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Density may alter diversity–productivity relationships in experimental plant communities

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Summary

Contemporary biodiversity experiments, in which plant species richness is manipulated and aboveground productivity of the system measured, generally demonstrate that lowering plant species richness reduces productivity. However, we propose that community density may in part compensate for this reduction of productivity at low diversity. We conducted a factorial experiment in which plant functional group richness was held constant at three, while plant species richness increased from three to six to 12 species and community density from 440 to 1050 to 2525 seedlings m^{-2}. Response variables included density, evenness and above- and belowground biomass at harvest. The density gradient converged slightly during the course of the experiment due to about 10% mortality at the highest sowing density. Evenness measured in terms of aboveground biomass at harvest significantly declined with density, but the effect was weak. Overall, aboveground, belowground and total biomass increased significantly with species richness and community density. However, a significant interaction between species richness and community density occurred for both total and aboveground biomass, indicating that the diversity–productivity relationship was flatter at higher than at lower density. Thus, high species richness enabled low-density communities to reach productivity levels otherwise seen only at high density. The relative contributions of the three functional groups C_3, C_4 and nitrogen-fixers to aboveground biomass were less influenced by community density at high than at low species richness. We interpret the interaction effects between community density and species richness on community biomass by expanding findings about constant yield and size variation from monocultures to plant mixtures.
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Zusammenfassung

Neuere Biodiversitätsxperimente, in denen die Anzahl Pflanzenarten variiert aber die Gesamtdichte konstant gehalten wurde, konnten einen positiven Zusammenhang zwischen pflanzlicher Diversität und Produktivität nachweisen. Unsere Hypothese war, dass eine erhöhte Gesamtdichte den negativen Effekt geringer Artenzahl teilweise kompensieren könnte. In einem faktoriellen Experiment untersuchten wir den Einfluss von drei Ausgangsdichten (440, 1050, 2525 Keimlinge pro Quadratmeter) und drei Diversitätsstufen (3, 6, 12 Arten) auf die oberirdische und unterirdische Pflanzenmasse sowie die Gesamtdichte der adulten Pflanzen und die Abundanzverteilung („evenness") der Arten nach vier Monaten Wachstum. Die positive Diversität–Produktivität-beziehung flachte mit zunehmender Gesamtdichte ab, während gleichzeitig die Dominanz einzelner Arten zunahm (geringere "evenness" bei hoher Dichte). Diese Ergebnisse zeigen, dass der negative Einfluss geringer Diversität auf die Produktivität durch eine höhere Gesamtdichte von Pflanzen teilweise kompensiert werden kann. Der relative Beitrag der drei funktionellen Gruppen C_3, C_4 und Stickstoff fixierender Pflanzen zur Biomasseproduktion war bei hoher Diversität weniger von der Dichte beeinflusst als bei geringer Diversität. Wir interpretieren die Interaktionen zwischen Gesamtdichte und Diversität, indem wir populationsbiologische Konzepte von Monokulturen auf gemischte Pflanzenbestände übertragen.

Introduction

Biodiversity experiments in which plant species richness is deliberately manipulated and aboveground productivity of the ecosystem measured, generally demonstrate that diversity is positively related to productivity (e.g., Naeem, Thompson, Lawler, Lawton, & Woodfin, 1994; Naeem, Hakansson, Lawton, Crawley, & Thompson, 1996; Tilman, Wedin, & Knops, 1996; Hector et al., 1999; Schläpfer & Schmid, 1999; Tilman, 1999; Dimitrakopoulos & Schmid, 2004; Pfisterer, Joshi, Schmid, & Fischer, 2004). However, a debate has ensued regarding the mechanism causing the relationship in these experimental systems (Grime, 1997; Huston, 1997; Lepš, 2004; Schmid & Hector, 2004) and questioning whether the observed positive relationship exists in natural systems with varying environmental conditions and density (Grime, 1997; Waide et al., 1999; Wardle et al., 2000). Here we propose that community density is a potentially confounding factor in the diversity–productivity relationship and may be responsible for some of the discrepancies observed between experimental and comparative studies (see Hooper et al., 2005 for a review).

In previous biodiversity experiments, community density was kept constant at a particular level. Because varying planting density over a large range does not affect stand biomass in monocultures (law of constant final yield, see e.g. Harper, 1977), it may indeed often be sufficient to test biodiversity effects on biomass production at a single level of community density. However, interactions between density and species richness may affect the potential of plant species mixtures to reach a level of biomass production that corresponds to constant final yield. In particular, we predict that high community density may in part compensate for low species richness and thus lead to a flattening of the diversity–productivity relationship with increasing community density.

To test our hypothesis we carried out an experiment in which early successional herbaceous species were grown in bins in a glasshouse for 4 months at three levels of community density factorially combined with three levels of species richness. To avoid confounding species richness with functional richness we planted species of the same three functional groups in each experimental community. In contrast to previous plant biodiversity experiments (see reviews in Schmid et al., 2002a; Schmid, Joshi, & Schläpfer, 2002b), the communities in our experiment were not subject to weed invasions and thus disturbance by repeated weeding was avoided. We measured the ecosystem response to the experimental treatments as both above- and belowground biomass production. Furthermore, for the aboveground biomass production we also tested the response of each of the three functional groups to species richness, community density and their interaction, to see if the contributions of different functional groups to community productivity changed with these factors.

Materials and methods

Experimental design and growth conditions

The study was conducted in an environmentally controlled glasshouse at Harvard University in Cambridge, Massachusetts. Experimental communities of three, six or 12 species were assembled from a pool of 15 early successional species of the Midwestern United States (Table 1). At each of the three species richness levels there were two different species combinations. Communities were sown to obtain densities corresponding to individual areas of 24.0, 8.0, or 2.4 cm^2 plant^{-1}. Germination ratios were used to calculate the mass of seeds needed to produce equal numbers of seedlings per community and equal partial densities per species within communities. There were six replicates for each factorial combination of species richness $\times$ species combination $\times$ community density, resulting in a total of 108 communities.

Each community contained the same three functional groups, i.e., C_3, C_4 and nitrogen-fixing plants. The six communities (three levels of species richness $\times$ two species combinations) were coded as treatments L1, L2; M1, M2; and H1, H2. The L1 and L2 treatments consisted of a single species from each functional group (C_3, C_4 and nitrogen-fixers), the M1 and M2 treatments consisted of two species from each functional group and the H1 and H2 treatments consisted of three species each of C_4 and nitrogen-fixers plus six C_3 species (see Table 2 for detailed list of the species assigned to each treatment).

While functional group richness was constant across the species richness gradient, functional group evenness was slightly lower at the highest than at the two lower species richness levels. The natural communities of annual species simulated in our experiment contain more C_3 species than C_4 or nitrogen-fixing species; therefore, it was most realistic to increase species richness by adding only C_3 species to the high level. We checked whether functional group evenness had higher explanatory power than species richness and found that the opposite was the case (the test was possible because functional richness and functional evenness were not fully confounded: there were two levels of richness for one evenness; see also "Statistical analyses" below).

The species composition of each community was determined based on a nested design like that used by Naeem et al. (1994) or by Niklaus, Leadley, Schmid, and Körner (2001); i.e., the community in L1 was a subset of M1, H1 and H2; L2 was a subset of M1, M2, H1, H2; and both M1 and M2 were subsets of H1 and H2. This means that as species richness increased, new species were added without replacing species already selected at lower levels. Thus, any effects of species richness are due to the addition of species. Because of restricted availabilities of species, the compositional differences at the two lower species richness levels were the presence (L1 and M1) or absence (L2 and M2) of *Ambrosia artemisiifolia* (hereafter referred to as *Ambrosia*).

Plant communities were grown in $28.5 \times 33.5 \times 20.0$ cm (length $\times$ width $\times$ height) plastic bins containing drainage holes in the bottom and filled with a 1:4 mixture of garden soil:Pro-Mix general-purpose growing medium (Red Hill, PA, USA). Each bin was fertilized with 15 g of Osmocote, a controlled-release fertilizer (N:P:K = 14%:14%:14%). Soon after germination, each bin was inoculated with both *Rhizobium leguminosarum* biovar *trifolli* and *R. leguminosarum* biovar *viciae* (MicroBio RhizoGen Corporation, Saskatoon, SK, Canada). Communities were randomly assigned to three zones (separate rooms) of an environmentally controlled glasshouse. Bins were arranged adjacent to each other to reduce edge effects within bins and to obtain a more realistic "larger-community" environment. In addition, green mesh netting was stretched around the outside of the bins in each zone, to mimic the effect of plant competition for light in bins located on the edges. To

Table 1. Pool of species used in the experiment and abbreviation for each as listed in Table 2

Species	Abbreviation
C_3 species	
Abutilon theophrasti Medikus.	At°
Ambrosia artemisiifolia L.	Ama°
Chenopodium album L.	Ca°
Conyza canadensis (L.) Cronq.	Cc°
Ipomoea hederacea Jacq.	Ih°
Poa annua L.	Pa°
Plantago lanceolata L.	Pl (°)
Bromus commutatus Schrader.	Bc°
C_4 species	
Amaranthus retroflexus L.	Am°
Setaria glauca (L.) P. Beauv.	Sg°
Setaria faberii R. Herm.	Sf°
Setaria italica (L.) P. Beauv.	Si°
Nitrogen-fixing species	
Vicia cracca L.	Vc
Trifolium pratense L.	Tp
Trifolium dubium Sibth.	Td°

Nomenclature follows Gleason and Cronquist (1991). Annual species are indicated by a circle (°) after the abbreviation (*Plantago lanceolata* can be annual or perennial).

Table 2.   Treatments used in the experiment and species assigned to each treatment

Species richness treatment	Community density	Species per community	Functional groups		
			C_3	C_4	N-fixers
L1	L, M, H	3	Ama	Si	Tp
L2	L, M, H	3	At	Si	Tp
M1	L, M, H	6	At, Ama	Si, Am	Tp, Vc
M2	L, M, H	6	At, Pl	Si, Am	Tp, Vc
H1	L, M, H	12	At, Ama, Ca, Ih, Pa, Pl	Si, Am, Sf	Tp, Vc, Td
H2	L, M, H	12	At, Ama, Ca, Cc, Pl, Bc	Si, Am, Sg	Tp, Vc, Td

The experiment consisted of communities planted at three levels of species richness, with two species combinations assigned to each species richness level, three densities and six replicates of each treatment.

minimize between-treatment carryover effects the bins were arranged at random within each zone and re-randomized each week during the course of the experiment. The temperature in all zones was maintained at 25 °C from 8:00–20:00 h during the day and 19 °C over night. Lighting was provided by natural sunlight filtered through the roof of the glasshouse, which reduced light levels by 28%. Bins were watered daily to keep the soil moist at all times.

Seeds were planted on 7 May 2001. The number of germinated seedlings was counted in each community of one zone to estimate the effective seedling densities. These averaged 42, 100 and 241 seedlings per bin, corresponding to densities of 440, 1050 and 2525 seedlings m^{-2} and individual areas of 22.9, 9.6 and 4.0 cm^2 $plant^{-1}$. At harvest, we counted the number of individuals in at least three of the six replicates of each treatment combination (62 bins in total) to estimate final plant densities. These final densities were 43, 103 and 219 plants per bin, corresponding to densities of 454, 1078 and 2291 plants m^{-2} and individual areas of 22.2, 9.3 and 4.4 cm^2 $plant^{-1}$. Thus, plant mortality was low and the differences we measured in response variables were mainly due to size plasticity as expected under the constant yield law rather than due to density-dependent thinning.

The harvest was conducted after four months, when plants had reached maximum biomass and just before the first individuals started to shed seeds. The duration of this experiment was similar to the growing-season length allowed until peak biomass harvest in experiments with perennial grassland species on relatively fertile soils (see e.g. Hector et al., 1999; Pfisterer et al., 2004). Owing to the optimal growing conditions in the glasshouse and the fact that plants could extend their aboveground parts beyond the bin and thus intercept light from a larger area, our experimental communities reached very high yield, in particular in relation to bin area.

Harvest measurements

At harvest, stems were cut at ground level and aboveground biomass of all plants per bin was collected. In at least three of the six replicates of each treatment combination (the same 62 bins for which the final plant density was determined) the aboveground biomass was separated into functional groups and species to obtain estimates of species evenness at harvest. Evenness was calculated using the following formula:

$$E = H' / \ln(S),$$

where E is the Shannon evenness index, H' the Shannon–Wiener diversity index and S the number of species (Pielou, 1969). To estimate total productivity of the root system, we took root subsamples from the middle of each bin using stainless steel cores (15 cm diameter × 20 cm deep). We also excavated 36 intact root systems of the whole community by carefully washing away the soil. A linear regression between root biomass of entire bins and core sub-samples was used to calculate the biomass of whole root systems of the remaining 72 bins. No attempt was made to separate roots by species or functional group. Above- and belowground biomass samples were dried at 65 °C for 1 week before measuring dry weight.

Statistical analyses

In this experiment, the dependent variables were total, above- and belowground biomass, root to shoot ratio (belowground biomass/aboveground biomass), aboveground biomass proportion of each

functional group (biomass of functional group/total biomass), final plant density and species evenness. All biomass variables and final plant density values were log-transformed to meet assumptions of normality and homogeneity of variances. We used the regression approach to the analysis of variance (ANOVA) as implemented in Genstat software (Payne et al., 1993). The full model included a term for zone (block, as a grouping variable), density (log seedling density of total community, as a continuous explanatory variable; see estimated values above), species richness (species richness, as a grouping variable, partitioned into a linear contrast and its deviation), density × species richness interaction, and species combination within species richness level (partitioned into a contrast comparing species combinations in which *Ambrosia* was present against those in which *Ambrosia* was absent). The untransformed means are presented in the graphs. The significance of effects was tested with the F-ratios between mean squares of effects and residuals. In the case of the species richness effect this was preferred over the alternative F-ratio calculated by dividing the mean square of species richness by the mean square of combination (Schmid et al., 2002a). The reason for this choice was the low number of combinations (two per species richness level). However, we present the mean squares in the tables allowing readers to calculate the alternative F-ratios for comparison.

In the analyses described above, functional group evenness was confounded with the species richness treatment (see above). In the low and medium species richness treatments, each functional group comprised 1/3 of the total species richness (proportion of species richness in each functional group was in the ratio 1:1:1 for C_3, C_4 and nitrogen-fixing species, respectively). In the high species richness treatment, however, C_4 plants and nitrogen-fixing species each comprised 1/4 of the total richness, while C_3 plants comprised 1/2 of the total richness (i.e., the representation of each functional group was in the ratio 2:1:1). To address this issue, we also analyzed the functional group biomasses on a per-seedling basis. Furthermore, we conducted an additional analysis in which we explicitly tested for the effects of functional group evenness, rather than species richness, on each dependent variable. In nearly all cases, functional group evenness explained less of the variance for each dependent variable than did species richness, indicating that functional group evenness was less important than species richness. Thus, for simplicity, we present here only the test results for the species richness effects.

Results

Relations between species richness, seedling density, final density and species evenness

The community density at harvest was affected by all experimental treatments (Table 3). Whereas all seedlings survived to adulthood in the low- and medium-density treatments, approximately 9.3% mortality occurred in the high-density treatment (see the section "Methods"). Species richness had an idiosyncratic effect on final density, which was depressed at the intermediate level of species richness. Species evenness declined with increasing seedling density and plant density at harvest (Table 3, Fig. 1). Because species evenness was measured in terms of aboveground biomass production, this result indicated that high community density promoted dominance of productive species.

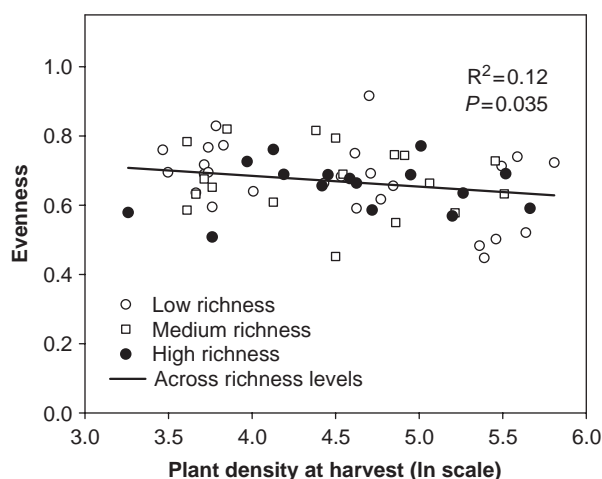## Effects of species richness, seedling density and species composition on biomass

Zone (block), density, species richness, density × species richness and combination within species richness were all significant predictors of total or aboveground biomass, explaining 34.1% or 35.8% of the total variation, respectively (Table 4). For belowground biomass, however, only species richness had a significant effect, explaining 17.3% of the total variation. Density, species richness and combination within species richness were significant predictors of root to shoot ratio, explaining 28.3% of the variation.

The effects of species richness were positive and linear (Fig. 2; see also small deviations from linearity in Table 4). Total and aboveground biomass also increased with density. However, because belowground (i.e., root) biomass remained constant, the root to shoot ratio declined with increasing density. These main effects of species richness and density must be viewed in light of the significant interactions between the two for both total biomass and aboveground biomass (Table 4). That is, the diversity–productivity relationship was more positive at low density and weakened with increasing density (Fig. 2). In other words, density effects on biomass production are most clearly seen at low species richness.

Communities with the C_3 species *Ambrosia* invested on average relatively less in above- and more in belowground biomass and, therefore, had a higher root to shoot ratio than communities lacking this species.

Table 3. Summary of analysis of variance (ANOVA) for final density (log-transformed prior to analysis) and species evenness

| Sources of variation	d.f.	S.S.	M.S.	F	P
Final density					
Zone	2	0.768	0.384	12.70	<0.001
Density	1	26.945	26.945	890.99	<0.001
Species richness	2	0.368	0.184	6.09	0.004
Linear	1	0.000	0.000	0.00	0.962
Deviation	1	0.368	0.368	12.18	0.001
Density × species richness	2	0.330	0.165	5.45	0.007
Linear	1	0.194	0.194	6.41	0.014
Deviation	1	0.136	0.136	4.50	0.039
Combination (Species richness)	3	0.381	0.127	4.20	0.010
Ambrosia	1	0.031	0.031	1.02	0.318
Combination (Species richness)	2	0.350	0.175	5.79	0.005
Residual	51	1.542	0.030		
Total	61	30.335	0.497		
Species evenness					
Zone	2	0.070	0.035	6.22	0.004
Density	1	0.049	0.049	8.74	0.005
Species richness	2	0.001	0.001	0.09	0.914
Linear	1	0.000	0.000	0.00	0.972
Deviation	1	0.001	0.001	0.18	0.674
Density × species richness	2	0.012	0.006	1.02	0.367
Linear	1	0.007	0.007	1.32	0.255
Deviation	1	0.004	0.004	0.72	0.400
Combination (Species richness)	3	0.141	0.047	8.35	<0.001
Ambrosia	1	0.005	0.005	0.97	0.329
Combination (Species richness)	2	0.136	0.068	12.04	<0.001
Residual	51	0.287	0.006		
Total	61	0.560	0.009		

Figure 1. Species evenness, calculated with the aboveground biomass proportions at harvest, plotted against total plant density in the experimental communities at harvest (ln(number of individuals)/bin). The different symbols refer to the three species richness levels (three, six, or 12 species).

Effects of species richness, seedling density and species composition on functional group proportions

The aboveground biomass proportions of the three functional groups did not remain constant across treatments (Table 5). The species richness effects were strongly linear for the C_3 and C_4 species but were not linear for the nitrogen-fixers. These effects were in part due to the experimental design, because C_4 and nitrogen-fixing plants were planted in proportions of 1/3, 1/3 and 1/4 and C_3 plants in proportions of 1/3, 1/3 and 1/2 at the low, medium and high levels of species richness, respectively. When the biomass of the different functional groups was calculated on a per-individual-germinated basis, i.e., the biomass of each functional group was divided by seedling density and then multiplied by sowing proportion (and the logarithm thereof analyzed), C_3 plants had the smallest individuals and C_4 plants had the largest

Table 4. Summary of analysis of variance (ANOVA) for total biomass, aboveground biomass, belowground biomass and root to shoot ratio (data were log-transformed prior to analysis)

Sources of variation	d.f.	S.S.	M.S.	F	P
Total biomass					
Zone	2	0.352	0.176	5.87	0.004
Density	1	0.279	0.279	9.29	0.003
Species richness	2	0.366	0.183	6.10	0.003
Linear	1	0.357	0.357	11.89	<0.001
Deviation	1	0.009	0.009	0.31	0.581
Density × species richness	2	0.241	0.120	4.01	0.021
Linear	1	0.126	0.126	4.18	0.044
Deviation	1	0.115	0.115	3.83	0.053
Combination (Species richness)	3	0.267	0.089	2.96	0.036
Ambrosia	1	0.076	0.076	2.55	0.114
Combination (Species richness)	2	0.190	0.095	3.17	0.046
Residual	97	2.912	0.030		
Total	107	4.417	0.041		
Aboveground biomass					
Zone	2	0.406	0.203	6.80	0.002
Density	1	0.426	0.426	14.30	<0.001
Species richness	2	0.181	0.090	3.03	0.053
Linear	1	0.161	0.161	5.40	0.022
Deviation	1	0.020	0.020	0.66	0.419
Density × species richness	2	0.218	0.109	3.65	0.030
Linear	1	0.128	0.128	4.30	0.041
Deviation	1	0.089	0.089	3.00	0.087
Combination (Species richness)	3	0.385	0.128	4.30	0.007
Ambrosia	1	0.158	0.158	5.28	0.024
Combination (Species richness)	2	0.227	0.113	3.80	0.026
Residual	97	2.894	0.030		
Total	107	4.509	0.042		
Belowground biomass					
Zone	2	0.064	0.032	0.19	0.828
Density	1	0.012	0.012	0.07	0.787
Species richness	2	3.787	1.893	11.21	<0.001
Linear	1	3.730	3.730	22.07	<0.001
Deviation	1	0.057	0.057	0.34	0.562
Density × species richness	2	0.387	0.193	1.14	0.323
Linear	1	0.079	0.079	0.47	0.496
Deviation	1	0.307	0.307	1.82	0.180
Combination (Species richness)	3	1.252	0.417	2.47	0.067
Ambrosia	1	0.413	0.413	2.45	0.121
Combination (Species richness)	2	0.838	0.419	2.48	0.089
Residual	97	16.390	0.169		
Total	107	21.891	0.205		
Root to shoot ratio					
Zone	2	0.267	0.134	0.90	0.409
Density	1	0.638	0.638	4.30	0.041
Species richness	2	2.498	1.249	8.42	<0.001
Linear	1	2.352	2.352	15.86	<0.001
Deviation	1	0.146	0.146	0.98	0.324
Density × species richness	2	0.085	0.043	0.29	0.751
Linear	1	0.010	0.010	0.07	0.796
Deviation	1	0.075	0.075	0.51	0.478
Combination (Species richness)	3	2.200	0.733	4.95	0.003
Ambrosia	1	0.997	0.997	6.73	0.011
Combination (Species richness)	2	1.203	0.601	4.05	0.020
Residual	97	14.385	0.148		
Total	107	20.074	0.188		
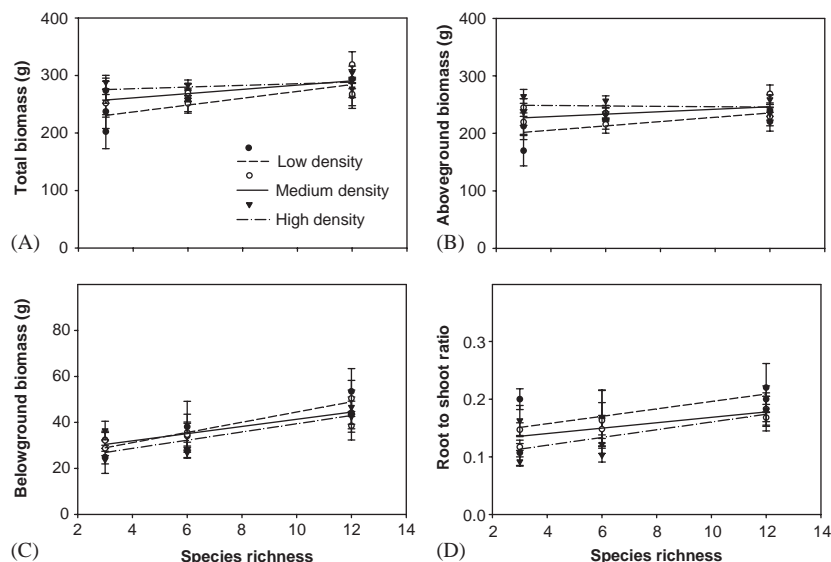
Figure 2. The species richness–productivity relationship as it is affected by total seedling density in the experimental communities: (A) Total biomass of each community, (B) aboveground biomass, (C) belowground biomass and (D) root to shoot ratio. Plants were grown at three species richness levels, containing three, six and 12 species and three density levels. Each species richness level contained two species combinations each replicated six times. Points in the graph are the mean $\pm$ 1 standard error for each species combination. The C_3, C_4 and nitrogen-fixing functional groups were all represented in each species richness treatment. Density levels were 440 (low), 1050 (medium) and 2525 seedlings m^{-2} (high).

individuals at the highest species richness. This could have been due to the relatively higher partial density stress for the C_3 functional group (planted at a proportion of 1/2 the species at the highest level of species richness) and lower partial density stress for the C_4 functional group (planted at a proportion of 1/4 the species at the highest level of species richness). The nitrogen-fixing plants had the largest individuals at intermediate species richness levels.

The biomass proportion of C_3 plants significantly decreased with density, while the proportion of C_4 plants increased (Fig. 3), although the latter was only marginally significant in the analysis (Table 5). The significant density × richness interactions for the aboveground biomass proportions of functional groups resembled that for the community biomass, i.e. density effects were stronger at low than at high species richness. However, the different functional groups showed different directions of density effects at low species richness. The proportion of C_3 species increased with density at low species richness, while C_4 and nitrogen-fixing species declined.

Species combination within species richness also significantly affected the aboveground biomass proportion of C_3 and C_4 plants (Table 5). These effects could be attributed to the presence of

Ambrosia: experimental communities of low and medium richness levels produced 36% more C_3 biomass when *Ambrosia* was absent and a 37% increase in C_4 biomass when *Ambrosia* was present.

Discussion

Diversity–productivity relationships at different levels of community density

Biodiversity experiments have repeatedly found positive relationships between plant species richness and biomass production or other ecosystem variables (Schmid et al., 2002b; Hooper et al., 2005). Variations in the strength of the relationship have been related to the specific species combinations used in the experiments or to abiotic factors such as site fertility (e.g., Dimitrakopoulos & Schmid, 2004). However, community density has received surprisingly little attention so far as a factor in such relationships. Thus, previous biodiversity experiments were carried out at a fixed community density (which has however varied between experiments, e.g., from 590 to 2307 plants or plantlets m^{-2} in Symstad, Tilman, Wilson, and Knops, 1998; Hector et al., 1999; Niklaus et al.,

Table 5. Summary of analysis of variance (ANOVA) for the aboveground biomass proportion of each functional group (data were log-transformed prior to analysis)

Sources of variation	d.f.	S.S.	M.S.	F	P
C_3 plants					
Zone	2	0.220	0.110	1.19	0.313
Density	1	0.427	0.427	4.61	0.037
Species richness	2	1.258	0.629	6.79	0.002
Linear	1	1.068	1.068	11.52	0.001
Deviation	1	0.191	0.191	2.06	0.157
Density × species richness	2	1.361	0.681	7.35	0.002
Linear	1	0.448	0.448	4.83	0.032
Deviation	1	0.914	0.914	9.86	0.003
Combination (Species richness)	3	1.852	0.617	6.66	<0.001
Ambrosia	1	1.616	1.616	17.44	<0.001
Combination (Species richness)	2	0.236	0.118	1.27	0.289
Residual	51	4.725	0.093		
Total	61	9.844	0.161		
C_4 plants					
Zone	2	0.070	0.035	0.30	0.745
Density	1	0.336	0.336	2.83	0.099
Species richness	2	0.855	0.428	3.60	0.035
Linear	1	0.557	0.557	4.69	0.035
Deviation	1	0.298	0.298	2.50	0.120
Density × species richness	2	0.876	0.438	3.69	0.032
Linear	1	0.109	0.109	0.91	0.343
Deviation	1	0.768	0.768	6.46	0.014
Combination (Species richness)	3	1.292	0.431	3.63	0.019
Ambrosia	1	0.865	0.865	7.28	0.009
Combination (Species richness)	2	0.428	0.214	1.80	0.176
Residual	51	6.061	0.119		
Total	61	9.491	0.156		
Nitrogen-fixing plants					
Zone	2	2.982	1.491	3.88	0.027
Density	1	0.000	0.000	0.00	0.976
Species richness	2	4.751	2.375	6.18	0.004
Linear	1	0.455	0.455	1.18	0.282
Deviation	1	4.296	4.296	11.18	0.002
Density × species richness	2	2.881	1.440	3.75	0.030
Linear	1	0.671	0.671	1.74	0.192
Deviation	1	2.210	2.210	5.75	0.020
Combination (Species richness)	3	0.596	0.199	0.52	0.673
Ambrosia	1	0.011	0.011	0.03	0.865
Combination (Species richness)	2	0.585	0.292	0.76	0.473
Residual	51	19.602	0.384		
Total	61	30.811	0.505		

2001; He, Bazzaz, & Schmid, 2002). Besides the experiment reported here we only know of one other current biodiversity experiment which includes a community density treatment (Roscher et al., 2004).

In our experiment, the diversity–productivity relationship was clearly positive, as reported in the previous experiments, at low community density. But at higher density the positive influence of species richness on total and aboveground biomass production was weakened. This supports our hypothesis that community density may in part compensate for species richness effects on productivity. Only the relationship between diversity and belowground biomass production was consistently positive at low and at high community density, leading to a positive effect of species richness on the community-wide root to shoot
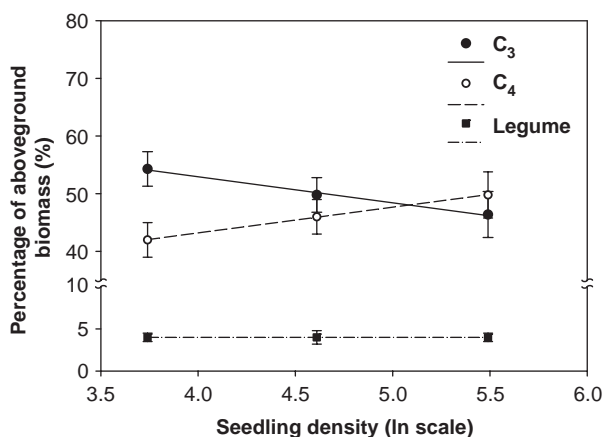
Figure 3. Effects of total seedling density (ln of mean number of individuals per bin at the three treatment levels) in experimental communities on the percentage of aboveground biomass of each plant functional group. Means and standard errors are shown ($n \geqslant 18$ for each point).

ratio. In contrast, other experiments (Spehn, Joshi, Schmid, Alphei, & Körner, 2000; Tilman et al., 2001; Schmid & Pfisterer, 2003) found weaker diversity—productivity relationships for below-ground than for aboveground biomass and a negative effect of species richness on the community-wide root to shoot ratio.

Separating the aboveground community biomass production into the three different functional groups showed that their relative contributions changed with species richness and community density and that again there was an interaction between the two experimental factors. The relative importance of C_4 plants increased and that of C_3 plants decreased with increasing community density and this was particularly pronounced at low species richness. Models have assumed that per capita productivity or nutrient cycling is similar at low and high population densities in monocultures. In light of our results (see also Cottingham, Brown, & Lennon, 2001), however, it seems likely that this assumption will not generally hold for communities representing mixtures of several species.

The particular species combinations of experimental communities within levels of species richness had a significant effect on community, C_3 and C_4 biomass. In part, these effects were due to the presence of a particular species, *Ambrosia artemisiifolia*, in some of the combinations but not in others. The C_3 plant *Ambrosia* explained approximately one-third of the combination effect with regard to community biomass and more than two-thirds of the combination effect with regard to biomass of C_3 and C_4 plants. Interestingly, experimental communities produced less C_3 but more C_4 biomass when *Ambrosia* was present, indicating that this C_3 species "helped" the C_4 functional group.

Population-biological explanations of density effects in species mixtures

High plasticity is a fundamental property of plants. In monocultures, this is reflected in a constant final yield over a range of densities (Kira, Ogawa, & Shinozaki, 1953; Harper, 1977). However, constant yield may also occur in mixed plant stands, i.e., at the level of the entire community. In this case, individuals of different species within the community may suffer density-dependent reductions in growth rate over a wide range of overall community densities. If these reductions in mean plant biomass compensate exactly for the increases in density, then a constant final yield at the community level should result. In our experiment, plant mortality occurred only at the highest density and even there it was less than 10%, so our densities were mostly within the range where the law of constant final yield would apply in monocultures. Averaged across levels of increasing species richness, belowground biomass was constant over the density range tested, whereas aboveground biomass increased slightly but significantly with community density. In terms of per-individual values this meant that decreases in community density were fully compensated by strong increases in individual belowground biomass, but only partly compensated by less strong increases in individual aboveground biomass. Therefore, we conclude that above- and belowground biomass in mixed communities may reach constant final yield at different rates: belowground biomass reached constant final yield by the end of the experiment, whereas aboveground biomass did not.

If the above considerations are now used to explain the significant interactions between community density and species richness on aboveground and total biomass, the stronger richness effects at relatively low density may be due to the potential of diverse mixtures to more rapidly fill aboveground space and attain constant final yield than less diverse mixtures or monocultures (Spehn et al., 2005) and that this difference can be reduced if communities are planted at high initial density.

However, changing community density may also affect the diversity—productivity relationship via changed species abundances and performances in mixtures. For example, competitive interactions

between species can change with density (Schmid & Harper, 1985). Under very high density, where in monocultures the law of constant yield gives way to the self-thinning law (Harper, 1977), differential mortality between species in mixtures can speed up the emergence of dominance (Bazzaz & Harper, 1976) and may even lead to the disappearance of entire species populations. But even when increasing density does not lead to mortality it can increase size variation among individuals in monocultures (Harper, 1977), and by extrapolation may increase size differences among species in mixtures, thereby reducing evenness. In our experiment, mortality was low even at high community density, but evenness in terms of aboveground biomasses of species did indeed decrease with density (see Fig. 1). Furthermore, evenness in terms of functional groups was affected via the differential effects of density on the C_3 and C_4 functional groups (see Fig. 3). Experiments specifically designed to test evenness effects found a positive influence on productivity (Chapin et al., 2000; Wilsey & Potvin, 2000; Polley, Wilsey, & Derner, 2003). Our results of reduced diversity effects at higher density and therefore lower evenness would be consistent with these previous findings, except that the flatter line for the response of productivity to species richness at higher density was actually above rather than below the steeper line for the response at lower density (see Fig. 2).

Conclusions

The traditional biodiversity experiments utilizing a substitutive design at one level of overall density (see review by Schmid et al., 2002b) assume that plots of all diversity treatments reach constant final yield at the density studied. However, the results of the present study show that constant final yield is reached for the low-density treatments only at high species richness, while the high-density treatments reached constant final yield at any level of species richness (see Fig. 2). This indicates that care should be taken when selecting an appropriate density for diversity experiments (see also Connolly, 1986; Taylor & Aarssen, 1989; Cousens & O'Neill, 1993 for discussions of density dependence in substitutive experiments).

Our experiment provides evidence that the rules of population biology can be extrapolated to entire plant communities. In populations, density influences size variation, effective population size and genetic diversity (Harper, 1977; Van Kleunen,

Fischer, & Schmid, 2005). In two-species mixtures, an early experiment showed that high density could lead to skewed size distributions and unequal abundances of species originally sown in equal proportions (Bazzaz & Harper, 1976). In the present experiment, we found that high density in multi-species communities also reduced species evenness and altered functional group abundances, in terms of biomass proportions. Due to such distortion effects of density on abundance distributions the consequences of variations in community density on ecosystem functioning are likely to be more far-reaching in multi-species communities than in populations of single species.

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