OIKOS 97: 337–348, 2002

Interactive effects of diversity, nutrients and elevated CO_2 on experimental plant communities

Jin-Sheng He, Fakhri A. Bazzaz and Bernhard Schmid

He, J.-S., Bazzaz, F. A. and Schmid, B. 2002. Interactive effects of diversity, nutrients and elevated CO_2 on experimental plant communities. – Oikos 97: 337–348.

The effects of species richness and elevated CO_2 on community productivity under altered nutrient levels were studied in experimental herbaceous communities composed of species from the Midwestern United States annual community, which consists of three functional groups C_3, C_4 and N-fixers. Aboveground and belowground biomass were measured at flowering stage and at the end of the experiment when fruits of most plants were ripe. At the low nutrient level, species richness did not have a significant effect on community productivity. However, at the high nutrient level, the community biomass decreased with decreasing species richness at both ambient and elevated CO_2 in the first harvest, and at elevated CO_2 in the second harvest. At low nutrient level, CO_2 slightly increased community biomass at medium and high species richness. At high nutrient level, CO_2 significantly increased community biomass in all species-richness treatments in the first harvest, but a significant response was observed only in the high richness treatment in the second harvest. At the functional group level, biomass of C_3 responded positively to CO_2, and C_4 responded very negatively to CO_2. The N-fixers responded positively to CO_2 at low and medium species richness, but negatively at high species richness, showing a $CO_2 \times$ richness interaction. CO_2 increased species evenness in the communities, depending on nutrient level. Species varied in the responses of light-saturated net photosynthesis (P_{max}) to elevated CO_2, even within functional groups. Our findings suggest that (1) the relationship between productivity and species diversity was dependent on nutrient levels. (2) Species diversity enhances responses of communities to elevated CO_2. (3) Harvest time can affect the results of diversity-productivity experiments. (4) Responses of C_3, C_4, and N-fixers to elevated CO_2 in communities did not follow the prediction based on functional groups or plants grown individually, rather it depended on species richness.

J.-S. He, F. A. Bazzaz, Dept of Organismic and Evolutionary Biology, Harvard Univ., Cambridge, MA 02138, USA (jhe@oeb.harvard.edu). – B. Schmid, Inst. für Umweltwissenschaften, Univ. Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.

The loss of biodiversity and rising atmospheric CO_2 concentration caused by the increased human impact on natural and managed ecosystems may substantially alter ecological functions and ecosystem services to humans (Schulze and Mooney 1993, Vitousek et al. 1997, Chapin et al. 2000). The economic significance of these losses might be considerable (Costanza et al. 1997), because ecosystem services are derived from the normal functioning of ecosystems. This raises the important issue of whether species-poor ecosystems perform differently or less efficiently than species-rich systems (Schulze and Mooney 1993, Chapin et al. 1998, Loreau 2000). Although this question dates back to Darwin (McNaughton 1993), it is in recent years that the so-called relationship between biodiversity and ecosystem functioning has emerged as a major scientific issue. A number of experiments have been devoted to this issue recently, some of them involving considerable effort in terms of equipment and personnel (Naeem et al. 1994, 1996, Tilman 1996, Hooper and Vitousek

Accepted 21 November 2001

ISSN 0030-1299

1997, 1998, Tilman et al. 1997, 2001b, Hector et al. 1999). However, these experiments generated considerable debate concerning which hypothesis is operating in nature (Grime 1997, Huston 1997, Kaiser 2000).

Most biodiversity experiments have focused on biodiversity-ecosystem functioning relationships under particular environmental conditions (Tilman et al. 1997, Hooper and Vitousek 1998), or across environments (Wardle et al. 1997, Hector et al. 1999). It is well known that fertilization can change community diversity and productivity (Austin and Austin 1980, Tilman 1996). Generally, nutrient enrichment increases net primary productivity (NPP) and decreases species richness, although the magnitude of the response was characterized by considerable variation (Tilman 1987, DiTommaso and Aarssen 1989, Gough et al. 2000). But effects of changing environmental conditions on the relationship between biodiversity and ecosystem functioning have rarely been addressed experimentally (Naeem et al. 1994, Stocker et al. 1999).

The interactions between diversity and elevated CO_2 are interwoven. On the one hand, rising CO_2 concentration may alter plant community structure and community diversity (Bazzaz 1990, Körner and Bazzaz 1996). On the other hand, declining species diversity might increase the rate at which atmospheric CO_2 concentration will rise (Naeem et al. 1994), since less diverse communities may take up less additional CO_2 in an elevated CO_2 environment (Stocker et al. 1999). Experiments with forest trees have shown that CO_2 responsive species will become dominant in a future landscape (Bazzaz and Miao 1993). Modeling has also shown that the species composition may change as the less responsive species are eliminated or their populations are reduced (Bolker et al. 1995). However, the remaining species-poor communities may be more productive than the original communities (Catovsky 2000). If this is the case, elevated CO_2 has the potential to modify the relationship between diversity and productivity. Addressing these interactions will require experiments in which diversity and CO_2 are manipulated in a factorial combination.

In this paper, using herbaceous plant communities, by changing species richness while keeping the number of functional groups constant, we attempt to determine (1) how soil nutrient levels affect the relationship between species richness and productivity of the communities; (2) if harvests at different phenology stages affect the results, and (3) how species diversity affects community responses to elevated CO_2.

Materials and methods

Experimental design

Species diversity was manipulated in herbaceous communities and combined with a factorial combination with two CO_2 (ambient and elevated) and two nutrient levels. We wanted to test the effects of species diversity, soil nutrient level and atmospheric CO_2 concentrations on community productivity. Because of the large number of replications, we did not use a full factorial design for the species-diversity treatment (neither have most other diversity studies, e.g. Hooper and Vitousek 1998). Two harvests were conducted to determine phenological effects on productivity. The CO_2 treatments (350 and 700 μmol mol^{-1}), diversity treatments (low, medium, and high), and soil nutrient treatments (low, 73 kg ha^{-1} nitrogen, and high, 210 kg ha^{-1} nitrogen) were replicated across three blocks, resulting in 120 stands (2 CO_2 levels × 2 nutrient levels × 5 diversity combinations × 2 harvest times × 3 replicates).

Diversity treatments

We conducted this experiment using assemblages of plants from a pool of 12 species from an annual plant community of the Midwestern United States (Table 1). The species represented three functional groups (C_3, C_4 and N-fixers). This experiment had 3 diversity levels, with 3, 6, and 12 species in each tub. There were 2 species combinations each at the 3- and 6-species levels. Thus, there were 5 treatments, coded L1, L2 (3 species per tub), M1, M2 (6 species per tub) and H (12 species

Table 1. Treatments and species in the experiment. Abbreviations for species are as follows: C_3 plants, At = *Abutilon theophrasti*, Ama = *Ambrosia artemisiifolia*, Amt = *Ambrosia trifida*, Ih = *Ipomoea hederacea*, Pl = *Plantago lanceolata*, Pa = *Poa annua*. C_4 plants, Am = *Amaranthus retroflexus*, Sf = *Setaria faberii*, Si = *Setaria italica*. N-fixer, Vc = *Vicia cracca*, Tp = *Trifolium pratense*, Td = *Trifolium dubium*. Monocultures of all species were grown in another experiment under the same environmental conditions.

Diversity	Code	Species/community	Functional groups		
			C_3	C_4	N-fixers
Low	L1	3	Ama	Si	Tp
	L2	3	At	Si	Tp
Medium	M1	6	At, Ama	Si, Am	Tp, Vc
	M2	6	At, Pl	Si, Am	Tp, Vc
High	H	12	At, Ama, Amt, Ih, Pl, Pa	Si, Am, Sf	Tp, Vc, Td

per tub). L1 and L2 treatments consisted of a single species out of the three functional groups, M1 and M2 treatments consisted of 2 species each of C_3, C_4 and N-fixers, and the H treatment consisted of 3 species each of C_4 and N-fixers plus 6 species of C_3.

We also grew each species in monoculture in another independent experiment, with 3 to 6 replicates per species, under the same environmental conditions. To keep the functional group composition constant, these monoculture data were only used to test sampling effects.

Experimental procedure

We used the germination rates to calculate the mass of seeds needed to produce 180 plants. This mass was then divided by the number of species in a given tub so that all species would be equally abundant and each container should have, on average, a total of 180 plants (about 5.3 cm^2 $plant^{-1}$) regardless of how many species it contained (Diemer et al. 1997). We planted the stands in $28.5 \times 33.5 \times 20$ cm deep plastic tubs filled with Pro-mix general-purpose growing medium (Red Hill, PA, USA). Four holes were drilled into the bottom of each tub to provide drainage. To these tubs were added either 5 g (low nutrient) or 15 g (high nutrient) Osmocote, a controlled release fertilizer (N:P:K = 14%:14%:14%). Soon after germination, we inoculated each pot in L1 and L2 treatments with *Rhizobium leguminosarum* biovar *trifolli*, while the other treatments were inoculated with both *Rhizobium leguminosarum* biovar *trifolli* and *Rhizobium leguminosarum* biovar *viciae* ((MicroBio RhizoGen Corporation, Saskatoon, SK, Canada).

As the order of germination may affect the competition and composition in a community (Bazzaz 1996), we simulated the early spring temperature rise by putting all tubs in two Environmental Growth Chambers (Chagrin Falls, OH, USA). In these two chambers, CO_2 concentration was maintained at 350 or at 700 μmol mol^{-1}. Both chambers were humidity and temperature controlled, with 100 m^{-2} s^{-1} of PAR via fluorescent lamps. We raised the temperature in the chambers from 8°C to 25°C in two weeks, while the humidity was kept constant at 60%.

After the temperatures reached 25°C, all tubs were moved to an environmentally controlled glasshouse at Harvard University (Cambridge, MA), which is divided into six separately controlled modules. In three modules, CO_2 concentration was maintained at 350 μmol mol^{-1}, while CO_2 was maintained at 700 μmol mol^{-1} in the other modules. The temperature in all modules was maintained at 25°C from 08.00 to 20.00 hours and at 19°C overnight. Lighting was provided by natural sunlight filtered through the roof of the glasshouse, which reduced light levels by $\approx 28\%$. Tubs were arranged randomly and positions were re-randomized once a week to reduce variation in growing conditions. Tubs were watered daily.

Measurements

Net photosynthesis at saturating light (P_{max}) was measured at the flowering stage, using an open path gas-exchange system (Li-Cor 6400) with a red-blue light source and a CO_2 mixer (Li-Cor Inc., Lincoln, NE, USA). For each species we measured 3 individuals in each treatment (2 $CO_2 \times 2$ nutrient, and diversity treatments in which species occurred). During all measurements, temperature in the leaf cuvette was maintained at 25°C and relative humidity was kept between 50–65%. The reference CO_2 concentration was 350 μmol mol^{-1} in ambient CO_2 and 700 μmol mol^{-1} in elevated CO_2. The saturating photosynthetic photon flux density (PPFD) was 1500 μmol m^{-2} s^{-1}.

The experiment lasted four months. The first harvest was conducted two months after the beginning of the experiment, when *Abutilon theophrasti*, *Ambrosia artemisiifolia*, *Ipomoea hederacea*, *Amaranthus retroflexus*, *Setaria faberii*, *Setaria italica* were at flowering stage, while *Ambrosia trifida*, *Plantago lanceolata*, *Poa annua*, *Vicia cracca*, *Trifolium pratense* and *Trifolium dubium* were still at the vegetative stage. The second harvest was conducted when fruits of most species were ripe but had not fallen. However, at this point *Plantago lanceolata*, *Poa annua*, *Vicia cracca*, *Trifolium pratense* and *Trifolium dubium* still had not flowered.

All plants were cut off at ground level and sorted to species. To estimate total productivity of the root system, we placed stainless steel cores (15 cm diameter × 20 cm deep) into the middle of each tub to obtain root subsamples. After sampling the soil cores, we obtained 20 intact root systems of the whole community in each harvest by carefully washing away the soil. A linear regression between root biomass of entire tubs and core subsamples was used to calculate the biomass of whole root systems of the remaining 40 tubs. No attempt was made to separate roots by species or functional group. Roots and shoots were dried at 65°C for one week before measuring dry weight.

Data analysis

A three-way analysis of variance (ANOVA) was used to test for the effects of CO_2, nutrient level, and species richness, with each main effect as a fixed factor. This experiment involves both a between chamber factor and within chamber treatment factors, so the data were analyzed with a split-plot design (Scheiner and Gurevitch 1993), in which the error term used to test the

Table 2. Results of analysis of variance (ANOVA) for the effects of species richness, nutrient level, and CO_2 on total, aboveground, and belowground biomass, and on root:shoot ratio (RSR). * $p<0.05$; ** $p<0.01$.

Sources of variation	d.f.	Harvest 1		Harvest 2	
		F	p	F	p
Total biomass					
CO_2	1	12.11	0.022*	3.79	0.117
Richness	2	5.07	0.010**	0.18	0.834
Nutrient	1	17.85	<0.001**	17.45	<0.001**
Richness × CO_2	2	0.55	0.580	2.23	0.119
Nutrient × CO_2	1	6.91	0.012*	3.22	0.080
Richness × nutrient	2	1.25	0.297	2.10	0.134
Richness × nutrient × CO_2	2	1.60	0.214	0.89	0.426
Aboveground biomass					
CO_2	1	18.32	0.009**	4.03	0.108
Richness	2	4.48	0.017*	0.37	0.693
Nutrient	1	25.51	<0.001**	22.52	<0.001**
Richness × CO_2	2	0.60	0.554	1.91	0.160
Nutrient × CO_2	1	7.91	0.007**	3.77	0.059
Richness × nutrient	2	1.75	0.186	1.87	0.166
Richness × nutrient × CO_2	2	1.78	0.180	1.39	0.260
Belowground biomass					
CO_2	1	1.59	0.270	2.79	0.165
Richness	2	2.38	0.104	0.19	0.831
Nutrient	1	0.84	0.364	0.28	0.601
Richness × CO_2	2	0.09	0.918	1.73	0.190
Nutrient × CO_2	1	0.18	0.671	0.30	0.588
Richness × nutrient	2	0.09	0.914	3.56	0.037*
Richness × nutrient × CO_2	2	0.69	0.509	0.21	0.809
RSR					
CO_2	1	0.02	0.902	1.04	0.361
Richness	2	0.46	0.636	0.77	0.468
Nutrient	1	7.19	0.010**	4.06	0.050*
Richness × CO_2	2	0.02	0.981	1.00	0.375
Nutrient × CO_2	1	0.41	0.524	0.12	0.728
Richness × nutrient	2	0.61	0.546	3.83	0.029*
Richness × nutrient × CO_2	2	0.91	0.408	1.01	0.373

effect of CO_2 is calculated in terms of the variation between the growth chambers, and the error term to test the nutrient and density effects is determined by the variation within the growth chambers. A General Linear Model procedure was used to perform ANOVA, employing type III sums of squares. Significant results were explored by Scheffé post hoc tests for subsequent multiple comparisons. The ANOVAs were performed using SAS version 8.01 (SAS Institute 1999). All biomass variables were $\log_{10}$-transformed to meet assumptions of normality and homogeneity of variances.

Community composition changes under elevated CO_2 were investigated using detrended correspondence analysis (DCA; ter Braak 1995). Community evenness (relative abundance of species) was measured by Pielou's J (Pielou 1966; see Huston 1994), which is the ratio of $H'/\log S$, where H' is the Shannon index $= -\Sigma (p_i \log p_i)$, S is the number of species in the community, p_i is the proportion of total biomass composed of species i.

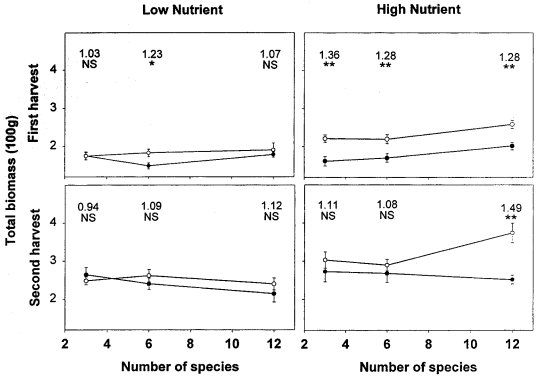

Fig. 1. Relationship between species richness and community total biomass at elevated (○, 700 μmol mol^{-1}) and ambient CO_2 (●, 350 μmol mol^{-1}). Means and standard errors (Mean ± 1 SEM) are shown (n = 3–6 for each datapoint). The results of the linear regression between total biomass and the species richness are shown in Table 3. CO_2 enhancement ratios (total biomass at 700 μmol mol^{-1}/350 μmol mol^{-1}) and their significance (NS, $p>0.05$, * $p<0.05$, ** $p<0.01$, based on post hoc comparisons) are shown above each point pair.

Table 3. Summary of linear regression analyses of the total community biomass with the species richness. n = 15 for each group.

	Nutrient	CO_2	R^2	p
First harvest	High	Ambient	0.20	0.054
	High	Elevated	0.19	0.057
	Low	Ambient	0.00	0.847
	Low	Elevated	0.00	0.367
Second harvest				
	High	Ambient	0.00	0.596
	High	Elevated	0.20	0.056
	Low	Ambient	0.13	0.102
	Low	Elevated	0.00	0.721

Results

Harvest time

In the first harvest, species richness, nutrient level and elevated CO_2 significantly increased total and aboveground biomass, with a significant interaction between nutrient level and CO_2 (Table 2, Fig. 1). The three factors did not have significant effects on belowground biomass. In the second harvest, however, only nutrient level significantly increased total and aboveground biomass. The interactive effects between species richness and nutrient level on belowground biomass and RSR were only observed in the second harvest. But these interactions were not observed for the aboveground biomass ($p = 0.166$), which resulted in the insignificant interactions between nutrient and richness for the total biomass (Table 2).

Effects of treatments on community productivity

The responses of total biomass to species richness were dependent on nutrient levels (Fig. 1). Variation of total biomass with species richness was greater at high nutrient level (CV = 19.75% in the first harvest, and 19.10% in the second harvest; CV = 100 × 1SD/Mean) than at low nutrient level (CV = 14.38% in the first harvest, and 14.89% in the second harvest). In the first harvest, the overall effect of increasing species richness at high nutrient level under both ambient and elevated CO_2, can be described by a linear relation between productivity and the number of plant species ($p = 0.054$ for the ambient CO_2 and 0.057 for the elevated CO_2). While at low nutrient level, the analysis showed no consistent change in productivity with species number under either ambient or elevated CO_2. In the second harvest, only communities with high nutrient level and under elevated CO_2 showed a linear relation between productivity and the number of plant species ($p = 0.056$) (Table 3).

Across nutrient and CO_2 treatments, the mean total biomass of the monocultures was 174.2 g, with a range of 77.2 to 210 g per tub, while the high diversity treatment had a mean total biomass of 270.0 g, with a range of 190.9 to 411.8 g per tub in the second harvest. These results indicated that there was no dominant species effect in the high diversity treatment, because sampling effects limit the maximal productivity of high diversity to that of the best monoculture (Tilman et al. 2001a). If the monocultures were included in the analysis, the linear relationships between productivity and the number of plant species were significant at high nutrient level ($p < 0.05$ for both ambient and elevated CO_2 in the first or second harvest).

The responses of total biomass to elevated CO_2 were also dependent on nutrient levels. At high nutrient level, the differences between elevated and ambient CO_2 were significant at all species-richness levels in the first harvest, and at the high richness levels in the second harvest. At low nutrient level, except for the treatment of medium species richness in the first harvest, there were no significant differences between elevated and ambient CO_2.

Effects of species composition

There were 2 species combinations each at the low and the medium richness treatments. The responses to elevated CO_2 and species richness were also dependent on species composition (the identity of the species present). For example, M2 (*Abutilon theophrasti*, *Plantago lanceolata* and *Setaria italica* dominated) is the only treatment that showed no significant response to elevated CO_2 in either harvest (Fig. 2). At low nutrient level, only M1 (*Abutilon theophrasti*, *Ambrosia artemisiifolia* and *Setaria italica* dominated) in the first harvest showed a significant increase in total biomass under elevated CO_2.

Response of community functional-group composition

In both the first harvest and the second harvest, C_3 responded positively to CO_2, and C_4 responded very negatively to CO_2 in all three species richness treatments (Fig. 3, Table 4). But only at high species richness, was the response of C_3 and C_4 significant in the second harvest. N-fixers significantly increased aboveground biomass under elevated CO_2 at low and medium species richness, while it decreased at high species richness (Fig. 3), showing a significant diversity × CO_2 interaction (Table 4).

From the first harvest to the second harvest, total biomass increase ratios were different for C_3, C_4 and N-fixers (Table 5). For C_3, there was no significant increase in total biomass from the first harvest to the second harvest, while total biomass significantly increased for C_4 plants (60.6%) and N-fixers (195.5%).

Response of community species composition to elevated CO_2

To examine changes in species composition in response to elevated CO_2, we conducted a DCA on the species-biomass data from the second harvest (Fig. 4). The results showed that CO_2-induced changes in community composition were highly dependent on species composition, and on nutrient level. For example, at high nutrient level, changes in community composition to elevated CO_2 in H (high species richness), M1 and M2 (both medium species richness) were larger than in L1 and L2 (both low species richness). At low nutrient level, however, changes in H and L1 were larger than in other treatments.

Elevated CO_2 increased species evenness J, in the communities ($p < 0.01$), especially at low nutrient level (Fig. 5), which led to a slight increase in species diversity as measured by the Shannon index H′ ($p < 0.0025$). H′ gave similar results as J, and is not presented here for brevity. This increase in species evenness was due to an increase in the relative contribution of C_3 and N-fixers, and a decrease in the dominance of C_4 at elevated CO_2.

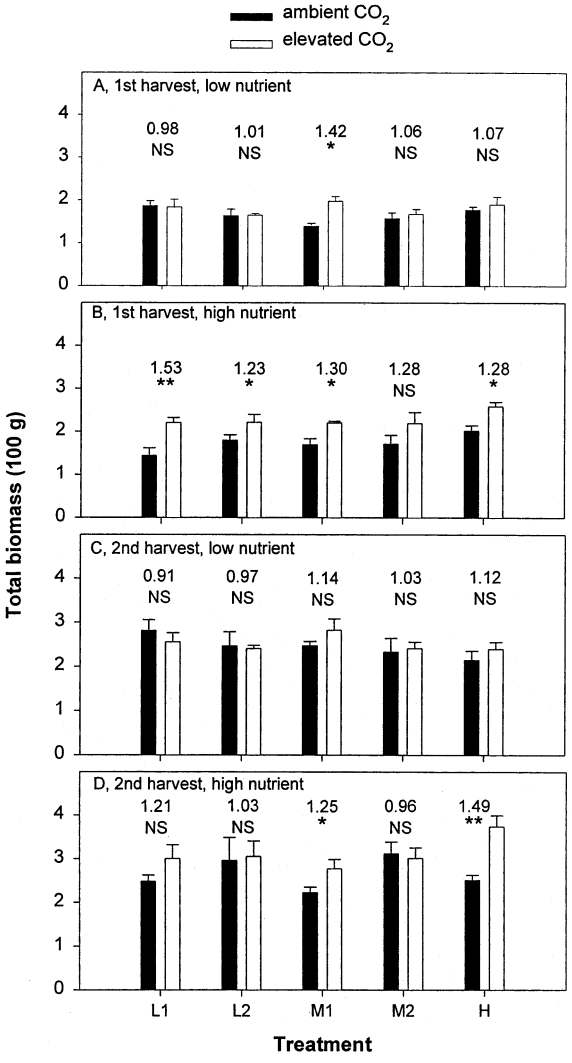

Fig. 2. Community total biomass at elevated (open, 700 μmol mol^{-1}) and ambient CO_2 (black, 350 μmol mol^{-1}), plotted by species composition (treatments) (n = 3 for each bar). Treatment designations are as in Table 1. Means and standard errors (Mean ± 1 SEM) are shown. CO_2 enhancement ratios (total biomass at 700 μmol mol^{-1}/350 μmol mol^{-1}) and their significance (NS, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, based on post hoc comparisons) are shown above each bar pair.

Response of leaf-level photosynthesis to elevated CO_2

Because there was no difference in P_{max} of species in the genera *Setaria* and *Trifolium*, the data for *Setaria faberii* and *Setaria italica*, *Trifolium pratense* and *Trifolium dubium* were averaged across the genus level. We found that nutrient level did not have a significant effect on P_{max} at the flowering stage ($p > 0.05$), but elevated CO_2 did increase P_{max} for all the species or genera with the exception of *Setaria* (Fig. 6). For C_3 plants and N-fixers, all species increased their P_{max} under elevated CO_2, but *Ambrosia artemisiifolia* showed no statistical difference. For C_4, *Setaria* did not show an obvious response to elevated CO_2, while *Amaranthus retroflexus* responded positively to elevated CO_2. Thus at leaf level, CO_2 responses are highly species-specific, even within functional groups.

Discussion

Manipulation of plant species diversity and productivity experiments

Two types of experimental ecosystems have been used to examine the relationship between species richness and ecosystem functions (see review by Schwartz et al. 2000). Some experiments manipulated species richness within semi-natural communities (Tilman 1996, Hooper and Vitousek 1997, 1998, Tilman et al. 1997, Hooper 1998, Hector et al. 1999, Leadley et al. 1999, Stocker et al. 1999). Others used artificial communities within controlled environments (Naeem et al. 1994, 1996, 2000, Leadley and Körner 1996, Naeem and Li 1997, Symstad et al. 1998). There are a number of issues associated with establishing stands of differing diversity (Leadley and Körner 1996, Grime 1997, Huston 1997, Huston et al. 2000). We feel that the initial condition may have an effect. In a controlled environment, when seeds of all species are sown at the same date under constant optimum temperature, they will germinate in a

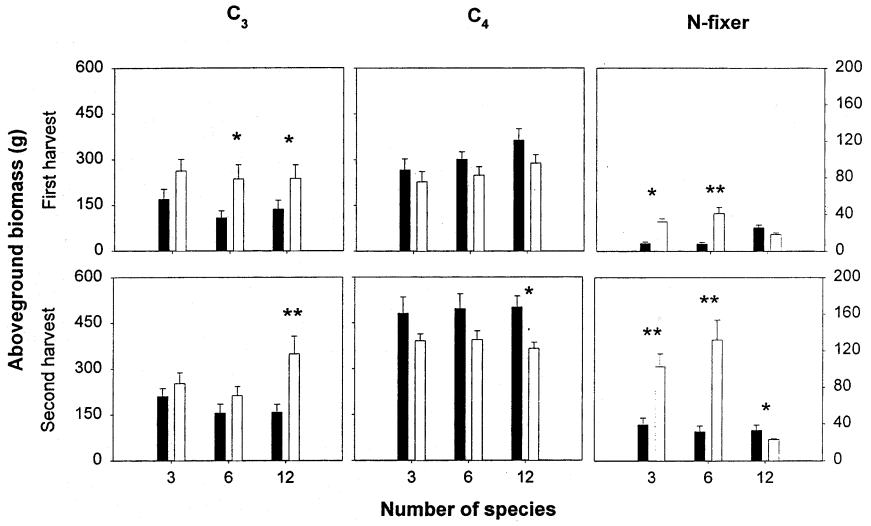

Fig. 3. The responses of biomass by functional group to species richness at elevated (open, 700 μmol mol^{-1}) and ambient CO_2 (black, 350 μmol mol^{-1}) in low and high nutrient treatments. Treatment designations are as in Table 1. We calculated for each functional group the biomass that we would have expected if only that group was present. Because C_4 and N-fixers have been planted in proportions of 1/3, 1/3, and 1/4, and C_3 in proportions of 1/3, 1/3, 1/2 in the low, medium and high species richness treatments, respectively, we divided the component biomass of the functional groups by these ratios. Means and standard errors (Mean ± 1 SEM) are shown (n = 6–12 for each bar). Means that were significantly different between elevated and ambient CO_2 are marked with asterisks (NS, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, based on post hoc comparisons).

certain order. But in the field, the species may germinate in a different sequence, as the temperature gradually increases during early spring (Bazzaz 1996). The differences in the time of germination result in different competitive outcomes in communities (Harper 1977, Bazzaz 1996). Therefore, we gradually raised the tem-

Table 4. Results of ANOVA for the effects of species richness, nutrient level, and CO_2 on C_3, C_4, and N-fixers. We calculated for each functional group the biomass that we would have expected if only that group was present. Because C_4 and N-fixers have been planted in proportions 1/3, 1/3, and 1/4, and C_3 in proportions 1/3, 1/3, 1/2 in the low, medium and high species richness treatments, respectively, we divided the component biomass of the functional groups by these ratios. * $p<0.05$; ** $p<0.01$.

Source of variation	d.f.	Harvest 1		Harvest 2	
		F	p	F	p
C_3					
CO_2	1	24.93	0.004**	24.1	0.005**
Richness	2	1.60	0.214	4.92	0.012*
Nutrient	1	47.53	<0.001**	70.5	<0.001**
Richness × CO_2	2	0.26	0.772	5.10	0.010**
Nutrient × CO_2	1	4.07	0.049*	0.76	0.389
Richness × nutrient	2	0.07	0.937	0.54	0.587
Richness × nutrient × CO_2	2	0.78	0.465	1.72	0.190
C_4					
CO_2	1	2.92	0.155	16.36	0.007**
Richness	2	3.16	0.052	0.04	0.958
Nutrient	1	15.53	<0.001**	0.61	0.439
Richness × CO_2	2	0.16	0.851	0.12	0.891
Nutrient × CO_2	1	0.15	0.697	1.80	0.187
Richness × nutrient	2	0.67	0.517	0.44	0.644
Richness × nutrient × CO_2	2	2.38	0.104	0.27	0.764
N-fixer					
CO_2	1	12.77	0.020*	27.79	0.003**
Richness	2	0.74	0.482	6.71	0.003**
Nutrient	1	3.55	0.066	4.83	0.033*
Richness × CO_2	2	12.41	<0.001**	6.88	0.003**
Nutrient × CO_2	1	0.33	0.567	0.52	0.473
Richness × nutrient	2	1.15	0.325	1.10	0.343
Richness × nutrient × CO_2	2	1.80	0.177	0.62	0.545

Table 5. Mean biomass and biomass increase ratios [100 × (biomass at second harvest – biomass at first harvest)/biomass at first harvest] for C_3, C_4 and N-fixer functional groups from the first harvest to the second harvest. Means followed by the same letter are not significantly different.

Total biomass (g)	C_3		C_4		N-fixer	
	Mean	SE	Mean	SE	Mean	SE
First harvest	192.8 a	16.6	273.6 a	13.7	22.5 a	2.31
Second harvest	216.3 a	15.0	439.3 b	17.5	66.6 b	7.72
Biomass increase ratio (%)	12.2		60.6		195.5	

perature in the growth chambers from 8°C to 25°C. This was different from other experiments.

The relationship between plant species richness and productivity: nutrient level and effect of harvest time

Our results showed that variation of total biomass with species richness was greater at high nutrient level than at low nutrient level, and the positive relationship between species richness and productivity was only found at high nutrient level. However, ANOVA showed that the interaction between nutrient and richness for total biomass was not significant, but was significant for root biomass (Table 2). As mentioned before, our experiment changed species richness while keeping the functional group richness constant, which may have caused the effect of diversity to be less pronounced. Our results suggest that nutrients may be part of the reasons why positive diversity effects were most consistently observed in grassland-type ecosystems and across experimental diversity gradients (Schläpfer and Schmid 1999, Waide et al. 1999), which are relatively uniform in soil nutrients. Similarly, the spatial scale of analysis – how the data are aggregated – can influence the form of the relationship between species richness and productivity (Gross et al. 2000, Mittelbach et al. 2001). The result that the effect of diversity was stronger at high nutrients seems to be unexpected according to conventional thinking about effects of fertilizer on species richness, but actually, these involve different processes. Nutrient addition can cause a decrease in species diversity, accompanied by shifts in species composition and changes in productivity (Tilman 1996). In our experiment, nutrition addition could not reduce species richness, which was itself a treatment factor.

At least two mechanisms have been proposed to explain observations of enhanced productivity in more diverse plant communities, i.e. niche complimentarity

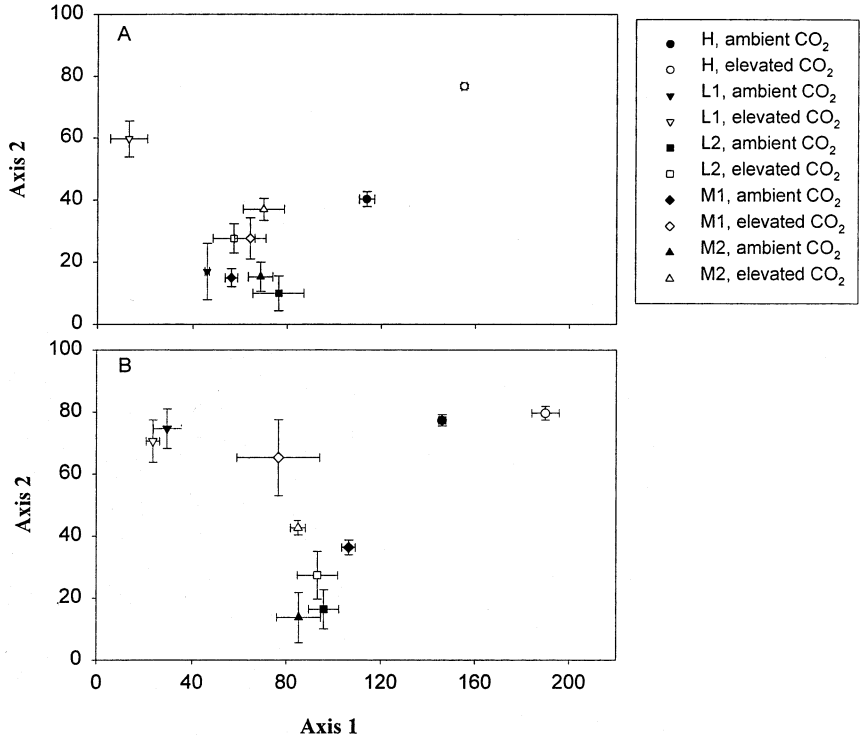

Fig. 4. Changes in community composition under ambient and elevated CO_2 in the second harvest at low (A) and high (B) nutrient levels, represented by mean plot scores ($\pm$ 1 SEM, n = 3 for each point) on the first two axes of detrended correspondence analysis (DCA), with axis 1 explaining 39% and axis 2 explaining 26% of the total variation.

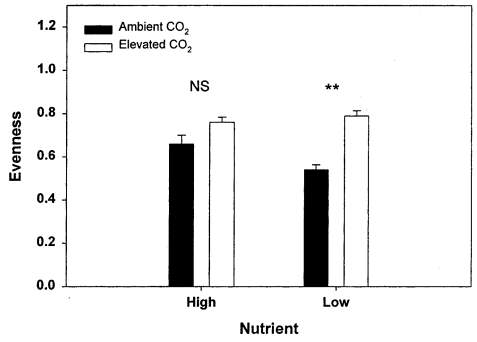

Fig. 5. Community evenness at elevated (open, 700 μmol mol^{-1}) and ambient CO_2 (black, 350 μmol mol^{-1}) in the low and high nutrient treatments. Means and standard errors (Mean ± 1 SEM, n = 30 for each bar) are shown. Means that were significantly different between elevated and ambient CO_2 are marked with asterisks (NS, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, based on post hoc comparisons). Community evenness was measured by Pielou's *J* (Pielou 1966; see Huston 1994), which is the ratio of H′/log S, where H′ is Shannon index = $-\Sigma (p_i \ln p_i)$, S is the number of species in the community, p_i is the proportion of total biomass composed of species *i*.

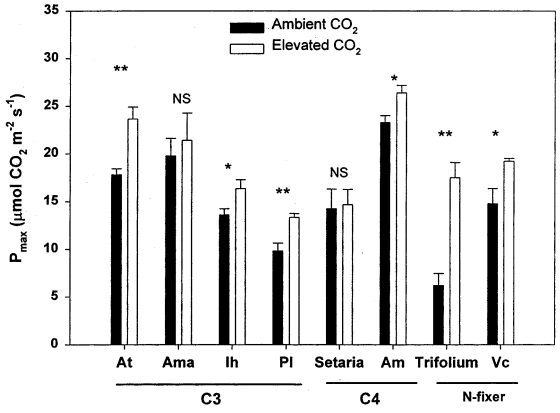

Fig. 6. In situ leaf maximum photosynthesis (P_{max}) of 8 species (genera) at elevated (open) and ambient CO_2 (black). Means and standard errors (Mean ± 1 SEM) are shown. Means that were significantly different between elevated and ambient CO_2 are marked with asterisks (NS, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, based on t-test). At = *Abutilon theophrasti*, Ama = *Ambrosia artemisiifolia*, Ih = *Ipomoea hederacea*, Pl = *Plantago lanceolata*, Ar = *Amaranthus retroflexus*, Setaria = *Setaria faberi* and *Setaria italica*, Vc = *Vicia cracca*, Trifolium = *Trifolium pratense* and *Trifolium dubium*.

effect and the sampling mechanism (Tilman 1999, Chapin et al. 2000). Ecosystem responses to plant richness could occur via complementary resource use if plant species differ in the way they take up nutrients, light, and water, either in time or space (Hooper and Vitousek 1997, Loreau 2000). If the nutrients in the soil are limited, species may not be able to express differences in resource capture and utilization, i.e. the effects of diversity can not "unfold" if the total biotope space is small, such as under stressful condition, which may thwart detection of significant ecosystems' response to species diversity (Schmid et al. 2002). These diversity-productivity relationships under varying environmental conditions or even stressful environments have previously been underemphasized (Mulder et al. 2001, Pfisterer and Schmid 2002). It is now believed that both the identity and number of species within a community could influence ecosystem processes (Hooper and Vitousek 1997, Hector et al. 1999). This study supports this belief because community productivity was also dependent on species composition. This makes the richness effects look smaller, because there are differences between different combinations of species (mixture) within the same level of richness.

Harvest time and method are crucial issues associated with diversity-productivity experiments. Some experiments harvested at the time of peak aboveground biomass in each stand (e.g. Tilman 1996) and others harvested at peak biomass for each functional group (e.g. Hooper and Vitousek 1997). In the present experiment, two harvests showed different results. On closer examination, the biomass dynamics of different functional groups were different. From the beginning of the fruit stage to the ripe fruit stage, the biomass of C_3 only increased 12.2%, while C_4 increased 60.6%. With the drop of the leaves of *Abutilon theophrasti*, the dominant C_3 species in the community, N-fixers took advantage of the changed light environment, increased about two-fold in biomass.

In most diversity manipulation experiments in the field, the productivity of plant communities has been estimated by measuring aboveground biomass production, because measurements for belowground biomass are more difficult to obtain. However, in temperate herbaceous communities about 60–80% of the biomass is produced below ground (Liira and Zobel 2000). Therefore, the richness-productivity relationship cannot be fully understood without paying attention to below ground biomass (Copley 2000). In this study we found that changes in aboveground and belowground biomass with regard to species diversity, nutrient level and CO_2 were different in both the first harvest and the second harvest. Other studies also found no effects of diversity on belowground but large effects on aboveground biomass (Spehn et al. 2000). The aboveground biomass showed a similar behavior as total biomass in this study (Table 2), just because belowground biomass only contributed about 8%–15% to total biomass. But in other ecosystems, belowground biomass can contribute to a large proportion of total biomass, therefore, incorporation of root biomass may produce different results.

Species diversity interacts with elevated CO_2

In the present study, we found that, in the second harvest, there was no response of biomass to elevated CO_2 at low or medium diversity, but there were signifi-

cant biomass responses to elevated CO_2 in the high diversity treatment. Leadley and Körner (1996) found similar results. Recently, a grassland field experiment in Minnesota, USA, using free air CO_2 enrichment, found that the enhanced biomass accumulation in response to elevated levels of CO_2 or nitrogen, or their combination, is less in species-poor than in species-rich communities (Reich et al. 2001). If these relationships hold true, we can predict that continuing loss of biodiversity may reduce the capacity of ecosystems to sequestrate additional carbon under elevated CO_2 concentration environment.

Plant communities in elevated CO_2 showed a shift in species evenness (Fig. 6). Changes in species evenness warrant increased attention, because they usually respond more rapidly to human activities than do changes in species richness (Chapin et al. 2000). These changes could represent an important mechanism by which environmental perturbations affect future ecosystem functioning (Bolker et al. 1995, Wedin and Tilman 1996), and could have important consequences to ecosystems long before a species is threatened by extinction (Chapin et al. 2000). Differences in species evenness could ultimately scale up to influence species' contributions to ecosystem-level processes, such as carbon gain and nutrient cycling (Reich et al. 1997). Wilsey and Potvin (2000) found that total and belowground biomass increased linearly with increasing levels of evenness after one growing season in communities with experimentally varied species evenness. Recently, a study in mixed forests in New England indicated that the predicted decrease of hemlock (*Tsuga canadensis*) from mixed temperate forests could increase whole-forest carbon gain by two- to four-fold (Catovsky 2000). These results are largely consistent with the idea that human-influenced reductions in small-scale plant diversity, in this case evenness, will lead to indirect changes in total primary production (Tilman 2000).

Scaling responses to elevated CO_2 from the individual to the community

Species-level responses to elevated CO_2 have been the focus of much global change research. A large proportion of such research has focused on how species differ in their response to rising CO_2 levels (Bazzaz 1990, Poorter et al. 1996, Wullschleger et al. 1997). It has been documented that C_3 plants have consistently higher CO_2 growth increases (47%) than either C_4 (10%) or CAM (19%) species (Poorter et al. 1996). Other studies have recorded CO_2-induced growth increases, with average enhancements of 25–50% for tree seedlings (Curtis and Wang 1998, Saxe et al. 1998, Norby et al. 1999), 35% for C3 wild herbaceous species and 40–60% for C3 crop species (Poorter et al. 1996). These responses were modified by abiotic factors, for example, low soil nutrient availability reduced plant responses to elevated CO_2 by half, from $+31\%$ under optimal conditions to 16%, while low light increased the response to $+52\%$ in a meta-analysis of CO_2 effects on tree species (Curtis and Wang 1998).

Our results suggest that responses of C_3, C_4, and N-fixers to elevated CO_2 in communities did not follow our predictions based on functional groups of species or responses of plants grown individually, depending on not only nutrient levels, but also species richness of the community. Similar results have been documented in other studies (Leadley and Stöcklin 1996, Roy et al. 1996, Navas et al. 1999). Mechanisms for community responses involve many higher-level interactions that need to be incorporated into the scaling. For example, when species are grown in mixture, competitive interaction generally changes the amounts they acquire of an available resource, relative to their acquisition in monoculture. Modeling has proved that such redistribution with increasing species can either increase or decrease productivity, even if the total absorbed resource remains the same (Nijs and Impens 2000).

Acknowledgements – The authors wish to thank summer students M. Villar, T. K. Ngodup, and E. Berdan for help carrying out the experiment, R. Stomberg for managing the glasshouse facilities, and G. Bauer and S. Kaufman for constructive comments on the manuscript. A grant from the Andrew Mellon Foundation to FAB supported this research.

References

Austin, M. P. and Austin, B. O. 1980. Behavior of experimental plant communities along a nutrient gradient. – J. Ecol. 68: 891–918.

Bazzaz, F. A. 1990. The response of natural ecosystems to the rising global CO_2 levels. – Annu. Rev. Ecol. Syst. 21: 167–196.

Bazzaz, F. A. 1996. Plant in changing environments: linking physiological, population, and community ecology. – Cambridge University Press.

Bazzaz, F. A. and Miao, S. L. 1993. Successional status, seed size, and responses of tree seedlings to CO_2, light, and nutrients. – Ecology 74: 104–112.

Bolker, B. M., Pacala, S. W., Bazzaz, F. A. et al. 1995. Species diversity and ecosystem response to carbon dioxide fertilization: conclusions from a temperate forest model. – Glob. Change Biol. 1: 373–381.

Catovsky, S. 2000. Linking community dynamics and ecosystem function in mixed conifer and broad-leaved forests. Ph.D. dissertation. – Harvard University.

Chapin, F. S., III, Sala, O. E., Burke, I. C. et al. 1998. Ecosystem consequences of changing biodiversity. – BioScience 48: 45–52.

Chapin, F. S., III, Zavaleta, E. S., Eviner, V. T. et al. 2000. Consequences of changing biodiversity. – Nature 405: 234–242.

Copley, J. 2000. Ecology goes underground. – Nature 406: 452–454.

Costanza, R., d'Arge, R., de Groot, R. et al. 1997. The value of the world's ecosystem services and natural capital. – Nature 387: 243–260.

Curtis, P. S. and Wang, X. 1998. A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. – Oecologia 113: 299–313.

Diemer, M., Joshi, J., Körner, C. et al. 1997. An experimental protocol to assess the effects of plant diversity on ecosystem functioning utilised in a European research network. – Bull. Geobot. Inst. ETH 63: 95–107.
DiTommaso, A. and Aarssen, L. W. 1989. Resource manipulations in natural vegetation: a review. – Vegetatio 84: 9–29.
Gough, L., Osenberg, C. W. O., Gross, K. L. and Collins, S. L. 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. – Oikos 89: 428–439.
Grime, J. P. 1997. Biodiversity and ecosystem function: the debate deepens. – Science 277: 1260–1261.
Gross, K. L., Willig, M. R., Gough, L. et al. 2000. Pattern of species density and productivity at different spatial scale in herbaceous plant communities. – Oikos 89: 47–427.
Harper, J. L. 1977. Population biology of plants. – Academic Press.
Hector, A., Schmid, B., Beierkuhnlein, C. et al. 1999. Plant diversity and productivity experiments in European grasslands. – Science 286: 1123–1127.
Hooper, D. U. 1998. The role of complementarity and competition in ecosystem responses to variation in plant diversity. – Ecology 79: 704–719.
Hooper, D. U. and Vitousek, P. M. 1997. The effects of plant composition and diversity on ecosystem processes. – Science 277: 1302–1305.
Hooper, D. U. and Vitousek, P. M. 1998. Effects of plant composition and diversity on nutrient cycling. – Ecol. Monogr. 68: 121–149.
Huston, M. A. 1994. Biological diversity. – Cambridge University Press.
Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. – Oecologia 110: 449–460.
Huston, M. A., Aarssen, L. W., Austin, M. P. et al. 2000. No consistent effect of plant diversity on productivity. – Science 289: 1255.
Kaiser, J. 2000. Rift over biodiversity divides ecologists. – Science 289: 1282–1283.
Körner, C. and Bazzaz, F. A. 1996. Carbon dioxide, population, and community. – Academic Press, San Diego, CA.
Leadley, P. W. and Körner, C. 1996. Effects of elevated CO_2 on plant spcies dominance in a highly diverse calcareous grassland. – In: Körner, C. and Bazzaz, F. A. (eds), Carbon dioxide, populations, and communities. Academic Press, pp. 159–175.
Leadley, P. W. and Stöcklin, J. 1996. Effects of elevated CO_2 on model calcareous grasslands: community, species, and genotype level responses. – Glob. Change Biol. 2: 389–397.
Leadley, P. W., Niklaus, P. A., Stocker, R. and Körner, C. 1999. A field study of the effects of elevated CO_2 on plant biomass and community structure in a calcareous grassland. – Oecologia 118: 39–49.
Liira, J. and Zobel, K. 2000. The species richness-biomass relationship in herbaceous plant communities: what difference does the incorporation of root biomass data make? – Oikos 91: 109–114.
Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. – Oikos 91: 3–17.
McNaughton, S. J. 1993. Biodiversity and function of grazing ecosystem. – In: Schulze, E. D. and Mooney, H. A. (eds), Biodiversity and ecosystem function. Springer, Berlin, pp. 361–383.
Mittelbach, G. G., Steiner, C. F., Scheiner, S. M. et al. 2001. What is the observed relationship between species richness and productivity? – Ecology 82: 2381–2396.
Mulder, C. P. H., Uliassi, D. D. and Doak, D. F. 2001. Physical stress and diversity-productivity relationships: The role of positive interactions. – Proc. Natl. Acad. Sci. U.S.A. 98: 6704–6708.
Naeem, S. and Li, S. 1997. Biodiversity enhances ecosystem reliability. – Nature 390: 507–509.
Naeem, S., Thompson, L. J., Lawler, S. P. and Woodfin, R. M. 1994. Declining biodiversity can alter the performance of ecosystems. – Nature 368: 734–737.
Naeem, S., Thompson, L. J., Jones, T. F. et al. 1996. Changing community composition and elevated CO_2. – In: Körner, C. and Bazzaz, F. A. (eds), Carbon dioxide, populations, and communities. Academic Press, pp. 159–175.
Naeem, S., Knops, J. M. H., Tilman, D. et al. 2000. Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. – Oikos 91: 97–108.
Navas, M.-L., Garnier, E., Austin, M. P. and Gifford, R. M. 1999. Effect of competition on the responses of grasses and legumes to elevated atmospheric CO_2 along a nitrogen gradient: differences between isolated plants, monocultures and multi-species mixtures. – New Phytol. 143: 323–331.
Nijs, I. and Impens, I. 2000. Underlying effects of resource use efficiency in diversity-productivity relationships. – Oikos 91: 204–208.
Norby, R. J., Wullschleger, S. D., Gunderson, C. A. et al. 1999. Tree responses to rising CO_2 in field experiments: implications for the future forest. – Plant Cell Environ. 22: 683–714.
Pfisterer, A. and Schmid, B. 2002. Density-dependent production can decrease the stability of ecosystem functioning. – Nature 416: 84–86.
Pielou, E. C. 1966. Measurement of diversity in different types of biological collections. – J. Theor. Biol. 13: 131–144.
Poorter, H., Roumet, C. and Campbell, B. D. 1996. Interspecific variation in the growth response of plants to elevated CO_2: a search for functional types. – In: Körner, C. and Bazzaz, F. A. (eds), Carbon dioxide, populations, and communities. Academic Press, pp. 375–412.
Reich, P. B., Grigal, D. F., Aber, J. D. and Gower, S. T. 1997. Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. – Ecology 78: 335–347.
Reich, P. B., Knops, J., Tilman, D. et al. 2001. Plant diversity enhances ecosystem responses to elevated CO_2 and nitrogen deposition. – Nature 410: 809–810.
Roy, J., Guillerm, J.-L., Navas, M.-L. and Dhillion, S. 1996. Response to elevated CO_2 in Mediterranean old-field microcosms: species, community, and ecosystem components. – In: Körner, C. and Bazzaz, F. A. (eds), Carbon dioxide, populations, and communities. Academic Press, pp. 123–138.
SAS Institute 1999. SAS/STAT User's guide, Version 8.01 (On-line Docs). – SAS Institute, Cary, NC.
Saxe, H., Ellsworth, D. S. and Heath, J. 1998. Tree and forest functioning in an enriched CO_2 atmosphere. – New Phytol. 139: 395–436.
Scheiner, S. M. and Gurevitch, J. 1993. Design and analysis of ecological experiments. – Chapman & Hall.
Schläpfer, F. and Schmid, B. 1999. Ecosystem effects of biodiversity: A classification of hypotheses and exploration of empirical results. – Ecol. Appl. 9: 893–912.
Schmid, B., Joshi, J. and Schläpfer, F. 2002. Empirical evidence for biodiversity-ecosystem functioning relationships. – In: Kinzig, A., Tilman, D. and Pacala, P. (eds), Functional consequences of biodiversity: experimental progress and theoretical extensions. Princeton Univ. Press, Princeton. In press.
Schulze, E. D. and Mooney, H. A. 1993. Biodiversity and ecosystem function. – Springer-Verlag.
Schwartz, M. W., Brigham, C. A., Hoeksema, J. D. et al. 2000. Linking biodiversity to ecosystem function: implications for conservation ecology. – Oecologia 122: 297–305.
Spehn, E. M., Joshi, J., Schmid, B. et al. 2000. Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. – Plant Soil 224: 217–230.
Stocker, R., Körner, C., Schmid, B. et al. 1999. A field study of the effects of elevated CO_2 and plant species diversity on

ecosystem-level gas exchange in a planted calcareous grassland. – Glob. Change Biol. 5: 95–105.
Symstad, A. J., Tilman, D., Willson, J. and Knops, J. M. H. 1998. Species loss and ecosystem functioning: effects of species identity and community composition. – Oikos: 389–397.
ter Braak, C. J. F. 1995. Ordination. – In: Jongman, R. H. G., ter Braak, C. J. F. and van Tongeren, O. F. R. (eds), Data analysis in community and landscape ecology, new edn. Cambridge University Press.
Tilman, D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. – Ecol. Monogr. 57: 189–214.
Tilman, D. 1996. Biodiversity: population versus ecosystem stability. – Ecology 77: 350–363.
Tilman, D. 1999. The ecological consequences of changes in biodiversity: a research for general principles. – Ecology 80: 1455–1474.
Tilman, D. 2000. Causes, consequences and ethics of biodiversity. – Nature 405: 208–211.
Tilman, D., Knops, J., Wedin, D. et al. 1997. The influence of functional diversity and composition on ecosystem processes. – Science 277: 1300–1302.
Tilman, D., Fargione, J., Wolff, B. et al. 2001a. Forecasting agriculturally driven global environmental change. – Science 292: 281–284.
Tilman, D., Reich, P. B., Knops, J. et al. 2001b. Diversity and productivity in a long-term grassland experiment. – Science 294: 843–845.
Vitousek, P. M., Mooney, H. A., Lubchenco, J. and Melillo, J. M. 1997. Human domination of Earth's ecosystems. – Science 277: 494–499.
Waide, R. B., Willig, M. R., Steiner, C. F. et al. 1999. The relationship between productivity and species richness. – Annu. Rev. Ecol. Syst. 30: 257–300.
Wardle, D. A., Zackrisson, O., Hornberg, G. and Gallet, C. 1997. The influence of island on ecosystem properties. – Science 277: 1296–1299.
Wedin, D. A. and Tilman, D. 1996. Influence of nitrogen loading and species composition on the carbon balance of grasslands. – Science 274: 1720–1723.
Wilsey, B. and Potvin, C. 2000. Biodiversity and ecosystem functioning: importance of species evenness in an old field. – Ecology 81: 887–892.
Wullschleger, S. D., Norby, R. J. and Gunderson, C. A. 1997. Forest trees and their response to atmospheric carbon dioxide enrichment: a complication of results. – In: Allen, L. H., Kirkham, M. B., Olszyk, D. M. and Whitman, C. E. (eds), Advances in carbon dioxide effects research. Am. Soc. Agron., Madison, Wisconsin, USA, pp. 79–100.