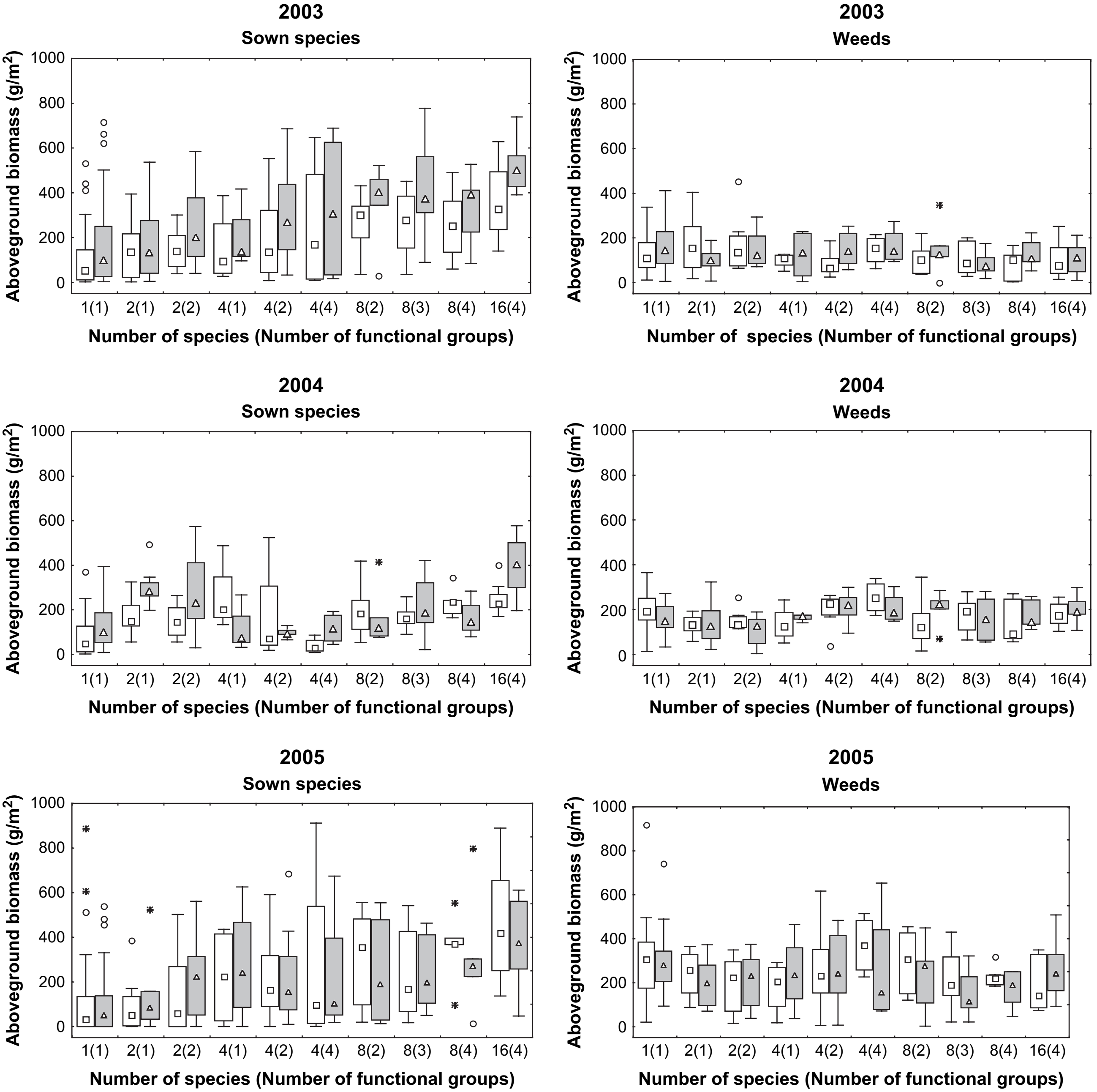

Fig. 1 – Relationships between aboveground biomass of sown species and weeds (colonizers) for years 2003, 2004, 2005 and species richness of the communities. Unfertilized plots: empty bars, fertilized plots: filled bars. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e., range of data within {lower quartile −1.5× interquartile range; upper quartile +1.5× interquartile range}), (o) – outliers, (*) – extremes.

combination, the standardized difference (D_{st}) and standardized absolute value of difference (AD_{st}) were calculated by dividing the respective differences by the average productivity in high and low nutrient treatments:

$$D_{st} = D/((W_f + W_c)/2) \quad AD_{st} = AD/((W_f + W_c)/2)$$

Each of the four variants captures other characteristics of productivity response to fertilization. Whereas plain difference is a measure of the mixture ability to take advantage of nutrient amendment, the absolute value is a measure of stability to a change of environment; for example, all the sown mixtures grow in competition with natural colonizers and consequently could be harmed by nutrient addition, when the natural colonizers have nutrient uptake capacity that the sown species. The standardization (of either plain or absolute difference) relates the magnitude of change to a potential productivity of the mixture.

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

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

where S is the number of species in the combination, $X_{f,i}$ and $X_{c,i}$ are the biomass values of the *i*-th species in control and fertilized plots 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

Biomass of sown species increased with the diversity of sown species in all the years. The effect of biodiversity (shared effect of species richness and functional group richness) was highly significant. Although in all the years only the partial effect of species richness was significant, the relative explanatory power of species richness decreased, and of functional group increased with time. Fertilized amendments had positive effects on the biomass of sown species in 2003 and 2004, but the differences between fertilized and control plots were not significant in 2005 (Table 2, Fig. 1). Biomass of colonizers significantly decreased with the increasing species richness of residents only in 2004 (Fig. 1), with the effect of functional group richness detected in 2004 and 2005 (Table 2). Biomass production of both sown species and colonizers significantly differed between years (Table 3); with decreasing trend in sown species and increasing in colonizers.

The biomass of colonizers decreased with increasing biomass of sown species, with the strength of the relationship increasing with time ($r_{2003} = -0.209$, $r_{2004} = -0.381$, $r_{2005} = -0.422$ for control plots, $r_{2003} = -0.076$, $r_{2004} = -0.164$, $r_{2005} = -0.494$ for fertilized plots), with all the relationships except for 2003 in fertilized plots being significant ($P < 0.05$).

The relationship between aboveground biomass production of a species in monoculture and its dominance in mixtures was significantly positive in both control and fertilized plots over all years ($P < 0.001$). We regressed dominance against monoculture biomass that was normalized to proportions of the maximum value observed at each control and fertilized treatment (Fig. 2; only for fertilized treatment; the results for control are similar). Aboveground biomass in

Table 3 – Results of repeated measures ANOVA for biomass of sown species and colonizers. Independent variable was fertilization; covariates were numbers of species and functional groups. Effect of both covariates was significant for both sown species (Rao's *R*, $F_{(6, 360)} = 16.29$, $P < 0.001$) and colonizers (Rao's *R*, $F_{(6, 356)} = 2.77$, $P = 0.012$)

	df	Sown species		Colonizers	
		F	*P*	*F*	*P*
Fertilization	1, 180	8.26	0.005	1.39	0.239
Time	2, 364	76.96	0.000	44.11	0.000
Interaction	2, 364	9.19	0.000	1.30	0.272

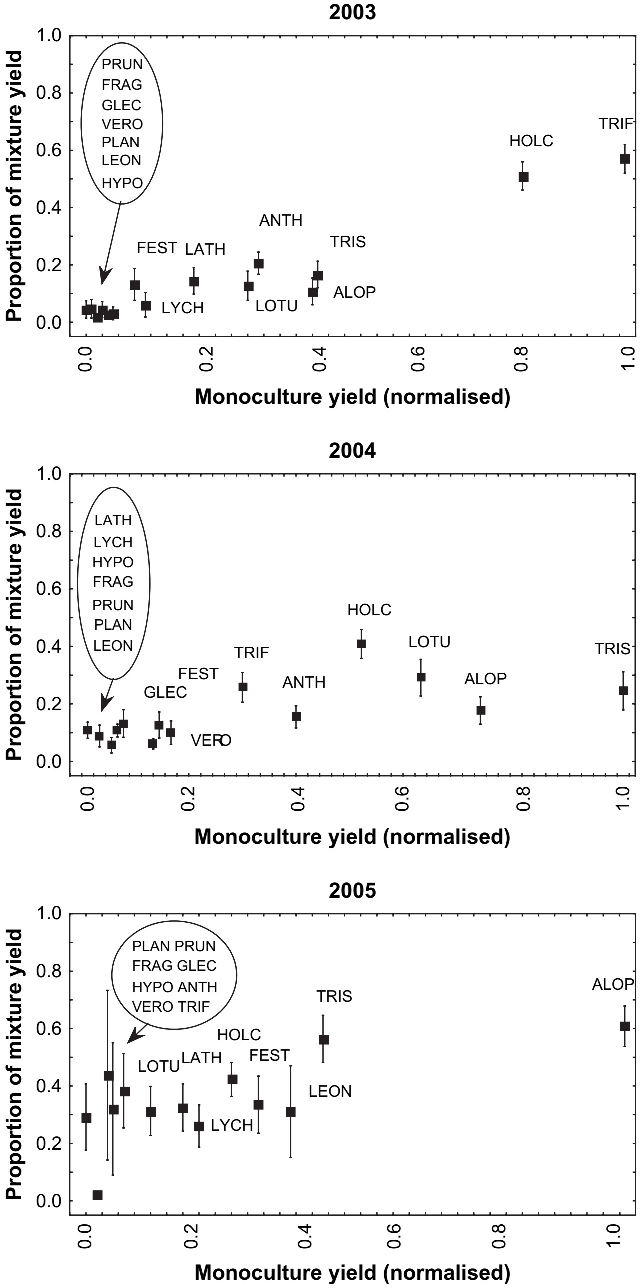

Fig. 2 – Dominance of species in individual mixtures (calculated as proportion of total aboveground biomass of a mixture) vs. average monoculture yields normalized to the maximum biomass of the most productive species. Results only for fertilized plots are shown. Boxes are means, whiskers s.e. Abbreviations: HOLC – *Holcus lanatus*, FEST – *Festuca rubra*, ALOP – *Alopecurus pratensis*, TRIS – *Trisetum flavescens*, HYPO – *Hypochaeris radicata*, LYCH – *Lychnis flos-cuculi*, LEON – *Leontodon hispidus*, PLAN – *Plantago media*, FRAG – *Fragaria vesca*, VERO – *Veronica officinalis*, PRUN – *Prunella vulgaris*, GLEC – *Glechoma hederacea*, TRIF – *Trifolium pratense*, LOTU – *Lotus corniculatus*, ANTH – *Anthyllis vulneraria*, LATH – *Lathyrus pratensis*.

monoculture was positively related ($r_{2003} = 0.55$, $r_{2004} = 0.32$, $r_{2005} = 0.37$ for control plots, $r_{2003} = 0.60$, $r_{2004} = 0.28$, $r_{2005} = 0.27$ for fertilized plots) to the success of a species in a mixture. Differences among species are much more pronounced in polycultures, suggesting that the variation is strongly affected by interspecific competition.

The analysis of OI showed significant relationships between indices values and species richness in 2005 (Table 4, Fig. 3). However, average values of log(OI) were lower than zero in most mixtures, showing that in majority of cases, the productivity of a mixture was lower than that of its most productive component. Values did not significantly differ among of study (Table 5). However, significant fertilization × time interaction indicated that values were higher for control plots in 2003, but were higher in fertilized plots in 2005.

The selection effect significantly increased with the number of functional groups in 2005. This effect significantly decreased with the number of species in 2004 (Table 4, Fig. 3). Fertilization positively influenced the values in 2003 and 2004. The second biodiversity term, complementarity effect, significantly increased with the number of species in 2004 and 2005, but significantly increased with the number of functional groups in 2003 (Table 4, Fig. 3). Fertilization influenced negatively its values in 2003 and 2004. Complementarity values were higher in control plots, while selection values were greater in fertilized plots. Values of selection and complementarity varied significantly among years, but the variation was not parallel in fertilized and control plots (Table 5).

The plain non-standardized difference (D) for biomass was independent of both the species number and functional group number, but the value strongly decreased with time (Fig. 4, Table 6). For some plots, the difference was negative, i.e. their biomass was higher in low nutrient treatments. The plain standardized difference (D_{st}) and absolute non-standardized difference (AD) also did not change with diversity, but varied with time. The standardized absolute value of the difference (AD_{st}) significantly decreased with diversity. Chord distance (CD) increased with diversity (Fig. 4, Table 6) and very strongly increased with time.

4. Discussion

Our results demonstrate that both plant species number and functional group richness affects aboveground biomass of sown residents and colonizers. Interestingly, the species number affects mainly the biomass of the sown species and its effect decreases with time, whereas the number of functional groups affects more the biomass of colonizers, and this effect increases with time. Biomass of colonizers was negatively influenced by the biomass of sown species. This agrees with previous biodiversity-productivity experiments (e.g., Hector et al., 2001; Symstad, 2000; Van Ruijven et al., 2003). These authors found a positive relationship between invasion resistance and increasing community diversity, which confirmed long-held theoretical expectations (Elton, 1958). The negative effect of diversity is often explained by increased resource use complementarity. When complementarity between species results in lower levels of available resources in high diversity, then invasion is inhibited (Knops et al., 1999).

In our case, the effect of functional group richness was relatively weak. However, as it was shown by Petchey et al. (2004), the poor performance does not necessarily mean no effect of functional diversity – the poor performance can be a consequence of the fact, that the definition of functional groups failed to capture the important features of functional differentiation. Defining the functional diversity is not a simple task. Number of functional groups is probably the simplest possibility, but this requires plants to be first classified into functional groups. We used an a priori classification based on functional and morphological characteristics, grouped the plants. The total morphological and functional variation was restricted by our decision to use plants these coexist in local grasslands: e.g. all the species were perennial, none of them possessed a ruderal character. We decided on four

Table 4 – Summary of biodiversity indices calculated for sown species and 3 consecutive years (2003, 2004 and 2005)

	df	2003		2004		2005	
		F	*P*	*F*	*P*	*F*	*P*
Complementarity							
Species richness	1, 116	3.02	0.085	30.14	0.000▲	51.44	0.000▲
Group richness	1, 116	5.26	0.024▲	0.28	0.597	3.23	0.075
Biodiversity	2, 116	14.01	0.000	34.73	0.000	36.59	0.000
Fertilization	1, 116	10.13	0.002▼	10.89	0.001▼	0.65	0.421
Selection							
Species richness	1, 116	0.01	0.498	7.09	0.009▼	0.022	0.883
Group richness	1, 116	0.46	0.908	0.03	0.854	3.986	0.048▲
Biodiversity	2, 116	0.59	0.555	7.87	0.001	3.61	0.030
Fertilization	1, 116	14.24	0.000▲	8.80	0.004▲	0.684	0.409
Log(OI)							
Species richness	1, 116	2.27	0.134	0.53	0.468	9.96	0.002▲
Group richness	1, 116	3.58	0.061	0.41	0.523	9.44	0.003▼
Biodiversity	2, 116	1.82	0.167	0.28	0.757	5.68	0.004
Fertilization	1, 116	0.02	0.876	5.79	0.177	3.15	0.078

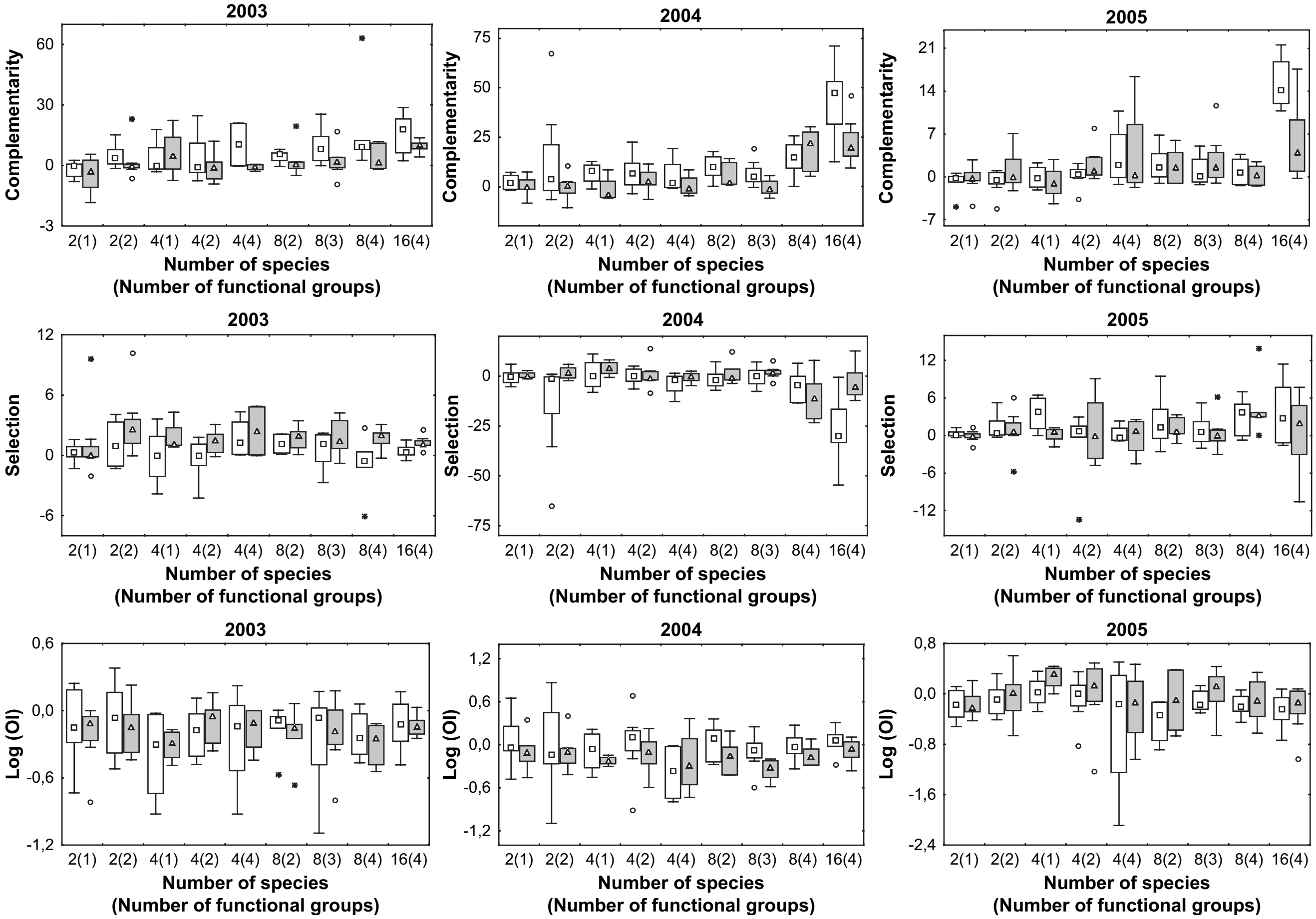

Fig. 3 – Selection, complementarity, and log(OI) regressed against biodiversity treatments used in the three year experiment. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e., range of data within {lower quartile −1.5× interquartile range; upper quartile +1.5× interquartile range}), (o) – outliers, (*) – extremes.

functional groups. Of them, grasses and legumes are generally considered to be distinct from the others, grasses by their unique root system and many other morphological features, legumes are N-fixers. Of the remaining forbs, we considered the distinction between rosette and creeping plants to be the most important – in particular in the competition for light. Rosette plants concentrate their leaves near the soil surface and during the growing season quickly form one stalk bearing reproductive organs. In contrast, creeping plants grow close to the soil surface and are considered poor competitors in our locality. Our classification partly corresponds with other studies conducted in grasslands (BIODEPTH in Europe or Cedar Creek experiments; in both sites plants were grouped on a subjective basis). However, it may be that some of the features important for community functioning are not reflected in our classification into functional groups, and that this might cause the rather weak explanatory power of functional group richness.

The experiment showed that a majority of sown species grown in monocultures had higher biomass in fertilized than in control plots. This contrasted with a previous pot experiment performed for one season in a greenhouse (Lanta and Lepš, 2006), where we also manipulated diversity in two nutrient levels. In this pot experiment, productivity was higher in lower nutrient treatments particularly in

Table 5 – Repeated measures ANOVA for complementarity and selection effects and Log(OI). Independent variable was fertilization. As covariates number of species and functional groups were used. Effect of both covariates is significant for complementarity (Rao's R, $F_{(6,\ 228)} = 20.99$, $P < 0.001$), selection (Rao's R, $F_{(6,\ 228)} = 4.02$, $P < 0.001$) and Log(OI) (Rao's R, $F_{(6,\ 228)} = 2.68$, $P = 0.016$)

	df	Compl		Select		Log (OI)	
		F	P	F	P	F	P
Fertilization	1, 116	19.22	0.000	10.94	0.001	0.03	0.857
Time	2, 236	19.10	0.000	16.32	0.000	2.72	0.068
Interaction	2, 236	3.95	0.000	6.20	0.002	5.04	0.007

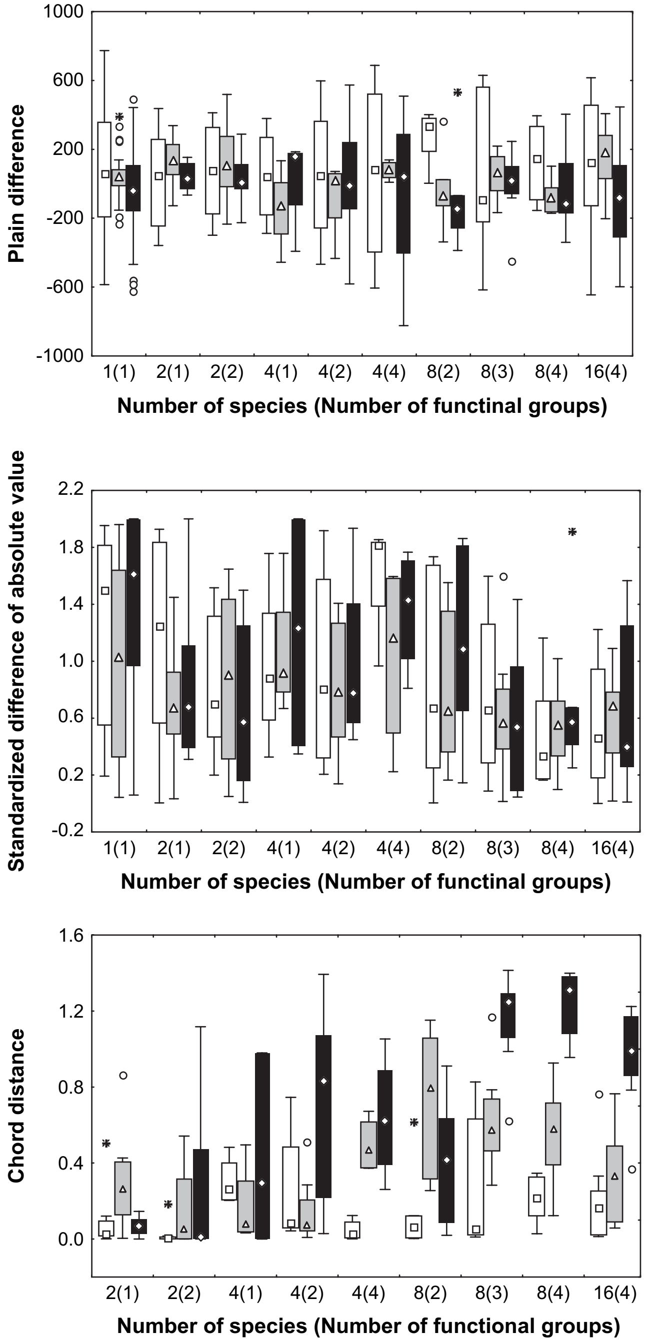

Fig. 4 – Plain difference, standardized difference of absolute value and chord distance between biomass of high and low nutrient levels. Differences for 2003 are shown by empty boxes, 2004 by gray boxes and 2005 by filled boxes. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e., range of data within {lower quartile −1.5× interquartile range; upper quartile +1.5× interquartile range}), (o) – outliers, (*) – extremes.

monocultures, while some species, often dominants, had higher productivity in mixtures than in monocultures. In pots, some species were apparently harmed by high nutrient levels, and profited from the presence of other species, which probably decreased the nutrients to acceptable levels. No such effects were found in the field experiment. This comparison demonstrates how important it is to compare pot experiments with real field situations.

Trifolium and *Holcus* dominated the mixtures in 2003. Both were also highly productive in our pot experiment (Lanta and Lepš, 2006). This might be a consequence of the high growth rate of *Trifolium*, as found in another experimental study (Spehn et al., 2002). However, this species gradually declined in the latter years of 2004 and 2005. This might have been partially caused by a rust fungus infection, which was observed on most plants of this species as very small gray-green spots. Again, the same effect was observed by Spehn et al. (2002). Another explanation is that some genotypes of this species are rather short-lived, and the seed regeneration was hindered in dense vegetation that developed in the site. *Holcus* is a major component of wet meadows (Lepš, 2004) that can quickly form very dense tussocks due to the production of extravaginal ramets (Grime et al., 1988). This species was very productive over the whole course of the experiment. Two other grasses, *Trisetum* and *Alopecurus* acquired very high biomass in 2004 and 2005 too, leading to the dominance of grasses in all plots, where they were sown. Accordingly, the values of both complementarity and selection effects change markedly over the course of the experiment. The dynamics of community composition and also of various indices during the first years demonstrate how caution is required in interpreting results of short term experiments with species mixtures, particularly when the aim is, as with this experiment, to generalize results to communities not undergoing transitional dynamics.

With the exception of 2005, selection was significantly higher in the fertilized plots, and complementarity significantly higher in the unfertilized controls. This supports the hypothesis that fertilization switches competition from underground to aboveground, and contradicts the assumption that increased plant height resulting from fertilization enables better partitioning of aboveground space. Fertilization partially releases plants from competition for nutrients, but, as a consequence, the competition for light increases. Taller plants are better competitors for light and usually take over in fertile conditions (Lepš, 1999). In fertile conditions with prevailing competition for light, which is more asymmetric than underground competition, the selection for highly productive species seems to be stronger. In contrast, in unfertilized plots with prevailing competition for underground resources, there is a better chance for complementary use of resources (e.g. due to heterogeneity of soil environment, different rooting depths of different species, etc.). Moreover, in additive partitioning, complementarity includes also facilitation, as it is impossible to distinguish it from complementarity only on the basis of compositional data (analysis of the mechanisms would be needed). Consequently, if there is an effect of legumes as nitrogen fixers (in our case mainly the effect of *Trifolium*), then this effect would be stronger in unfertilized plots. Generally, the positive effect of legumes on the productivity of the entire community is a well known phenomenon; consequently, some authors (e.g. Huston et al., 2000) consider the increase of productivity with diversity in this situation, where increasing diversity increases the probability of including a legume, as trivial.

Table 6 – Results of repeated measures ANOVA for *D*, AD, D_{st}, AD_{st} and CD. Time was only one variable. As covariates number of species and functional groups were used

	df	D		AD		D_{st}		AD_{st}		df	CD	
		F	P	F	P	F	P	F	P		F	P
Time	2, 182	5.20	0.006	24.29	0.000	3.54	0.031	2.69	0.071	2, 112	31.69	0.000
Covariates	6, 174	1.10	0.362	0.66	0.685	1.29	0.262	4.23	0.001	6,104	4.93	0.000

During the three years, the plots undergone a type of secondary succession (typical old-field succession), with gradual replacement of competitively weak annual weeds with large numbers of seeds in the permanent seed bank by competitively stronger colonizers. Interestingly, although all the sown species belong to the latter successional stages, some of them were outcompeted by the growing biomass of natural colonizers (this was particularly true for the creeping species). As a consequence, the average biomass of monocultures decreased with time, and the plots sown with monocultures of weak competitors undergone normal secondary succession. In contrast, the competitively strong grasses (*Trisetum*, *Alopecurus*) increased their biomass over time, as did the mixtures, whenever these strong grasses were present.

When the control and fertilized plots with the same species composition were compared, several trends were observed. First, whereas the differences in species composition increased with time, most measures of differences in productivity decreased. The increase in compositional difference with time suggests that nutrient status is an important determinant of competitive equilibria, which are being reached step by step over time. However, this increase in compositional difference signifies that the most appropriate species are selected for a given environment. This might cause the decrease in differences in productivity. In particular, the standardized absolute difference decreased both with time and also with species richness, whereas the chord distance increased. This corresponds to the theoretical predictions of Tilman (1999) that the stability of aggregated characteristics (such as total productivity) might increase with diversity as a consequence of greater changes in individual populations (changes in individual populations are reflected by chord distance). This shows that changes in species composition toward the one best suited to a given environment can lead to optimal resource use and, in consequence, diminish the variation in community functioning in a changing environment.

This experiment demonstrated that, on average, productivity increases with the diversity (with number of species, number of functional groups or both), but that transgressive overyielding was generally not found. This suggests that the use of resources by a mixture is usually better than the average of its constituent species (due to a mix of complementarity and sampling effects). Nevertheless, the mixture rarely reaches the efficiency of its most efficient component. The biodiversity effects were modified by the productivity of environment: The selection was relatively more important and the complementarity less important under high nutrients. Finally, three years are not enough to separate the successional trends from the interannual variation. Nevertheless, all the metrics we have applied had shown some variation with time – and only part of this variation could be interpreted as successional trend.

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