

# Complementarity among four highly productive grassland species depends on resource availability

Christiane Roscher<sup>1,2</sup> · Bernhard Schmid<sup>3</sup> · Olaf Kolle<sup>4</sup> · Ernst-Detlef Schulze<sup>4</sup>

Received: 20 July 2015 / Accepted: 8 February 2016 / Published online: 1 March 2016  
© Springer-Verlag Berlin Heidelberg 2016

**Abstract** Positive species richness–productivity relationships are common in biodiversity experiments, but how resource availability modifies biodiversity effects in grass–legume mixtures composed of highly productive species is yet to be explicitly tested. We addressed this question by choosing two grasses (*Arrhenatherum elatius* and *Dactylis glomerata*) and two legumes (*Medicago × varia* and *Onobrychis viciifolia*) which are highly productive in monocultures and dominant in mixtures (the Jena Experiment). We established monocultures, all possible two- and three-species mixtures, and the four-species mixture under three different resource supply conditions (control, fertilization, and shading). Compared to the control, community biomass production decreased under shading (−56 %) and increased under fertilization (+12 %). Net diversity effects (i.e., mixture minus mean monoculture biomass) were

positive in the control and under shading (on average +15 and +72 %, respectively) and negative under fertilization (−10 %). Positive complementarity effects in the control suggested resource partitioning and facilitation of growth through symbiotic N<sub>2</sub> fixation by legumes. Positive complementarity effects under shading indicated that resource partitioning is also possible when growth is carbon-limited. Negative complementarity effects under fertilization suggested that external nutrient supply depressed facilitative grass–legume interactions due to increased competition for light. Selection effects, which quantify the dominance of species with particularly high monoculture biomasses in the mixture, were generally small compared to complementarity effects, and indicated that these species had comparable competitive strengths in the mixture. Our study shows that resource availability has a strong impact on the occurrence of positive diversity effects among tall and highly productive grass and legume species.

Communicated by Scott Collins.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00442-016-3587-4) contains supplementary material, which is available to authorized users.

✉ Christiane Roscher  
christiane.roscher@ufz.de

<sup>1</sup> UFZ, Helmholtz Centre for Environmental Research, Physiological Diversity, Permoserstrasse 15, 04318 Leipzig, Germany

<sup>2</sup> German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

<sup>3</sup> Institute of Evolutionary Biology and Environmental Studies and Zurich-Basel Plant Science Center, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

<sup>4</sup> Max Planck Institute for Biogeochemistry, POB 100164, 07701 Jena, Germany

**Keywords** Biodiversity effects · Fertilization · Grass · Legume · Shading

## Introduction

Experimental studies have demonstrated that biodiversity and ecosystem functions, such as aboveground primary productivity, are predominantly positively related (Hooper et al. 2005; Cardinale et al. 2012). Explanations for increased biomass production in mixtures have been proposed and discussed vigorously, and center around two hypotheses which are not mutually exclusive. The complementarity effect hypothesis is based on the assumption that more diverse communities use available resources more completely due to greater complementarity in the

acquisition and use of resources or more opportunities for facilitative interactions (Tilman et al. 1997). The so-called sampling (or selection) effect hypothesis states that, in randomly constructed communities, species-rich communities have a higher probability of containing a species with particularly large individual effects on biomass production (Huston 1997; Tilman et al. 1997). However, the physiological species-specific mechanisms underlying both hypotheses are poorly understood.

Natural grassland communities are usually a mixture of a few tall-growing species, which make up the largest fraction of community biomass, and a larger number of small species contributing a minor proportion to community biomass (Grime 1998). Light and nutrient availability are the most limiting resources for plant growth in temperate grasslands in humid regions. Competition for light is asymmetric due to the unidirectional supply of incoming radiation. Taller growth provides a disproportionate advantage in stands of vegetation by simultaneously increasing light capture and shading smaller competitors (Schwinning and Weiner 1998). Although competition for soil resources is generally thought to be size-symmetric, it also has been demonstrated that belowground competition may be size-asymmetric if soil resources are heterogeneously distributed (Rajaniemi 2003).

Most biodiversity experiments have focused on biodiversity–ecosystem functioning relationships under particular environmental conditions. Effects of varying environmental conditions have only been addressed in some experimental studies. Those studies provided evidence that varying levels of nutrient availability (e.g., Reich et al. 2001; Fridley 2002; He et al. 2002; Lanta and Lepš 2007; Nyfeler et al. 2009; Wacker et al. 2009) may impact diversity–productivity relationships. For example, Nyfeler et al. (2009) have shown that in fertilized agricultural grasslands composed of legumes and grasses, diversity–productivity effects were reduced and rapidly disappeared at high levels of N fertilization. It is common knowledge that plant productivity and plant–plant interactions are strongly controlled by resource availability (Grime 2001) and that the close interaction between carbon and nutrient metabolism requires a balance between nutrient and light supply for growth. However, we are aware of just one biodiversity experiment that manipulated soil resources by fertilizer addition and light availability by shading, and that experiment used annual forb species (Fridley 2003). It showed that overyielding, i.e., greater biomass in mixtures compared with monocultures, was highest under fertilization and full light.

Although it has been demonstrated that particular species or functional groups are important for the observed extent of complementarity and selection effects, to our knowledge biodiversity experiments have never been

restricted to tall and dominant species. Here, we present results from an experiment specifically testing for diversity effects among four grassland species (two grasses, two legumes) with tall growth stature, high productivity in monoculture, and the ability to rapidly establish (Heisse et al. 2007). These species were identified as dominant species in the mixtures of a large biodiversity experiment (the Jena Experiment) over several years (Roscher et al. 2005, 2011). However, these species do not occur in combination in the randomly assembled communities of the Jena Experiment (Roscher et al. 2004). We grew monocultures and all possible mixtures of these species in three replicates, which were assigned to different treatments after 1 year of growth to manipulate the availability of the most important resources limiting plant growth in temperate grasslands (control vs. fertilization vs. shading).

Fertilizer addition increases the availability of soil resources, but it usually also enhances competition for light due to increased plant biomass and canopy shading. Manipulation of light availability by shading is likely to decrease productivity due to light limitation, but it does not necessarily increase competition for light when canopy shading declines with decreased plant biomass. In addition, lower productivity under low-light conditions may attenuate competition for soil resources due to lower demands for plant growth. Both manipulations, fertilization and shading, are likely to favor the growth of grasses over legumes due to the high energetic costs of N<sub>2</sub> fixation, which reduces legume performance in low-light conditions (Vitousek and Howarth 1991). Beneficial effects of legumes on the community-level productivity of grass–legume mixtures are most likely under conditions where nutrients are the major growth-limiting resource and symbiotic N<sub>2</sub> fixation of legumes facilitates the growth of grasses in the mixtures. We measured aboveground biomass production as well as plant and soil variables related to nitrogen acquisition to test the following specific hypotheses:

1. Selection effects among tall and dominant grassland species are small compared to complementarity effects because they have similar competitive strengths as mixture species
2. Complementarity effects are positive in the control without resource manipulation due to nutrient partitioning and facilitation of grasses through N<sub>2</sub>-fixing legumes
3. Complementarity effects become negative under fertilization when external nutrient supply suppresses facilitative grass–legume interactions due to increased competition for light
4. Complementarity effects do not occur under shading because growth is generally limited by reduced light availability

These hypotheses imply that resource availability strongly impacts the possibility of positive diversity effects among tall and dominant grass and legume species.

## Materials and methods

### Experimental design

The experiment was established at the field site of the Jena Experiment (Roscher et al. 2004), located in the floodplain of the River Saale north of the city of Jena (Germany, 50°55'N, 11°35'E, 130 m a.s.l.). The area around Jena has a mean annual air temperature of 9.3 °C, and annual precipitation is 587 mm (Kluge and Müller-Westermeier 2000). The soil is a loamy Eutric Fluvisol. The experimental area was used as a fertilized agricultural field for about 40 years before a grass mixture was sown in spring 2002. After 2 years, in autumn 2004, the area was ploughed to a depth of 30 cm and covered with a black perforated foil (Aquafool, Hermann Meyer KG, Rellingen, Germany) until 2007. In spring 2008, the area was harrowed and raked several times to remove emerging weed seedlings before the experiment was sown on plots of size 2 × 2 m on 5 May 2008.

We selected two perennial legume species (*Medicago × varia* Martyn and *Onobrychis viciifolia* Scop.) and two perennial grass species (*Arrhenatherum elatius* (L.) J. et C. Presl and *Dactylis glomerata* L.) for our experiment. These experimental species, which were among the tallest and most productive species in the Jena Experiment, were established as monocultures, all possible two- and three-species combinations, and the full four-species mixture. The experiment was arranged in three blocks, each comprising a randomly positioned replicate of each monoculture, two- and three-species mixture, and two replicates of the four-species mixture (=16 plots per block and 48 plots in total). Seeds were purchased from the same commercial supplier as used to establish the Jena Experiment (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany). Plots were sown at a total density of 1000 viable seeds per m<sup>2</sup> in a substitutive design in which constant total density was achieved by reducing the sowing densities of individual species according to the number of species in the mixture. Number of sown seeds was adjusted for germination rates obtained from laboratory tests. After mowing in September 2008, all plots were re-sown with a total density of 500 viable seeds per m<sup>2</sup> to mimic a diverse natural age structure for all species. In the second year, blocks were allocated to experimental treatments (1) manipulating light availability through shading, (2) manipulating nutrient availability through fertilization, and (3) acting as a control. In the fertilizer treatment, fertilizer was applied as commercial NPK pellets (slow-release NPK fertilizer

150:65.4:124.5 kg ha<sup>-1</sup> a<sup>-1</sup>) divided into two equal portions in early spring (31 March 2009) and after the first mowing (21 June 2009). The amount of fertilizer used was equivalent to the usual fertilizer intensities employed in agriculturally managed semi-natural grasslands in Europe (Olf et al. 1990). In the shade treatment, a reduction in photosynthetically active radiation was achieved by fixing two layers of green shading cloth (polyethylene, aperture size 2 × 10 mm, Hermann Meyer KG, Rellingen, Germany) on a roof (2.5 m height) and covering all sides; this apparatus was installed in spring (14 April 2009). To assess the effects of shading on climatic conditions, a weather station was established under the roof to measure the following variables: air temperature and relative humidity (KPK1/5-ME humidity/temperature sensor, Mela Sensortechnik, Mohlsdorf-Teichwolframsdorf, Germany), photosynthetically active radiation (LI-90SA quantum sensor, LI-COR, Lincoln, NE, USA) at 2 m height, precipitation (precipitation transmitter, Thies GmbH, Göttingen, Germany), and soil moisture at 8, 16, and 32 depth (ThetaProbe ML2X, Delta-T Devices Ltd., Cambridge, UK). Data were stored with a data logger (CR10X, Campbell Scientific, Logan, UT, USA). A comparison with data from the central weather station of the Jena Experiment field site showed that shading reduced photosynthetically active radiation by 82 % and precipitation by 40 %, while soil moisture was increased on average by 20 % at depths of 8 and 16 cm and 40 % at a depth of 32 cm. Air temperature and relative humidity did not deviate from ambient conditions (see Figs. S1 and S2 in the Electronic supplementary material, ESM).

Plots were regularly weeded to remove species that did not belong to the sown species combinations. Following the typical management of extensively used hay meadows and the usual management of the Jena Experiment (Roscher et al. 2004), plots were mown in early June and September 2009, and mown plant material was removed.

### Data collection

Aboveground plant biomass was harvested in one sample quadrat (50 × 50 cm size) in the central area of each plot at estimated peak biomass before mowing on 3 June 2009 and 2 September 2009. Plant material was clipped 3 cm above ground level and sorted to species, separating out unsown species and detached dead material. Samples were dried at 70 °C (48 h) and weighed. Dry plant material per plot and species was shredded and a subsample was ground with a ball mill to a fine powder. Approximately 20 mg were used for elemental C and N analyses with an elemental analyzer (Vario EL element analyzer, Elementar, Hanau, Germany).

During the spring harvest, fully developed leaves of all species were sampled in all plots to determine N-isotope

ratios. Isotope ratios were measured from approximately 3 mg of dried and finely milled leaf material with an isotope-ratio mass spectrometer (Delta C prototype IRMS, Finnigan MAT, Bremen, Germany). The  $\delta^{15}\text{N}$  values were calculated relative to the international standard, the atmospheric nitrogen isotope ratio:

$$\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000, \quad (1)$$

where  $R$  represents the molar ratio of  $^{15}\text{N}$  to  $^{14}\text{N}$  in a sample. The  $\delta^{15}\text{N}$  values of actively  $\text{N}_2$ -fixing legumes are close to the atmospheric  $\delta^{15}\text{N}$  signal ( $\sim 0\%$ ) because the fractionation of N isotopes during symbiotic  $\text{N}_2$  fixation is very small. Plants that depend on soil nitrogen generally show higher  $\delta^{15}\text{N}$  values. Variation in the natural abundances of stable  $^{15}\text{N}$  isotopes in nonlegume species may roughly indicate shifts in the uptake of different N sources or the supply of depleted legume-derived N in communities with legumes (Högberg 1997).

Soil samples were taken on 21 June 2009 (after the first mowing and before the second fertilization) and 13 October 2009. Three soil cores 1 cm in diameter (0–15 cm depth) were sampled in each plot and pooled. The fresh soil samples were sieved (mesh size of 2 mm) and visible root parts were removed before extraction with 1 M KCl (50 ml on 5 g of soil material). The filtered extracts were frozen until analysis for nitrate and ammonium with a continuous flow analyzer (SAN Plus, Skalar, Erkelenz, Germany). Inorganic nitrogen concentrations were expressed in  $\mu\text{g}$  per g dry soil after determining the water contents of the soil samples gravimetrically (72 h, 105 °C), and then averaged across sampling dates to compare different resource manipulation treatments.

### Calculations of overyielding

The relative yield total (RYT, de Wit and van den Bergh 1965) was calculated as a measure of overyielding in relative terms. The relative yield ( $\text{RY}_i$ ) of species  $i$  with respect to biomass is the quotient of the species' biomass in a mixture ( $O_i$ ) and the monoculture biomass of the species ( $M_i$ ). The RYT of a mixture is the sum of the RYs of all of its component species.  $\text{RYT} > 1$  indicates overyielding. The RYT is directly linked to “nontransgressive overyielding,” quantified by the proportional index  $D_{\text{mean}}$

$$D_{\text{mean}} = (O_{\text{T}} - \overline{M}) / \overline{M}, \quad (2)$$

where  $O_{\text{T}}$  is the observed biomass of a given mixture, and  $\overline{M}$  is the average monoculture biomass of all species in this mixture ( $\overline{M} = M_i/N$ ;  $N$  = species richness); i.e.,  $D_{\text{mean}}$  indicates whether a mixture outperforms the average of its component monocultures ( $D_{\text{mean}} = \text{RYT} - 1$ ; Loreau 1998). Transgressive overyielding ( $D_{\text{max}} > 0$ ), indicating

that the biomass produced by a mixture is greater than the biomass produced by the most productive monoculture of any of the species in this mixture ( $M_{\text{max}}$ ), was calculated by replacing  $\overline{M}$  by  $M_{\text{max}}$  in Eq. 2 (Loreau 1998).

The ratio of the observed to the expected biomass of an individual species corrected for sowing proportion (i.e., multiplied with species richness  $N$ ) was used to compare species performance and determine the contribution of each species to overyielding. A value of  $(N \times \text{RY}_i) > 1$  suggests that the species performs better in a mixture than in monoculture, a value of  $N \times \text{RY}_i = 1$  indicates that the species presents the same performance in a mixture and in monoculture, and  $(N \times \text{RY}_i) < 1$  indicates that the species performs worse in a mixture than in monoculture.

To evaluate whether mixed communities depleted soil mineral nitrogen (ammonium and nitrate) more efficiently than monocultures, the proportional index  $D_{\text{mean}}$  (Loreau 1998) was modified by taking  $O_{\text{T}}$  as the observed soil mineral nitrogen in mixture and  $\overline{M}$  as the expected soil mineral nitrogen based on monocultures of the component species weighted by the biomass proportion of each species in the mixture. Values of  $D_{\text{mean}} < 0$  indicate that mixtures reduce soil mineral nitrogen to lower levels than would be expected from the component monocultures, suggesting complementary resource use (Palmborg et al. 2005).

Furthermore, the additive partitioning method (Loreau and Hector 2001) was applied to assess diversity effects on biomass production. According to Loreau and Hector (2001), the net diversity effect (NE) on the biomass of a given mixture is the difference between the observed total mixture biomass and the expected total mixture biomass based on the average of the monoculture biomasses of the component species. The net diversity effect is the sum of two effects generated by species interactions in a mixture: selection effects (SE) and complementarity effects (CE)

$$\text{NE} = \text{SE} + \text{CE}. \quad (3)$$

Selection effects (SE) were quantified as the standard statistical covariance

$$\text{SE} = N \text{cov}(\Delta \text{RY}, M), \quad (4)$$

where  $N$  is the number of species in the mixture,  $M$  is a species' monoculture biomass, and  $\Delta \text{RY}$  is the difference between the observed relative yield and the expected relative yield (i.e., its sown proportion  $I/N$ ). Positive (negative) selection effects occur if species with higher-than-average (lower-than-average) monoculture biomasses dominate mixtures. Complementarity effects (CE) measure the degree to which all species similarly contribute to a higher (or lower) total mixture biomass than the average monoculture, and are calculated as

$$\text{CE} = N \overline{\Delta \text{RY} M}, \quad (5)$$

where  $\overline{\Delta RY}$  is the average  $\Delta RY$  of all species in the mixture and  $\overline{M}$  is the average monoculture biomass of all species in this mixture. Positive complementarity effects occur when increases in the biomasses of some species are not completely compensated for by decreases in others, and may indicate niche differentiation such as resource partitioning.

All calculations were done separately for each resource treatment.

### Statistical analyses

Linear mixed-effects models using the *lmer* function in the library *lme4* (Bates et al. 2012) of the statistical software R (version 3.0.2, R Development Core Team, <http://www.R-project.org>) were used to analyze the influence of each experimental factor on the measured variables. Community-level variables were analyzed starting from a constant null model with mixture identity as a random effect. Then the fixed effects—resource (as a factor with three levels: control, fertilization, shading), sown species richness (SR; as a log-linear term), and the interaction between these terms (resource  $\times$  SR)—were entered stepwise. In alternative models, the presence/absence of each species and its interaction with resource was included to test for species identity effects. In analyses of species-level variables, mixture and plot were modeled as random effects, and species identity (species ID) as well as the interaction resource  $\times$  species ID were fitted as additional fixed effects. The maximum likelihood method (ML) and likelihood ratio tests ( $X^2$ ) were applied to assess model improvement and the statistical significance ( $\alpha \leq 0.05$ ) of the fixed effects. The *glht* function in the R library *multcomp* (Hothorn et al. 2008) was used to identify differences between the resource treatments and species (in analyses of species-level variables) with Tukey's HSD test in models fitted with the restricted maximum likelihood method (REML). Separate analyses of variance (ANOVA) were conducted for each resource treatment for measures of overyielding and diversity effects in order to test grand means (i.e., overall means) against hypothetical values.

If necessary, data were log-transformed to meet the assumptions of linear mixed-effects models. Because the resource treatments had to be applied at a block level, it is conceivable that resource treatment effects reflect block effects to some extent. This could not be avoided, and is mentioned here as a caveat. Another shortcoming of our experimental design (and many other biodiversity experiments) is the lack of identical replicates of each species combination in the different resource treatments, which would allow us to test for mixture identity effects and obtain a more robust estimate of diversity effects.

## Results

### Community biomass production

Community biomass production varied with resource availability (Table 1; Fig. 1a). Averaged across species-richness levels, biomass production under fertilization was higher and biomass production under shading was lower than in the control (mean  $\pm$  SD; control =  $1195 \pm 45 \text{ g}_{\text{dw}} \text{ m}^{-2}$ , fertilized =  $1350 \pm 48 \text{ g}_{\text{dw}} \text{ m}^{-2}$ , shaded =  $503 \pm 42 \text{ g}_{\text{dw}} \text{ m}^{-2}$ ). On average, community biomass production increased with species richness, but species richness effects depended on resource availability (significant resource  $\times$  SR interaction; Table 1). Positive species-richness effects on community biomass production were larger under shading than in the control, while increasing species richness did not increase community biomass production under fertilization (Table S1 in the ESM). The presence/absence of *A. elatius*, *D. glomerata*, or *M. varia* did not affect community-level biomass production, while the effects of *O. viciifolia* were dependent on resource availability (significant resource  $\times$  Ov interaction; Table 1). The presence of *O. viciifolia* had positive effects on community biomass production in the control and under fertilization, but negative effects under shading (Table S1 in the ESM).

### Nontransgressive and transgressive overyielding

The relative yield totals (RYTs) varied with resource availability (Table 1). The RYTs were smaller under fertilization and greater under shading than in the control (Fig. 1b). The overall mean RYTs across all species-richness levels were  $>1$  (i.e.,  $D_{\text{mean}} > 0$ ) in the control and under shading (mean  $\pm$  SD; control =  $1.186 \pm 0.152$ , shaded =  $1.608 \pm 0.413$ ), indicating nontransgressive overyielding. Under fertilization, the overall mean RYTs were  $<1$  ( $0.877 \pm 0.154$ ). The RYTs did not change with increasing species richness. The presence of *D. glomerata* had negative effects on the RYTs (Table 1), which varied with resource availability. Under fertilization and shading, the RYTs were smaller in mixtures with *D. glomerata* than in mixtures without this species, while this was not the case in the control (Table S1 in the ESM).

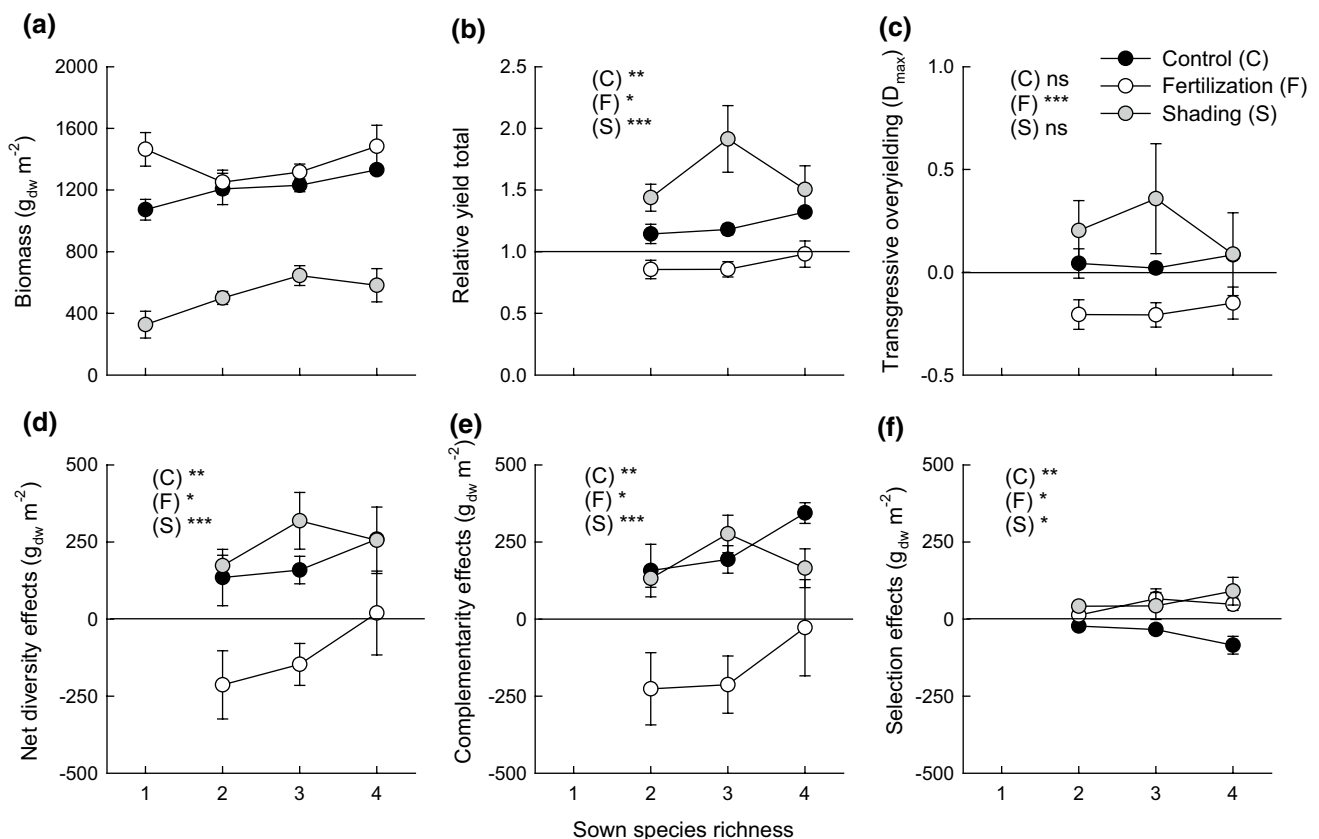
Transgressive overyielding ( $D_{\text{max}}$ ) also varied with resource availability (Table 1; Fig. 1c), but, on average, mixtures did not outperform the best monoculture in the control and under shading (mean  $\pm$  SD; control =  $0.042 \pm 0.123$ , shaded =  $0.235 \pm 0.392$ ) or even underyielded ( $D_{\text{max}} < 0$ ) under fertilization ( $-0.197 \pm 0.140$ ).

**Table 1** Summary of mixed-effects model analyses of community biomass production: relative yield total (RYT; or nontransgressive overyielding ( $D_{\text{mean}} = \text{RYT} - 1$ )), transgressive overyielding ( $D_{\text{max}}$ ),

net diversity effects (NE), complementarity effects (CE), and selection effects (SE) calculated for biomass production in mixtures

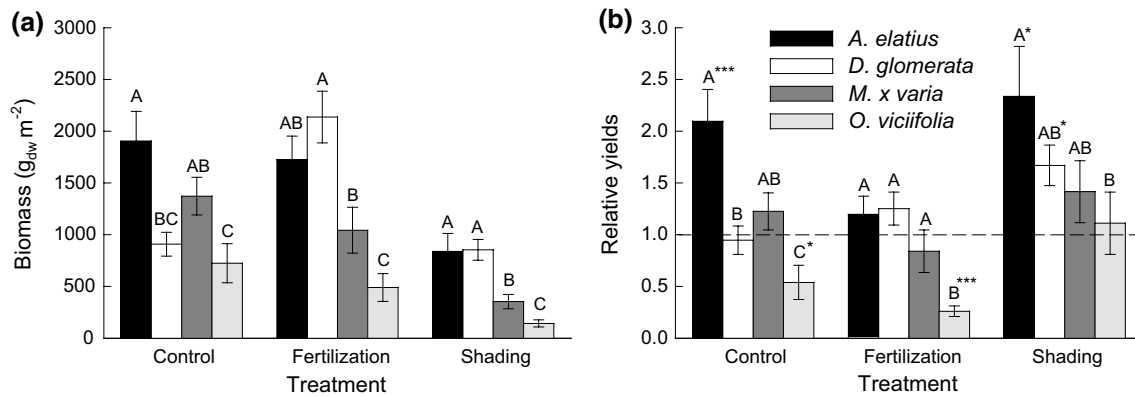
Source of variation	Community biomass		RYT		$D_{\text{max}}$		NE		CE		SE	
	$X^2$	$P$	$X^2$	$P$	$X^2$	$P$	$X^2$	$P$	$X^2$	$P$	$X^2$	$P$
Resource	81.20	<0.001	36.72	<0.001	18.64	<0.001	25.36	<0.001	26.92	<0.001	16.65	<0.001
Species richness (SR)	4.91	0.027 $\uparrow$	3.25	0.071	0.13	0.714	3.13	0.077	3.32	0.068	0.32	0.572
Resource $\times$ SR	6.11	0.047	0.10	0.950	0.10	0.950	0.35	0.839	0.13	0.937	4.64	0.098
<i>Arrhenatherum elatius</i> (Ae)	0.18	0.672	0.04	0.833	0.12	0.733	0.12	0.727	0.02	0.892	0.54	0.461
Resource $\times$ Ae	3.69	0.158	0.64	0.725	0.78	0.676	0.96	0.619	1.30	0.523	0.65	0.722
<i>Dactylis glomerata</i> (Dg)	0.01	0.929	10.10	0.001 $\downarrow$	11.93	0.001 $\downarrow$	5.78	0.016 $\downarrow$	5.89	0.015 $\downarrow$	0.03	0.862
Resource $\times$ Dg	0.18	0.913	9.62	0.008	15.23	<0.001	11.99	0.002	12.20	0.002	11.24	0.004
<i>Medicago x varia</i> (Mv)	0.02	0.875	2.66	0.103	3.31	0.069	1.14	0.286	1.03	0.309	0.02	0.898
Resource $\times$ Mv	0.69	0.710	3.87	0.144	5.67	0.059	3.04	0.219	2.89	0.236	2.26	0.324
<i>Onobrychis viciifolia</i> (Ov)	1.41	0.234	2.26	0.133	1.04	0.309	2.73	0.099	2.33	0.127	0.12	0.733
Resource $\times$ Ov	7.83	0.020	0.15	0.926	1.07	0.586	0.91	0.636	1.54	0.463	3.19	0.203

Models were fitted by stepwise inclusion of fixed effects. Shown are the results of likelihood ratio tests ( $X^2$ ) that were applied to assess model improvement and the statistical significance of the fixed effects ( $P$  values). Note that the presence/absence of individual species and their interactions with resource availability were fitted in separate models. Arrows indicate an increase ( $\uparrow$ ) or a decrease ( $\downarrow$ ) of the variable in response to increasing species richness or the presence of particular species



**Fig. 1** Effects of sown species richness and experimental treatments (control, fertilization, and shading) on **a** community biomass production, **b** relative yield total (or nontransgressive overyielding,  $D_{\text{mean}} = \text{RYT} - 1$ ), **c**  $D_{\text{max}}$  (transgressive overyielding), **d** net diversity effects (NE), **e** complementarity effects (CE), and **f** selec-

tion effects (SE). Shown are the means ( $\pm$  standard error) per species-richness level and treatment. Results of tests for overall means of  $\text{RYT} \neq 1$  and  $D_{\text{max}}$ , NE, CE and SE  $\neq 0$ , respectively, for each resource treatment are indicated by *ns* nonsignificant,  $*P \leq 0.05$ ,  $**P \leq 0.01$ , or  $***P \leq 0.001$



**Fig. 2** Effects of experimental treatments (control, fertilization, and shading) on **a** species-level biomass production and **b** relative yields (RYs). Shown are means ( $\pm$  standard error) per treatment across species-richness levels. Species biomass in mixtures was corrected for sowing proportions (i.e., multiplied by species number) before averaging. Significant deviations of the observed from the expected val-

ues for species-level relative yields (i.e.,  $RY \neq 1$ ) across all species-richness levels are indicated by  $*P \leq 0.05$  and  $***P \leq 0.001$ , where  $RY > 1$  indicates overyielding. Results of Tukey's test applied to test for significant differences in biomass production and relative yields among species for each resource treatment are indicated by letters

### Net diversity, complementarity, and selection effects

Resource availability also influenced net diversity effects (NE), complementarity effects (CE), and selection effects (SE) (Table 1). NE and CE did not differ between the control and the shade treatment and were positive (Fig. 1d, e). Due to the lower productivity under shading, net diversity effects increased biomass in the mixtures by 72 % under shading. The average increase in biomass amounted to 15 % in the control. NE and CE were negative under fertilization, i.e., on average, net diversity effects decreased biomass by 10 %. SE were small compared to CE (Fig. 1f); the SE were negative in the control (-4 %) and positive under shading (+15 %) and fertilization (+2 %). Irrespective of the resource treatment, NE, CE, and SE did not change with species richness. The presence of *D. glomerata* in the mixtures decreased NE and CE, while the presences/absences of other species had no additional effects on NE, CE, or SE (Table 1). However, effects of *D. glomerata* varied with resource availability. The species had strong negative effects on NE and CE under fertilization and mainly caused the negative NE and CE in this treatment (Table S1 in the ESM). The NE and CE were also smaller in mixtures with *D. glomerata* than without this species under shading, while *D. glomerata* tended to have positive effects on NE and CE in the control (Table S1 in the ESM). Depending on resource availability, the presence/absence of *D. glomerata* also influenced SE. The SE tended to be more positive in mixtures with *D. glomerata* in the control and under fertilization, while SE were lower in mixtures with *D. glomerata* than without this species under shading (Table S1 in the ESM).

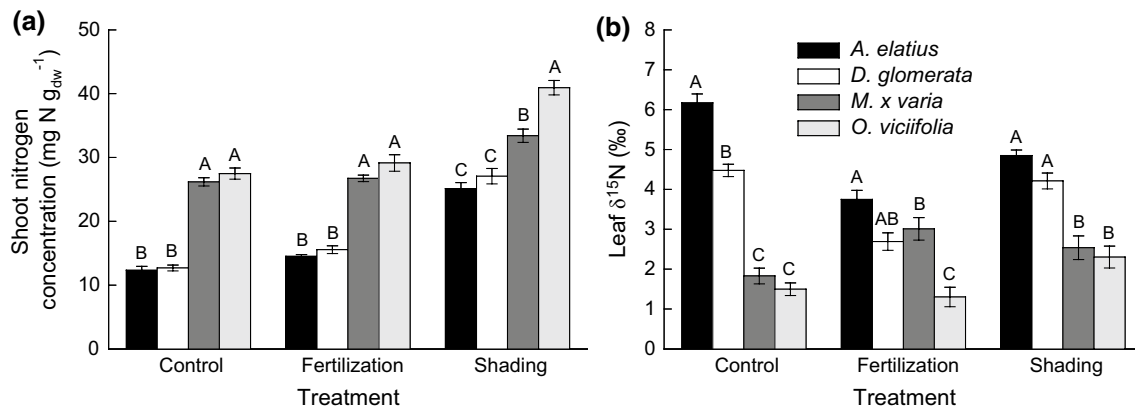
### Species-level biomass production and relative yields

On average, the grass species (*A. elatius* and *D. glomerata*) and the legume *M. x varia* produced more biomass than the legume *O. viciifolia* (Fig. 2a). Differences in biomass production among the grass species and the legume *M. x varia* depended on resource availability (Table S2 in the ESM). In the control, species biomass decreased in the order *A. elatius*, *M. x varia*, *D. glomerata*. In contrast, *D. glomerata* was more productive than *M. x varia* under fertilization (Fig. 2a). Under shading, the biomasses of *D. glomerata* and *A. elatius* did not differ and the grasses were more productive than the legume *M. x varia*.

Relative yields (RY) of individual species also varied with resource availability (Table S2 in the ESM; Fig. 2b). *Arrhenatherum elatius* had a significant  $RY > 1$  in the control and under shading, but not under fertilization. *Dactylis glomerata* achieved  $RY > 1$  only under shading. The legume species did not achieve  $RY > 1$ , and *O. viciifolia* even underyielded ( $RY < 1$ ) in the control and under fertilization. In general, increasing species richness did not affect species-level RY with the exception of positive species richness effects on RY of *A. elatius* in the control (analyses not shown).

### Shoot nitrogen concentrations and leaf $\delta^{15}\text{N}$

Shoot nitrogen concentrations ( $N_{\text{Shoot}}$ ) varied with resource availability, while increasing species richness did not affect  $N_{\text{Shoot}}$  (Table S2 in the ESM). On average,  $N_{\text{Shoot}}$  was lowest in the control, slightly larger under fertilization, and largest under shading (Fig. 3a).  $N_{\text{Shoot}}$  values of the legume species were higher than those in the grass species.



**Fig. 3** Effects of experimental treatments (control, fertilization, and shading) on species-level **a** shoot nitrogen concentrations and **b** leaf  $\delta^{15}\text{N}$ . Shown are means ( $\pm$  standard error) per treatment across spe-

cies-richness levels. Results of Tukey's test applied to test for significant differences in shoot nitrogen concentrations and leaf  $\delta^{15}\text{N}$  among species for each resource treatment are indicated by letters

The  $N_{\text{Shoot}}$  values were not different between the two grass species. The  $N_{\text{Shoot}}$  values also did not differ between both legume species, except that  $N_{\text{Shoot}}$  of *M. x varia* was significantly lower than *O. viciifolia* under shading (Fig. 3a).

The  $\delta^{15}\text{N}$  measured in leaf samples collected in spring before first mowing varied with resource availability. On average, values under fertilization were reduced compared to the control and the values obtained with shading, but the effects of resource treatment depended on species identity (Table S2 in the ESM). Increased species richness did not alter leaf  $\delta^{15}\text{N}$ . Although leaf  $\delta^{15}\text{N}$  values of legumes were larger than zero in all samples, legume species (except for *M. x varia* under fertilization) generally had lower leaf  $\delta^{15}\text{N}$  values than the grass species, suggesting that a portion of the legume N was derived from symbiotic  $\text{N}_2$  fixation (Fig. 3b). Additional analyses testing for the dependence of differences in leaf  $\delta^{15}\text{N}$  between grasses and legumes on resource availability showed that differences between functional groups were largest in the control and smallest under fertilization (estimated coefficients from linear mixed-effects models: control = 3.62 ‰, fertilization = 1.02 ‰, shading = 2.07 ‰).

### Soil mineral nitrogen concentrations

Soil mineral nitrogen concentrations ( $N_{\text{min}}$ ) differed with resource availability (Fig. 4a; Table S3 in the ESM).  $N_{\text{min}}$  values were lowest in the control and largest under shading. The effects of increasing species richness on  $N_{\text{min}}$  were not significant. The presence of *D. glomerata* led to a decrease in  $N_{\text{min}}$ , while the presence of *O. viciifolia* caused an increase. The ratio of observed to expected mineral soil nitrogen concentrations ( $D_{\text{mean}}$ ) also varied with resource availability. The overall means across all species richness

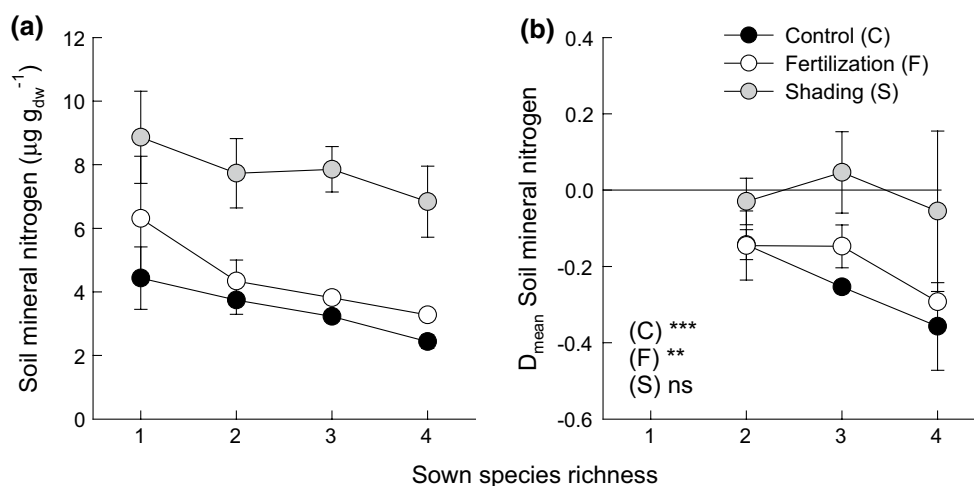
levels did not differ between the control and the fertilized treatment, and both had values that were significantly lower than zero (Fig. 4b). Values observed under shading did not differ from expected values.

### Discussion

Biodiversity experiments have been repeatedly criticized for their random scenarios of species loss, ignoring the impact of environmental and biotic filters which constrain species diversity and affect community assembly in natural systems (e.g., Aarssen 1997; Lepš 2004). In this study, we took advantage of the long-term Jena Experiment, which is based on a pool of 60 grassland species (Roscher et al. 2004). We chose four tall-statured species that had highly productive monocultures and were dominant in mixtures of the Jena Experiment. Thus, our additional experiment complements the so-called “dominance experiment” in the framework of the Jena Experiment. In contrast to our additional experiment, the species pool for the dominance experiment was selected based on the criterion that it should consist of potentially dominant species in semi-natural grasslands of the study region (Roscher et al. 2004), but these species showed different levels of monoculture productivity (Roscher et al. 2005, 2007).

The main results of our additional experiment on the site of the Jena Experiment were that even tall and dominant species show higher productivity in mixtures than expected from their monocultures. However, our experimental results clearly indicated that resource availability had strong impacts on diversity effects among these species.





**Fig. 4** Effects of sown species richness and experimental treatments (control, fertilization, and shading) on **a** soil mineral nitrogen concentrations, and **b**  $D_{\text{mean}}$  of soil mineral nitrogen concentrations. Shown are means ( $\pm$  standard error) per species-richness level and treatment.  $D_{\text{mean}} < 0$  indicates that mineral soil nitrogen concentrations in the

mixture were lower than the biomass-weighted means of the component monocultures. The significance of  $D_{\text{mean}} \neq 0$  across all species-richness levels was tested separately for each resource treatment. ns nonsignificant,  $**P \leq 0.01$  and  $***P \leq 0.001$

### Selection effects among tall and dominant grassland species

While complementary resource use and facilitation (quantified as positive “complementarity effects” in the additive partitioning method of Loreau and Hector 2001) have been identified as the mechanism behind overyielding in numerous biodiversity experiments, the contribution of selection effects ranges from negative (van Ruijven and Berendse 2003) to positive (e.g., Dimitrakopoulos and Schmid 2004; Roscher et al. 2005). In line with our hypothesis 1, selection effects were small compared to complementarity effects, and they were negative in the control and positive under shading and fertilization. The small magnitude of the selection effects (Fig. 1f) indicated that no single species achieved a higher biomass production in the mixtures at the expense of others. Together with the lack of positive transgressive overyielding (Fig. 1c), these results suggest that our experimental species were comparable in their competitive strengths as mixture species. Nevertheless, we also found evidence for the influence of a single species, namely *D. glomerata*, on selection effects. *Dactylis glomerata* appeared to benefit most from the supply of additional nutrients; the species had the most productive monoculture and accelerated positive selection effects under fertilization. Interestingly, *D. glomerata*, which also had the most productive monoculture under shading, decreased the generally positive selection effects in this treatment. Thus, it is likely that growth was most controlled by the reduced light supply through the shading and not by species interactions. In the control, *D. glomerata* had the least productive

monoculture. Selection effects became less negative in mixtures with this species, probably due to the use of the additional nitrogen provided by  $\text{N}_2$ -fixing legumes.

### Complementarity effects among tall and dominant grassland species

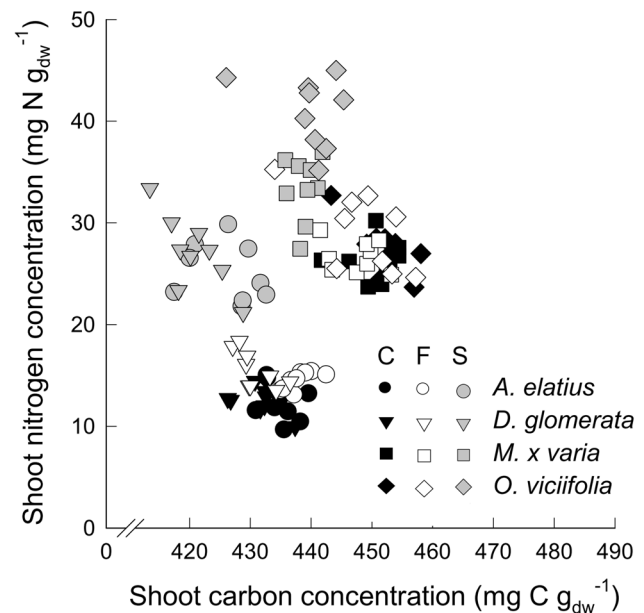
In line with our expectations (hypotheses 2–4), we found that complementarity effects varied greatly with resource availability (Fig. 1e), which was consistent with results of RYT analyses (Fig. 1b). In the control without resource manipulation, complementarity effects were positive, confirming hypothesis 2. In many biodiversity experiments, the positive interactions between  $\text{N}_2$ -fixing legumes and non- $\text{N}_2$ -fixing species were important contributors to positive complementarity effects (e.g., Spehn et al. 2005; Marquard et al. 2009). In the design of our experiment, it was unavoidable that all mixtures (with the exception of the two-species mixture of *A. elatius* and *D. glomerata*) contained legumes. Although leaf  $\delta^{15}\text{N}$  values of both legumes were above zero in all resource treatments, legumes mostly had lower  $\delta^{15}\text{N}$  and higher shoot N concentrations than grasses, suggesting that legumes at least partly relied on symbiotic  $\text{N}_2$  fixation (Fig. 3b).

Confirming our hypothesis 3, complementarity effects became negative under fertilization. Nyfeler et al. (2009) also reported that positive complementarity effects and transgressive overyielding in four-species grass–legume mixtures disappeared at very high levels of N fertilization ( $450 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ). Negative complementarity effects under fertilization were greatest in the mixture of

the two grass species ( $-685 \text{ g m}^{-2}$ ); average complementarity effects across the mixtures with legumes were  $-144 (\pm 57) \text{ g m}^{-2}$ . Differences in leaf  $\delta^{15}\text{N}$  between grasses and legumes were smaller under fertilization than in the control (Fig. 3b). It is well known that the physiologically costly symbiotic  $\text{N}_2$  fixation is suppressed when mineral soil N availability is increased through fertilization (Hartwig 1998). Analyses of species-level relative yields showed that under fertilization, no single species reached higher RYs in biomass production than expected from monocultures, and the legume *O. viciifolia* even underyielded (Fig. 2b).

In contrast to our expectations (hypothesis 4), complementarity effects were positive under shading (Fig. 1e), and none of the species underyielded in this treatment (Fig. 2b). It is likely that nutrients were taken up and used for growth less completely under shading than in the other treatments. In contrast to the control and under fertilization, mineral soil nitrogen concentrations were higher and not depleted to lower levels in the mixtures than expected from the monocultures (Fig. 4). Leaf  $\delta^{15}\text{N}$  values for legume species were higher and differences in leaf  $\delta^{15}\text{N}$  between legume and grass species were smaller under shading than in the control (Fig. 3b). Both of these observations suggest that there is less reliance of legumes on symbiotic  $\text{N}_2$  fixation under shading. A greater availability of mineral soil N in this treatment due to limited plant growth and therefore lower consumption of soil N or increased soil N mineralization rates at higher soil moisture (Wang et al. 2006) are possible explanations for why legumes were less dependent on symbiotic  $\text{N}_2$  fixation in this treatment. Furthermore, higher species-level shoot N concentrations and lower biomass production under shading indicated that the plant growth of all species was carbon-limited. The fact that high shoot N concentrations occurred at lower shoot C concentrations under shading than in the other treatments (Fig. 5) also suggests that growth was carbon-limited in this treatment (Stitt and Schulze 1994). However, severe shading is clearly not a realistic scenario for tall-growing grassland species under natural conditions, so a limited ability to tolerate such shade may be expected in the chosen species. The unexpected positive complementarity effects under shading, however, imply that plant growth was not completely controlled by external resource supply. Possibly, a more diverse canopy structure of plant mixtures compared to the monocultures allowed for a complementary and more complete use of the available light in the mixtures.

Our experiment only addressed short-term changes in community responses to the manipulation of resource availability. Several long-term grassland biodiversity experiments have shown that the strength of diversity–productivity relationships and positive complementarity effects increase through time, while selection effects decrease



**Fig. 5** Shoot nitrogen concentration plotted against shoot carbon concentration for the four experimental species in each treatment (control C, fertilization F, shading S)

(Fargione et al. 2007; Marquard et al. 2009). It also has been reported that diversity effects among the species sown in fertilized and unfertilized experimental grasslands showed great variation during a 3-year study period (Lanta and Lepš 2007). Transient dynamics following a perturbation such as environmental changes may differ from conditions when communities reach equilibrium (Tilman 1988). Thus, the observed effects of fertilization or shading in a single year of treatment application may change over a longer time span. Nevertheless, our short-term experiment clearly demonstrated that selection effects are small among highly productive and dominant grass and legume species, while the strength and direction of complementarity effects varies greatly with resource availability.

**Acknowledgments** This study was part of the Jena Experiment and supported by the German Science Foundation (RO2397/1 within FOR456). We acknowledge A. Weigelt for field coordination. We thank U. Gerighausen, S. Ferber, C. Kups, the gardeners, and several student helpers for their support with the establishment and management of the four-species experiment and during sampling; I. Hilke, S. Matthei, and H. Geilmann for conducting chemical and stable isotope analyses; F. Voigt and B. Schlöffel for the construction of the shade roof; K. Hippler, M. Hertel, and A. Fastnacht for setting up and maintaining the weather station; and three anonymous reviewers for their helpful comments on a previous version of the manuscript.

**Author contribution statement** CR, E-DS, BS, and OK conceived and designed the experiment. CR and OK performed the experiment. CR analyzed the data and wrote the manuscript; E-DS and BS discussed the results and contributed to writing the manuscript; OK provided editorial advice.

## References

- Aarssen LW (1997) High productivity in grassland ecosystems: effected by species diversity or productive species? *Oikos* 80:183–184
- Bates D, Maechler M, Bolker B (2012) lme4: linear mixed-effects models using Eigen and Eigen. <http://www.r-project.org>
- Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivastava DS, Naeem S (2012) Biodiversity loss and its impact on humanity. *Nature* 486:59–67
- de Wit CT, van den Bergh JP (1965) Competition between herbage plants. *Neth J Agric Sci* 13:212–221
- Dimitrakopoulos PG, Schmid B (2004) Biodiversity effects increase linearly with biotope space. *Ecol Lett* 7:574–583
- Fargione J, Tilman D, Dybzinski R, Hille Ris Lambers J, Clark C, Harpole WS, Knops JMH, Reich PB, Loreau M (2007) From selection to complementarity: shifts in the causes of biodiversity–productivity relationships in a long-term biodiversity experiment. *Proc R Soc Lond B* 274:871–876
- Fridley JD (2002) Resource availability dominates and alters the relationship between species diversity and ecosystem productivity in experimental plant communities. *Oecologia* 132:271–277
- Fridley JD (2003) Diversity effects on production in different light and fertility environments: an experiment with communities of annual plants. *J Ecol* 91:396–406
- Grime JP (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *J Ecol* 86:902–910
- Grime JP (2001) Plant strategies, vegetation processes and ecosystem properties. Wiley, Chichester
- Hartwig UA (1998) The regulation of symbiotic N<sub>2</sub> fixation: a conceptual model of N feedback from the ecosystem to the gene expression level. *Perspect Plant Ecol Evol Syst* 1:92–120
- He J-S, Bazzaz FA, Schmid B (2002) Interactive effects of diversity, nutrients and elevated CO<sub>2</sub> on experimental plant communities. *Oikos* 97:337–348
- Hesse K, Roscher C, Schumacher J, Schulze E-D (2007) Establishment of grassland species in monocultures: different strategies lead to success. *Oecologia* 152:435–447
- Högberg P (1997) <sup>15</sup>N natural abundance in soil–plant systems. *New Phytol* 137:179–203
- Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setälä H, Symstad AJ, Vandermeer J, Wardle DA (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol Monogr* 75:3–35
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom J* 50:346–363
- Huston MA (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* 110:449–460
- Kluge G, Müller-Westermeier G (2000) Das Klima ausgewählter Orte der Bundesrepublik Deutschland. Jena. *Ber Deutsch Wetterdienst* 213:1–290
- Lanta V, Lepš J (2007) Effects of species and functional group richness on production in two fertility environments: an experiment with communities of perennial plants. *Acta Oecol* 32:93–103
- Lepš J (2004) What do the biodiversity experiments tell us about consequences of plant species loss in the real world? *Basic Appl Ecol* 5:529–534
- Loreau M (1998) Separating sampling and other effects in biodiversity experiments. *Oikos* 82:600–602
- Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature* 412:72–76 [Erratum 413:548]
- Marquard E, Weigelt A, Temperton VM, Roscher C, Schumacher J, Buchmann N, Fischer M, Weisser WW, Schmid B (2009) Plant species richness and functional composition drive overyielding in a 6-year grassland experiment. *Ecology* 90:3290–3302
- Nyfelner D, Huguenin-Elie O, Suter M, Frossard E, Connolly J, Lüscher A (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *J Appl Ecol* 46:683–691
- Olf H, van Andel J, Bakker JP (1990) Biomass and shoot/root allocation of five species from a grassland succession series at different combinations of light and nutrient supply. *Funct Ecol* 4:193–200
- Palmborg C, Scherer-Lorenzen M, Jumpponen A, Carlsson G, Huss-Danell K, Högberg P (2005) Inorganic soil nitrogen under grassland plant communities of different species composition and diversity. *Oikos* 110:271–281
- Rajaniemi TK (2003) Evidence for size asymmetry of belowground competition. *Basic Appl Ecol* 4:239–247
- Reich PB, Knops J, Tilman D, Craine J, Ellsworth D, Tjoelker M, Lee T, Wedin D, Naeem S, Bahaeddin D, Hendrey G, Jose S, Wrage K, Goth J, Bengston W (2001) Plant diversity enhances ecosystem responses to elevated CO<sub>2</sub> and nitrogen deposition. *Nature* 410:809–810
- Roscher C, Schumacher J, Baade J, Wilcke W, Gleixner G, Weisser WW, Schmid B, Schulze E-D (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic Appl Ecol* 5:107–121
- Roscher C, Temperton VM, Scherer-Lorenzen M, Schmitz M, Schumacher J, Schmid B, Buchmann N, Weisser WW, Schulze E-D (2005) Overyielding in experimental grassland communities—irrespective of species pool or spatial scale. *Ecol Lett* 8:419–429
- Roscher C, Schumacher J, Weisser WW, Schmid B, Schulze E-D (2007) Detecting the role of individual species for overyielding in experimental grassland communities composed of potentially dominant species. *Oecologia* 154:535–549
- Roscher C, Scherer-Lorenzen M, Schumacher J, Temperton VM, Buchmann N, Schulze E-D (2011) Plant resource-use characteristics as predictors for species contribution to community biomass in experimental grasslands. *Perspect Plant Ecol Evol Syst* 13:1–13
- Schwinning S, Weiner J (1998) Mechanisms determining the degree of size asymmetry in competition among plants. *Oecologia* 113:447–455
- Spehn EM, Hector A, Joshi J, Scherer-Lorenzen M, Schmid B, Bazeley-White E, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Högberg P, Huss-Danell K, Jumpponen A, Koricheva J, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Palmborg C, Pereira JS, Pfisterer AB, Prinz A, Read DJ, Schulze E-D, Siamantziouras A-SD, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH (2005) Ecosystem effects of biodiversity manipulations in European grasslands. *Ecol Monogr* 75:37–63
- Stitt M, Schulze E-D (1994) Plant growth, storage and resource allocation: from flux control in a metabolic chain to the whole-plant level. In: Schulze E-D (ed) Flux control in biological systems: from enzymes to populations and ecosystems. Academic, San Diego, pp 57–118
- Tilman D (1988) Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton
- Tilman D, Knops J, Wedin D, Reich P, Ritchie M, Siemann E (1997) The influence of functional diversity and composition on ecosystem processes. *Science* 277:1300–1302
- van Ruijven J, Berendse F (2003) Positive effects of plant species diversity on productivity in the absence of legumes. *Ecol Lett* 6:170–175

- Vitousek PM, Howarth RW (1991) Nitrogen limitation on land use and in the sea: how can it occur? *Biogeochemistry* 13:87–115
- Wacker L, Baudois O, Eichenberger-Glinz S, Schmid B (2009) Diversity effects in early- and mid-successional species pools along a nitrogen gradient. *Ecology* 90:637–648
- Wang C, Wan S, Xing X, Zhang L, Han X (2006) Temperature and soil moisture interactively affected soil net N mineralization in temperate grasslands in Northern China. *Soil Biol Biochem* 5:1101–1110