Consistent Effects of Biodiversity on Ecosystem Functioning Under Varying Density and Evenness

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Abstract Biodiversity experiments typically vary only species richness and composition, yet the generality of their results relies on consistent effects of these factors even under varying starting conditions of density and evenness. We tested this assumption in a factorial species richness x density x evenness experiment using a pool of 60 common grassland species divided into four functional groups (grasses, legumes, tall herbs and short herbs). Richness varied from 1, 2, 4, 8 to 16 species, total planting density was 1,000 or 2,000 seeds/m^2, and species were sown in even or uneven proportions, where one functional group was made dominant. Aboveground plant biomass increased linearly with the logarithm of species richness in all density and evenness treatments during all three years of the experiment. This was due to a convergence of realized density and evenness within species richness levels, although functional groups which were initially made dominant retained their dominance. Between species richness levels, realized density increased, and realized evenness decreased with species richness. Thus, more individuals could coexist if they belonged to different species. Within species richness levels, higher biomass values were correlated with lower density, suggesting an underlying thinning process. However, communities with low realized evenness also had low biomass values; thus high biomass did not result from species dominance. So-called complementarity and selection effects were similar across density and evenness treatments, indicating that the mechanisms underpinning the biodiversity effects were

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not altered. Species richness was the dominant driver of aboveground biomass, irrespective of variations in total densities and species abundance distributions at the start of the experiment; rejecting the hypothesis that initial differences in species abundance distributions might lead to different "stable states" in community structure or biomass. Thus, results from previous biodiversity experiments that only manipulated species richness and composition should be quite robust and broadly generalizable.

Keywords Biodiversity-productivity relationship · Multiple stable states · Richness × density × evenness experiment

Plant nomenclature Jäger and Werner (eds) (2002)

Introduction

Positive relationships between species richness and ecosystem productivity have now been established as general features of many ecosystems, particularly when richness of primary producers is modified (Balvanera et al. 2006; Cardinale et al. 2012; Naeem et al. 2012). Complementary resource use between species as well as dominance of particular species can drive these relationships (Hooper et al. 2005). However, while these relationships have over the years been observed in many experimental studies, the influence of variation in total density and in species abundance distributions have rarely been investigated. Greater species richness leads to greater density (Marquard et al. 2009a), and evenness has been shown to modify the relationship between species richness and ecosystem functioning in field and microcosm experiments (Wilsey and Potvin 2000; Maestre et al. 2012), but the interactive effects of species richness, total density and evenness on community productivity have not been examined so far. In particular, we expect that planting density should strengthen the biodiversity effect at high richness (Roscher et al. 2007), although perhaps only at high evenness, if dominance at high density of one species leads to rapid exclusion of other species.

Evenness is the similarity of the proportions of component species and is inversely related to dominance (Whittaker 1975). This community attribute is often held constant in plant diversity-productivity studies (Guo 2011). However, experiments have shown that variation in the evenness of mixtures of plant species can reduce community productivity – for example, when one species out of three reduces it by becoming dominant (Wilsey and Potvin 2000) – and that overyielding is increased at high evenness (Isbell et al. 2009). An analysis of a multi-site grassland biodiversity experiment (BIODEPTH) revealed that plots with the same species composition but lower evenness produced more biomass, and that higher species richness decreased evenness but increased biomass production (Mulder et al. 2004). At the same time, the relationship between species richness and productivity was steeper for communities with higher than for communities with lower evenness (Hector et al. 2002).

Like evenness, total density may also influence the diversity-productivity relationship in experimental plant communities. For example, He et al. (2005) showed that at low sowing density, constant final yield was only reached in plant communities with high species richness, while at high sowing density, communities reached constant final yield at any level of species richness. Constant final yield refers to the plateauing of

biomass production as density increases (Weiner and Freckleton 2010), and arises in part because size distributions of individuals in monocultures are more skewed at higher density (Harper 1977). By extrapolation it might be predicted that in mixtures, species abundance distributions become more rapidly uneven the higher the total plant density in a community is at the beginning of an experiment. However, this prediction has not been tested so far. Species deliberately made dominant or gaining dominance due to high community density may subsequently determine aggregated ecosystem measures, as suggested by the sampling effect.

To study density and evenness effects in connection with species richness, we manipulated these three variables in a factorial biodiversity experiment. This experiment was part of a large integrated biodiversity project (The Jena Experiment, Roscher et al. 2004). To compare the initial differences in community density and evenness with realized density and evenness values, we recorded plant densities for each species in each treatment combination over time. We expected the evenness and total density at the beginning of the experiment to affect the species richness–productivity relationship of the plant community. We, however, also expected that the effects of initial evenness and density might disappear over time if realized density and evenness converged. This would suggest that variations in community composition, and principally in species richness, have stronger effects than variations in species abundance distributions, i.e. community structure, on aboveground plant community productivity and ecosystem functioning in general.

Material and Methods

Experimental Design

This experiment was an additional component of the Jena Experiment, which had been set up to study the relationship between plant diversity and ecosystem processes (Roscher et al. 2004). The experiment was established on a former agricultural field on the floodplain of the Saale river near the city of Jena, Germany (50°55′ N, 11°35′ E, 130 m above sea level; mean annual temperature 9.3°C, mean annual precipitation 587 mm). We created a gradient of plant species richness with mixtures of 1, 2, 4, 8, and 16 species, stratified into 1–4 functional groups (grasses, legumes, tall herbs, short herbs) which were obtained by ordination of species traits (Roscher et al. 2004). We assembled experimental plant communities from seeds in May 2002 by random selection from a pool of 60 typical grassland species of Central Europe. Random selection was constrained by the requirement for even representation of all functional groups at each level of species richness. In addition, we avoided confounding of species richness and functional group richness as far as possible. For example, communities with one functional group varied in species richness from 1–16, communities with two functional groups from 2–16 and communities with three or four functional groups from 4–16 (see Roscher et al. 2004 for details). For the manipulation of density and evenness, we divided plots of 3.5 × 3.5 m into four subplots of 1.75 × 1.75 m. Buffer zones of 1 m separated subplots. Subplots within a plot had the same species composition but differed in sowing density and evenness. In total, the experiment consisted of 280 subplots because we could not impose evenness treatments in monocultures. The density and evenness treatments were (see also Table 1):

Table 1 Species and functional groups that were made numerically dominant in the uneven treatment of the experiment (see text for more detail)

Number of species	2 species	4 species	8 species	16 species
Proportion of seeds	3,1	5,1,1,1	5,5,1,1,1,1,1,1	5,5,5,5,1,1,1,1,1,1,1,1,1,1,1,1
Grasses	*Dactylis glomerata*	*Arrhenatherum elatius*	*Bromus hordeaceus, Festuca rubra*	*Alopecurus pratensis, Festuca pratensis, Holcus lanatus, Phleum pratense*
	Festuca rubra	*Bromus erectus*	*Cynosurus cristatus, Dactylis glomerata*	*Alopecurus pratensis, Bromus hordeaceus, Cynosurus cristatus, Luzula campestris*
	Poa pratensis	*Bromus hordeaceus*	*Phleum pratense, Luzula campestris*	*Arrhenatherum elatius, Cynosurus cristatus, Phleum pratense, Poa pratensis*
	Trisetum flavescens	*Luzula campestris*	*Trisetum flavescens, Phleum pratense*	*Holcus lanatus, Dactylis glomerata, Trisetum flavescens, Avenula pubescens*
Legumes	*Medicago lupulina*	*Lathyrus pratensis*	*Lathyrus pratensis, Trifolium dubium*	*Medicago sativa, Trifolium campestre, Trifolium hybridum, Trifolium repens*
	Medicago sativa	*Medicago lupulina*	*Lotus corniculatus, Trifolium hybridum*	*Onobrychis viciifolia, Trifolium dubium, Trifolium fragiferum, Vicia cracca,*
	Trifolium dubium	*Medicago sativa*	*Medicago lupulina, Onobrychis viciifolia*	*Lotus corniculatus, Medicago sativa, Trifolium hybridum, Trifolium repens*
	Trifolium pratense	*Trifolium pratense*	*Onobrychis viciifolia, Trifolium hybridum*	
Small herbs	*Plantago lanceolata*	*Plantago lanceolata*	*Glechoma hederacea, Leontodon autumnalis*	*Ajuga reptans, Prunella vulgaris, Ranunculus repens, Taraxacum officinale*
	Ranunculus repens	*Plantago lanceolata*	*Leontodon hispidus, Plantago lanceolata*	*Ajuga reptans, Plantago lanceolata, Ranunculus repens, Taraxacum officinale*
	Taraxacum officinale	*Plantago media*	*Plantago media, Primula veris*	*Leontodon hispidus, Prunella vulgaris, Ranunculus repens, Taraxacum officinale*
		Primula veris	*Taraxacum officinale, Veronica chamaedrys*	
Tall herbs	*Carum carvi*	*Anthriscus sylvestris*	*Anthriscus sylvestris, Sanguisorba officinalis*	*Anthriscus sylvestris, Campanula patula, Cardamine pratensis, Geranium pratense*
	Daucus carota	*Cardamine pratensis*	*Cardamine pratensis, Ranunculus acris*	*Achillea millefolium, Campanula patula, Rumex acetosa, Sanguisorba officinalis*
	Heracleum sphondylium	*Knautia arvensis*	*Crepis biennis, Sanguisorba officinalis*	*Anthriscus sylvestris, Campanula patula, Heracleum sphondylium, Leucanthemum vulgare*
	Ranunculus acris	*Knautia arvensis*	*Galium mollugo, Knautia arvensis*	*Crepis biennis, Daucus carota, Sanguisorba officinalis, Tragopogon pratensis, Sanguisorba officinalis*

i) Low sowing density and even abundance distribution (control). In these sub-plots, the total density of 1,000 viable seeds per m^2 was divided equally among the component species (same as in a parallel set of 20 × 20 m plots of the Jena Experiment, see Roscher et al. (2004)). The number of viable seeds was adjusted according to germination pre-tests.

ii) Low sowing density, uneven abundance distribution (for monocultures *ii* = *i*). These subplots had the same total density as *i*), and we obtained uneven species abundance distributions by increasing the density of only one species or functional group and decreasing the density of all others. The uneven distributions were 3:1 (2-species mixtures), 5:1:1:1 (4-species mixtures), 5:5:1:1:1:1:1:1 (8-species mixtures), 5:5:5:5:1:1:1:1:1:1:1:1:1:1:1:1 (16-species mixtures). In the 8- and 16-species mixtures, the two and four dominant species, respectively, belonged to the same functional group. The reason for making more than one species numerically dominant in the high diversity mixtures (the 8 and 16 species mixtures) was to maintain a fixed ratio between the numbers of dominant and subordinate species. The functional group and the species within functional groups which were made numerically dominant were selected randomly, with the restriction that every functional group was made dominant in the same number of replicates at each species richness level (Table 1; a few exceptions to this rule occurred at the 2- and 16-species richness level).

iii) High sowing density, even abundance distribution. In these subplots, the component density of each species was doubled, yielding a total density of 2,000 viable seeds per m^2.

iv) High sowing density, uneven abundance distribution (for monocultures *iv* = *iii*). These subplots had the same total density as *iii*) and the same uneven species abundance distribution as *ii*). The dominants were also the same as in *ii*).

The initial proportion of a species was the number of seeds of that species divided by the total number of viable seeds in the subplot. Later, during the course of the experiment, we calculated the proportion of a species from the relative densities of surviving individuals. We removed weeds twice a year in April and July. After establishment in the fall of 2002, we mowed the experimental plots twice a year in June and September, simulating the traditional management of hay meadows (see Roscher et al. 2004). The high sowing-density treatments were monitored from 2002–2003, while the other treatments were observed until late summer 2004.

We assessed population dynamics of all species by recording population densities (number of individuals per species) and sampling individual species biomass along transects of 10 × 100 cm (0.1 m^2). Identification of individuals follows the definitions described in Marquard et al. (2009b). These assessments were done twice in late spring of 2003 and 2004. Plants were cut at ground level, dried at 70°C for about 48 h to a constant mass and weighed. In accordance with the harvest protocols of other subprojects of the Jena Experiment, we also harvested community biomass in 20 × 50 cm (0.1 m^2) quadrats at 3 cm above ground (see Roscher et al. 2005) in late summer 2002 and 2003 (all subplots), in late spring 2003 and 2004 (subplots with low sowing density and even abundance distribution) and in late summer 2004 (subplots with low sowing density).

Calculations

We used either initial seed proportions (initial evenness as an experimental factor) or the number of individuals of each species (realized evenness) as the basis for calculations of evenness. We assigned all monocultures to the initial low evenness treatment category in a statistical model. This was justified by extrapolation of the regression lines for community biomass in even and uneven treatments from 16-, 8-, 4- and 2-species richness levels to monocultures (log-richness scale): the monoculture values were laying exactly on the regression line for the uneven treatment and distant from the regression line of the even treatment. If realized evenness was included in a model, monocultures were given missing values because, by definition, they cannot vary in evenness. To separate effects of evenness from effects of species richness, we used the index $E_{1/D}$, which is mathematically independent of species richness (Smith and Wilson 1996). The index is based on Simpson's dominance index D (Simpson 1949) and is calculated as follows:

$$E_{1/D} = \frac{1/_D}{S} = \frac{1}{S \sum_{i=1}^{S} p_i^2} \cdot \frac{1}{S}$$

Here p_i is the proportion of individuals from species i and S is the number of sown species in the sample. The index is 0 when all species except one in a mixture have no individuals. It is 1 when individuals are equally distributed among all species of the sample. The disadvantage of this index is that it is also 1 for monocultures, which in fact we considered as most uneven (one species fully dominant, see previous paragraph).

To calculate mechanisms underlying the biodiversity effect, we followed the method of Loreau and Hector (2001) to partition the net biodiversity effect into a complementarity and a selection effect. A further extension of this method has been proposed to split the selection effect into a "trait-dependent complementarity effect" and a "dominance effect" (Fox 2005), but this tripartite partitioning method requires a diversity gradient where all lower-richness plots are a subset of one high-richness plot, which is not the case for our experimental design. The Loreau and Hector method relies on comparisons of the performance of each species in a particular mixture *versus* the expected performance from the combination of monocultures. Thus, monoculture performance data were required for all 60 species. These data were available for the 2003 and 2004 harvests.

Statistical Analysis

We analyzed the data using general linear models, with the design variables used as treatment factors (Schmid et al. 2002a). We eliminated environmental heterogeneity of the field site by block and plot effects using a split-plot design with the error strata plot and subplot (cf. Roscher et al. 2004). Species richness as a continuous variable and as a factor was tested in the plot stratum (plots as error term) whereas density and evenness were tested in the subplot stratum (subplots as error term). Corresponding interactions were also tested in these error strata. Log-linear contrasts were fitted for species richness and its interactions. Changes in biomass (2002–2004) and evenness (2003–2004) between years were tested using a repeated-measures analysis of variance (ANOVA)

for the data obtained in the low sowing-density subplots (Meyer and Schmid 1999). The high sowing-density treatment was discontinued after the second harvest in spring 2003 and used for a different experimental study. Data were log-transformed where necessary to achieve normality and homoscedasticity of residuals. All analyses were done using the statistical software GenStat (Payne et al. 1993).

Results

Species Richness Effects

Aboveground biomass production, recorded for all subplots until late spring 2003 and for low sowing-density subplots until 2004, increased linearly with the logarithm of plant species richness in all analyses (all subplots 2002/2003: $F_{1,70}$=22.80, P<0.001; low sowing density subplots 2002–2004: Table 2; Fig. 1a–g). A repeated-measures ANOVA of the biomass data from the low sowing-density treatment showed that the positive effect of species richness did not significantly change over the years (non-significant interaction between log species richness and year in Table 2; Fig. 1a,c,e,g,h) or between harvest methods (harvest at 3 vs 0 cm above ground; interaction between log species richness and harvest with $F_{1,66}$=1.98 and P=0.164, in Table 2 pooled with residual). However, the species richness effect on aboveground biomass production was stronger at the time of peak biomass in late spring than in late summer (significant interaction between log species richness and season in Table 2).

Species richness positively affected realized community density (counts of individuals that survived into the second year, 2003, in high and low sowing density subplots: $F_{1,73}$=28.35, P<0.001; counts of individuals that survived into the third year, 2004, in low sowing-density subplots only: $F_{1,72}$=4.15, P=0.045; Fig. 2a–c). At the same time, species richness negatively affected realized evenness, $E_{1/D}$, calculated with the number of surviving individuals in low sowing-density subplots (2003/2004 without monocultures: $F_{1,57}$=130.87, P<0.001, Fig. 3a–c).

Density Effects

The sowing-density treatment affected aboveground biomass production of the entire plant communities only in the first year (2002), when all treatment categories were analyzed ($F_{1,176}$=4.02, P=0.047). In the first year (2002), subplots with high sowing density produced on average 10% more biomass than subplots with low sowing density. By the second year (2003), sowing density no longer influenced biomass production ($F_{1,198}$=0.24, P=0.625). Realized community density, however, still differed between the two sowing-density treatments. An average of 294 (SE ± 13.69) individuals occurred per m² in subplots with low sowing density (initially 1,000 seeds m⁻²) and 383 (SE ± 14.78) individuals in subplots with high sowing density (initially 2,000 seeds m⁻²; $F_{1,73}$=53.55, P<0.001; Fig. 2a,b).

Using realized density instead of sowing density as an explanatory variable showed a reversal of the density effect on aboveground biomass in the second year, 2003. Subplots with high realized community density within species richness levels (realized density was fitted after species richness) had significantly lower aboveground biomass production than subplots with lower realized community density (Table 3, Fig. 1c,d). This can

Table 2 Repeated-measures ANOVA of aboveground community biomass production 2002–2004 (low sowing density treatment; the high-sowing density treatment was only followed from 2002–2003)

Source	d.f.	MS	F	P
Block	3	325584	0.77	0.514
Species richness (log-linear)	1	9312340	22.05	**<0.001**
Species richness (deviation)	3	403602	0.96	0.419
Initial evenness	1	499454	7.93	**0.007**
Species richness (log-linear) × initial evenness	1	133745	2.12	0.150
Plot	70	422257	6.70	**<0.001**
Plot × subplot	60	63019	0.88	0.682
Year	1	3589744	21.86	**<0.001**
Season	1	63808517	272.00	**<0.001**
Year × season	1	1591718	14.13	**<0.001**
Harvest	1	41173	0.37	0.546
Species richness (log-linear) × year	1	448792	2.73	0.103
Species richness (deviation) × year	3	102925	0.63	0.600
Initial evenness × year	1	135	0.00	0.965
Species richness (log-linear) × initial evenness × year	1	142013	1.99	0.163
Plot × year	73	164205	2.30	**0.001**
Plot × subplot × year	60	71240	0.63	0.982
Species richness (log-linear) × season	1	2609300	11.12	**0.001**
Species richness (deviation) × season	3	340065	1.45	0.236
Initial evenness × season	1	352859	3.58	0.063
Species richness (log-linear) × initial evenness × season	1	87399	0.89	0.350
Plot × season	73	234589	2.38	**<0.001**
Plot × subplot × season	60	98502	0.87	0.729
Residual	258	112656		
Total	679	276634		

Values in bold are statistically significant.

be expected when self-thinning of communities allows surviving individuals to increase in biomass by a larger amount than the biomass lost from dying individuals. The negative relationship between realized community density and aboveground biomass production within species richness levels did not occur at the highest diversity levels (significant interaction between log species richness and realized density, Table 3), indicating that self- or alien-thinning in species-rich experimental communities did not allow for an increase in total aboveground biomass.

In contrast to our prediction, high sowing density did not decrease the realized evenness calculated with the number of surviving individuals (2003: $F_{1,183}=0.222$, $P=0.638$; Fig. 3a,b). This suggests that species with small individuals did not suffer higher mortality in subplots with high initial community density. In other words, self- and alien-thinning in communities did not affect species differentially. Furthermore, there was no significant relationship
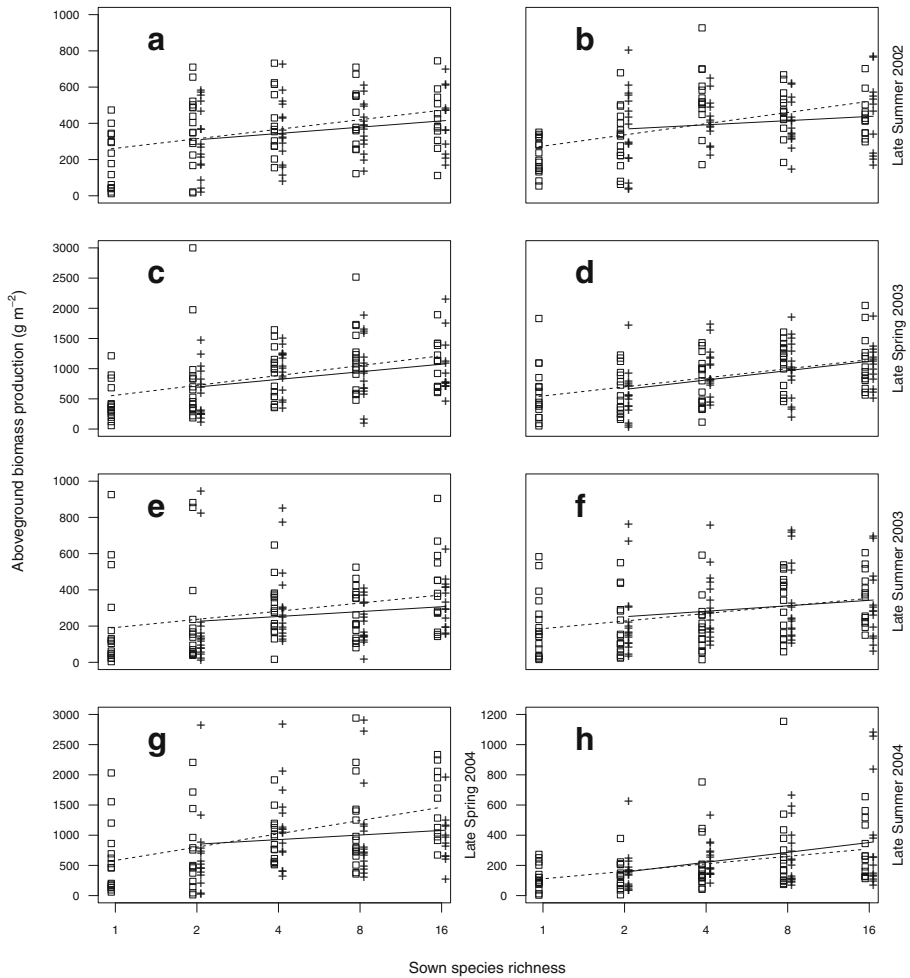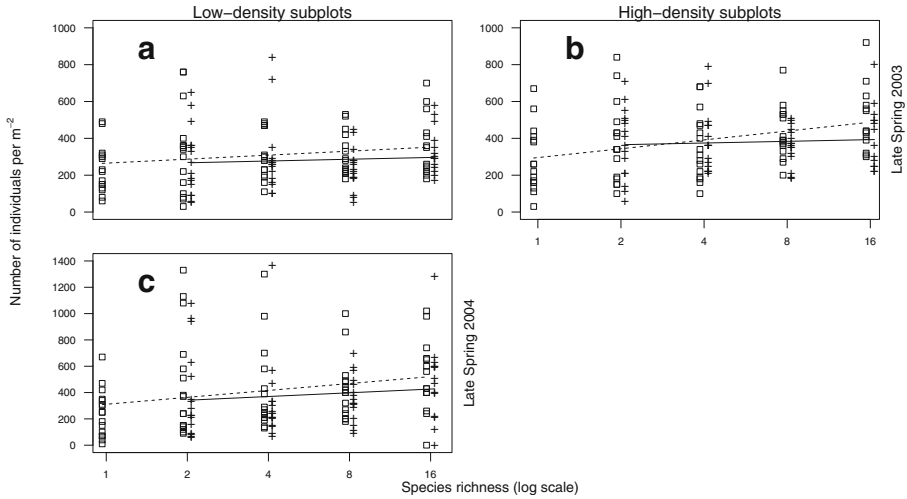
Fig. 1 Aboveground community biomass production as a function of species richness in uneven (□) and even subplots (+) started from low (**a**, **c**, **e**, **g**, **h**) or high sowing density (**b**, **d**, **f**) at five different harvest dates. Monocultures are considered uneven (see Material and Methods). Lines represent regression slopes from the statistical model. Solid lines illustrate even subplots and dotted lines uneven subplots

between realized density and realized evenness in the low sowing-density treatment for which we had data for both 2003 and 2004 (2003/2004: $F_{1,62}=0.14$, $P=0.712$).

Evenness Effects

Dominance treatments were successful in creating plots dominated by one particular functional group, and the dominance treatments were maintained over the years. In the second year of the treatments, grasses increased in dominance slightly whereas tall herbs tended to decline (Table 4). A repeated-measures ANOVA of available data from the low sowing-density treatment from 2002–2004 showed that subplots sown with an even initial species

Fig. 2 Realized community density (number of surviving individuals per m^2) as a function of species richness in uneven (□) and even subplots (+) started from low (**a**, **c**) or high sowing density (**b**) at two different harvest dates. Monocultures are considered uneven (see Material and Methods). Lines represent regression slopes from the statistical model. Solid lines illustrate even subplots and dotted lines uneven subplots

abundance distribution produced significantly more aboveground biomass (14 %) than subplots sown with an uneven initial species abundance distribution (Table 2; Fig. 1a,c,e,g,h). Although separate analyses for each year suggested that this positive effect of initial evenness on biomass production declined over time (being significant in the first but not in the second and third year), interactions
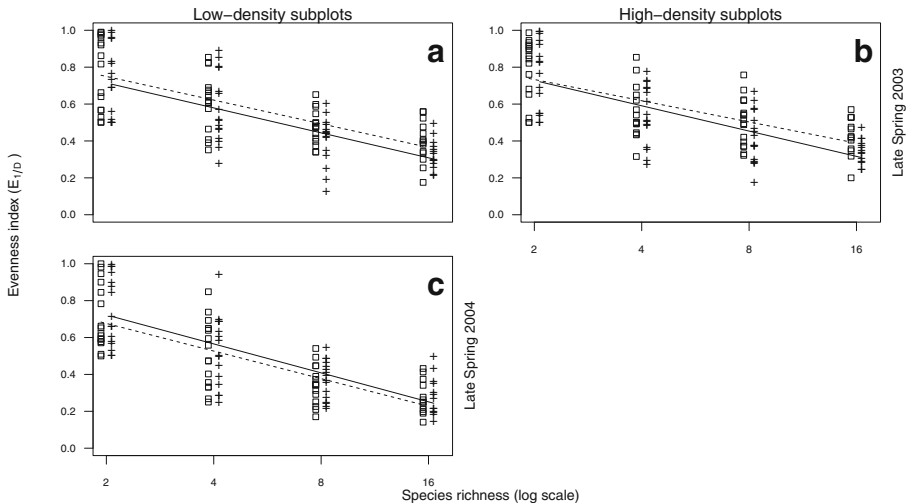
Fig. 3 Realized evenness (E$_{1/D}$-values based on number of individuals, see Material and Methods) as a function of species richness in uneven (□) and even subplots (+) started from low (**a**, **c**) or high sowing density (**b**) at two different harvest dates. Dash-dot lines represent evenness at the time of sowing for uneven subplots (lower line) and even subplots (upper line). Monocultures are omitted (see Material and Methods). Lines represent regression slopes from the statistical model. Solid lines illustrate even subplots and dotted lines uneven subplots

Table 3 Repeated-measures ANOVA of aboveground community biomass production 2003–2004, with realized density as a covariate (low sowing density treatment; in the high sowing density treatment realized density was only assessed in 2003)

Source	d.f.	MS	F	P
Block	3	338764	0.57	0.634
Species richness (log-linear)	1	10893680	18.47	**<0.001**
Species richness (deviation)	3	619100	1.05	0.376
Realized density	1	2108266	12.91	**0.001**
Species richness (log-linear) × realized density	1	1862225	11.41	**0.001**
Plot	70	589649	3.61	**<0.001**
Plot × subplot	62	163263	0.89	0.674
Year	1	1046048	3.74	0.057
Block × year	3	1024842	3.67	**0.017**
Species richness (log-linear) × year	1	9921	0.04	0.851
Species richness (deviation) × year	3	145284	0.52	0.670
Realized density × year	1	252725	1.38	0.246
Species richness (log-linear) × realized density × year	1	269770	1.47	0.231
Block × plot × year	66	279393	1.52	0.056
Residual	54	183565		
Total	271	378512		

Values in bold are statistically significant.

between year, season or harvest type and evenness were not significant in the repeated-measures ANOVA (Table 2). This indicates that initial evenness had a longer-lasting effect on biomass production than did the sowing density.

As a dependent variable, realized evenness ($E_{1/D}$) was strongly influenced by initial evenness in summer 2003 ($F_{1,123}=16.97$, $P<0.001$; Fig. 3a,b). However, compared with the index value based on the sowing proportions, realized evenness was reduced in all treatments, albeit least in the low species-richness with uneven-sowing combinations (Fig. 3a,b). After two years, in spring 2004, subplots with even and uneven sowing had converged to essentially the same realized evenness (low sowing density subplots: $F_{1,121}=0.94$, $P=0.337$); although functional groups which were initially made dominant retained their dominance. In the repeated-measures ANOVA of the years 2003 and 2004 for realized evenness as dependent variable, the corresponding year by evenness interaction was highly significant (low sowing density subplots: $F_{1,59}=8.80$, $P=0.004$). Apart from this, realized evenness was almost perfectly linearly related to the log of species richness across both years (low sowing density subplots: $F_{1,60}=109.74$, $P<0.001$; Fig. 3c).

As an explanatory variable, realized evenness had a consistent positive effect on aboveground biomass in 2003 and 2004 ($F_{1,62}=6.40$, $P=0.014$ in repeated-measures analysis of the two harvests in 2003 and 2004; Fig. 4). This indicates that sampling effects, which would coincide with reductions in realized evenness, were not the major cause of high aboveground biomass production (see also next paragraph). The slope of the relationship increased with the log of species richness (interaction

Table 4 Proportional abundance (mean ± 1 s.d.) of each of the four functional groups in plots of varying dominance treatments, all within the normal density treatments. Diagonals in bold show the resulting dominance of each functional group within the plots where that group was experimentally made dominant

Year	Plot subset	Grasses	Short herbs	Tall herbs	Legumes
2003	Grass dominant	**0.52±0.38**	0.28±0.41	0.14±0.28	0.06±0.13
	Short herb dominant	0.20±0.36	**0.48±0.43**	0.09±0.29	0.23±0.30
	Tall herb dominant	0.35±0.38	0.06±0.11	**0.50±0.40**	0.10±0.15
	Legume dominant	0.10±0.19	0.19±0.40	0.16±0.17	**0.54±0.34**
	Even plots	0.26±0.34	0.23±0.35	0.27±0.33	0.24±0.29
	Overall	0.28±0.35	0.24±0.36	0.25±0.33	0.23±0.30
2004	Grass dominant	**0.64±0.32**	0.15±0.21	0.18±0.33	0.02±0.07
	Short herb dominant	0.25±0.30	**0.55±0.36**	0.08±0.23	0.12±0.23
	Tall herb dominant	0.48±0.47	0.05±0.11	**0.29±0.42**	0.18±0.35
	Legume dominant	0.05±0.11	0.20±0.39	0.20±0.32	**0.55±0.38**
	Even plots	0.35±0.38	0.24±0.34	0.18±0.31	0.23±0.34
	Overall	0.36±0.38	0.23±0.33	0.19±0.32	0.22±0.34

between log species richness and realized evenness $F_{1,62}$=6.89, P=0.011; Fig. 4), indicating that evenness plays a greater role in species-rich communities. At the same time, the mean of realized evenness in species-rich communities was less variable and lower than the mean of realized evenness in species-poor communities (Figs. 3a–c and 4). This indicates that there is a trade-off or balance between the effects of species richness and realized evenness on aboveground biomass production of plant communities.

Complementarity and Selection Effects

When the net biodiversity effect was partitioned into complementarity (overyielding due to many species performing better than in monoculture) and selection effects (overyielding due to inclusion of single or few highly productive species), equivalent patterns were observed over all treatments (Fig. 5, Table 5). Partitioning was done for all treatments in 2003, and for all plots with involving normal density treatments in 2004. In all cases, complementarity effects increased with sown species richness and were larger than selection effects.

Discussion

Similar Species Richness-Productivity Relationships Under Different Evenness and Density Treatments

The positive relationship between plant species richness and aboveground biomass production in grassland plant communities was not affected by the additional
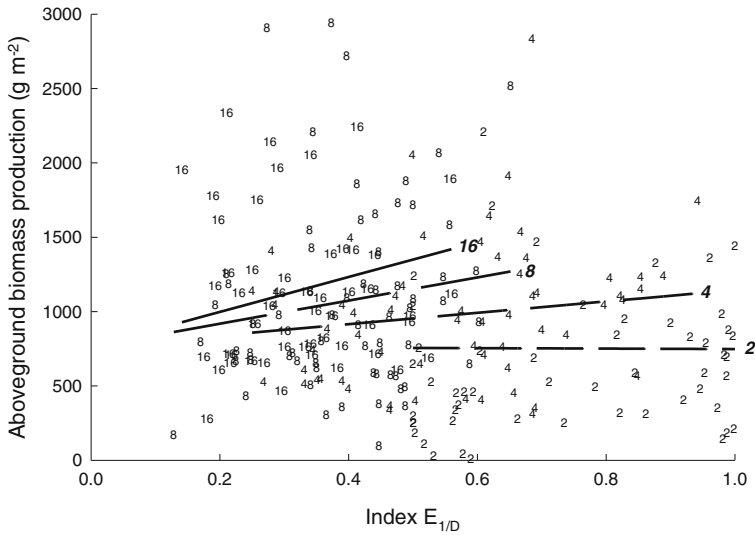
Fig. 4 Relationship between realized evenness (index $E_{1/D}$) and aboveground community biomass production in low sowing density subplots of both evenness treatments for plant communities of 2, 4, 8 and 16 species in late spring 2003 and late spring 2004. Lines represent regression slopes from the statistical model. Solid line – regression slope for 16-species mixtures (**16**), long dashed line – 8-species mixtures (**8**), medium dashed line – 4-species mixtures (**4**), short dashed line – 2-species mixtures (**2**). Small numbers indicate one subplot of the species richness treatments at the time of harvest

experimental manipulations of density and evenness beyond the establishment phase of experimental communities. In the even treatment, we theoretically maximized evenness by sowing equal proportions of the species, as in most biodiversity experiments. By the end of the experiment, the resulting communities were no longer at maximum evenness. In fact, by the first year, they showed rank-abundance distributions similar to semi-natural grasslands (Roscher et al. 2005). Similar patterns of species abundance distributions were also obtained in the uneven treatments, even though the identity of the dominant species was maintained (Table 6). This means that experimental communities sown at maximum evenness have a more realistic species abundance distribution than previously thought (Grime 1998; Schmid et al. 2002b; Lepš 2004; Wilsey and Polley 2004). It also suggests that varying only species richness is possibly a sufficient manipulation to study general patterns of biodiversity-ecosystem functioning relationships in experiments. In fact, if different starting proportions and densities always converge to similar species abundance distributions after the initial phase of an experiment, other designs that vary species proportions, e.g. so-called simplex designs (Nyfeler et al. 2009; Suter et al. 2010), may not be more realistic than designs that simply vary species richness to study biodiversity effects. Nevertheless, the convergence of rank-abundance distributions was not functional-group specific: functional groups whose species were initially made dominant retained their dominance during the entire experiment, although the identity of the dominant functional did not affect the species richness-aboveground biomass production relationship (data not show).
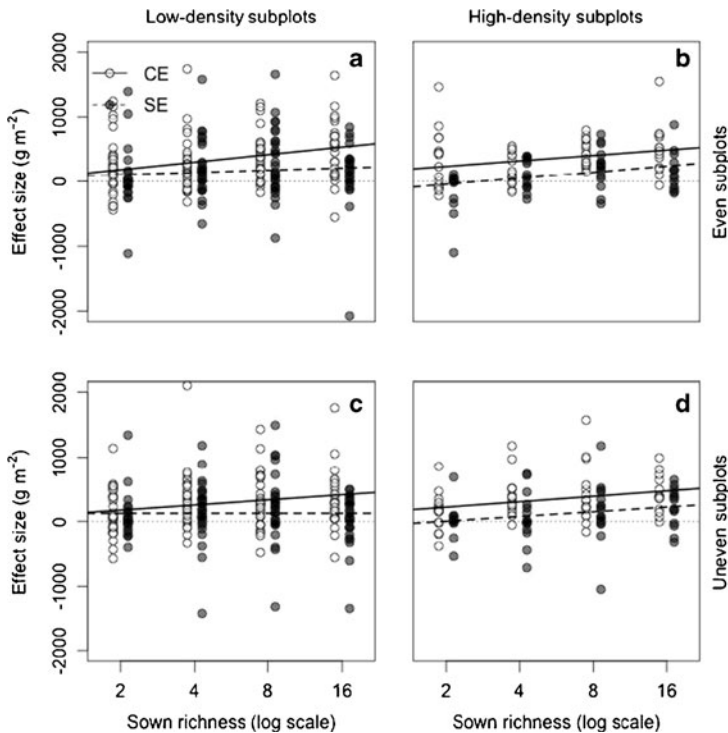
Fig. 5 Selection and complementarity effects for mixture plots in density and evenness treatments. Data are pooled across years for simplicity. In all treatments, complementarity effects increased with species richness, irrespective of density or evenness treatments. In high-density plots (**b** and **d**), selection effects also tended to increase with species richness

Density Effects

The loss of the initial effect of the density treatment on species richness–productivity relationship suggests that the communities were below constant yield during the establishment phase. Thus, higher community densities yielded a higher biomass production (Harper 1977; He et al. 2005). Although initial density did not affect community biomass production in the longer term, the realized density remained somewhat higher in plots with high sowing density than in plots with lower sowing density in the second year after establishment (2003). The community had to have around constant yield in the second year because the low sowing-density treatment on average yielded the same community biomass with fewer individuals as the high sowing-density treatment. In fact, within treatments there was a negative correlation between realized density and community biomass at low species richness levels. This indicates that some degree of community-level thinning may allow higher community biomass, which is in accordance with the self-thinning rule in single-species stands (Harper 1977; Weiner and Freckleton 2010). Apparently, such community-level thinning was more pronounced in low- than in high-species richness plots: plant species richness had a positive effect on the realized density of individuals in the community, an effect also observed in the main plots of the Jena Experiment (Marquard et al. 2009b). This suggests that high species richness enhances

Table 5 Summary of ANOVA results for partitioned biodiversity effects. Both complementarity effects (top sub-table) and selection effects (bottom sub-table) increased consistently with species richness, but in neither case did planted evenness or density directly or interactively alter the magnitude of the biodiversity effects

Complementarity effect				
Source	d.f.	MS	F	P
Block	3	325029	1.80	0.146
Species richness (log-linear)	1	4128509	22.91	**<0.001**
Species richness (deviation)	2	15909	0.09	0.916
Initial evenness	1	248605	1.38	0.241
Initial density	1	65565	0.36	0.547
Year	1	848685	4.71	**0.031**
Species richness (log-linear) × evenness	1	71965	0.40	0.528
Species richness (deviation) × evenness	2	528446	2.93	0.055
Species richness (log-linear) × density	1	19785	0.11	0.741
Species richness (deviation) × density	2	67035	0.37	0.690
Evenness × density	1	48877	0.27	0.603
Species richness (log-linear) × year	1	50974	0.28	0.595
Species richness (deviation) × year	2	32834	0.18	0.834
Evenness × year	1	21294	0.12	0.731
Residuals	351	180222		
Selection effect				
Source	d.f.	MS	F	P
Block	3	53574	0.33	0.803
Species richness (log-linear)	1	739915	4.57	**0.033**
Species richness (deviation)	2	683170	4.22	**0.015**
Initial evenness	1	32314	0.20	0.655
Initial density	1	141534	0.87	0.350
Year	1	259646	1.60	0.206
Species richness (log-linear) × evenness	1	106285	0.66	0.418
Species richness (deviation) × evenness	2	41530	0.26	0.774
Species richness (log-linear) × density	1	461471	2.85	0.092
Species richness (deviation) × density	2	56938	0.35	0.704
Evenness × density	1	52666	0.33	0.569
Species richness (log-linear) × year	1	13579	0.08	0.772
Species richness (deviation) × year	2	131708	0.81	0.444
Evenness × year	1	2817	0.02	0.895
Residuals	351	161833		

Values in bold are statistically significant.

establishment and survival of individual plants. Thus, species-rich communities used local resources more efficiently, which suggests a mechanism for complementarity effects in biodiversity experiments (Naeem et al. 1994; Loreau 2000; Silvertown 2004).

Partitioning selection (or "chance") effects from complementarity effects is critical when evaluating the mechanisms underlying the biodiversity effect (Lepš et al. 2001).

Table 6 Proportional abundance (mean ± 1 s.d.) of each of the four functional groups in plots along the sown diversity gradient and across evenness treatments, all within the normal density treatments. The biodiversity effects found in this experiment did not arise from an overwhelming increase in the dominance of legumes

Evenness	Sown diversity	Grasses	Short herbs	Tall herbs	Legumes
Even	2	0.27±0.41	0.32±0.45	0.22±0.39	0.20±0.38
	4	0.32±0.40	0.20±0.35	0.28±0.39	0.20±0.34
	8	0.28±0.38	0.25±0.38	0.21±0.34	0.27±0.38
	16	0.32±0.37	0.15±0.23	0.26±0.34	0.26±0.28
Uneven	2	0.27±0.41	0.32±0.46	0.22±0.40	0.18±0.39
	4	0.36±0.42	0.16±0.33	0.27±0.39	0.21±0.34
	8	0.30±0.39	0.25±0.38	0.20±0.34	0.24±0.37
	16	0.33±0.38	0.18±0.28	0.23±0.37	0.25±0.31

Indeed, complementarity effects were stronger than selection effects in all cases, and increased with species richness (Fig. 5). However, in contrast to some previous results, finding increased complementarity effects in even-abundance communities (Isbell et al. 2009) or increased complementarity and selection effects with greater density (Stachová et al. 2012), we found no change in the strength of the complementarity or selection effects with evenness or density (Table 5).

Extrapolating constant-yield and self-thinning rules from single- to multispecies stands (Bazzaz and Harper 1976; He et al. 2005) would also lead to the prediction that community-level thinning should remove subordinate species and thus high sowing density should lead to faster gains of dominant species than low sowing density. Interestingly, however, varying initial community density did not affect realized evenness in our communities. This demonstrates that the self-thinning rule cannot be directly extrapolated to multispecies communities because the effects of community-level thinning are not indifferent to species identities. This contradicts the results of a two-species self-thinning experiment of Bazzaz and Harper (1976), which to our knowledge is the only multi-species thinning experiment carried out so far.

Evenness Effects

The experimental communities were sown in May 2002 and first harvested in late summer 2002. Thus, there was not much time for single species to develop dominance according to their competitive abilities. Nevertheless, the analysis of realized evenness using the index $E_{1/D}$ showed that both evenness treatments had already departed from their initial sowing proportions. Yet, the treatment with even initial proportions still had a higher realized evenness than the treatment with uneven initial proportions (see Fig. 3a,b). This was combined with a positive effect of evenness on biomass production at the time of the first harvest in 2002. Wilsey and Polley (2004) also found short-term benefits of evenness for productivity, but only in deep-rooting plants.

As the two evenness treatments continued to converge in realized evenness, differences in initial evenness no longer affected community biomass production in the second and third year. The continuous decline of the evenness index $E_{1/D}$ over time

indicates that some species were still gaining dominance in the experimental communities. Mulder et al. (2004) showed in a path analysis that such dominance developments can eventually weaken the positive effect of evenness on biomass production as suggested by Nijs and Roy (2000) with a simulation model. However, Mulder et al. (2004) found no evidence for a consistent decline in evenness over two years in their analysis of a multi-site biodiversity experiment. Although they found their results difficult to interpret without knowing to what extend growing conditions may have differed over time, the results indicate that dominance and complementarity may reach some balance in the longer term. Such a balance between dominance and complementarity may also be inferred from the fact that community biomass production increased with realized evenness at each level of species richness – an observation also made in the BIODEPTH multi-site experiment (Hector et al. 2002), but realized evenness decreased with increasing species richness (see Figs. 3a–c and 4). Thus, higher biomass production was reached at lower levels of realized evenness if the community contained more species. The more species a community has, the higher is the chance that the community will incorporate highly productive species, which will then gain dominance and thereby reduce realized evenness. It is conceivable that the species gaining dominance at high diversity are not those that perform best in monocultures or in low diversity mixtures (Lambers et al. 2004; Schläpfer et al. 2005). Lambers et al. (2004) and Mulder et al. (2004) also showed that dominance of particular species with high yield in mixtures generally does not lead to the displacement over time of other particular species with lower yield. This is also consistent with our finding that species or functional groups which were arbitrarily made dominant could maintain this dominance during the course of the experiment.

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