NO EVIDENCE FOR A POSITIVE RESPONSE OF BIOMASS PRODUCTION TO SPECIES DIVERSITY IN AN EXPERIMENTAL GRASSLAND

ABSTRACT: Plant diversity is generally thought to enhance productivity, driven by either (1) chance inclusion of highly productive species in more diverse communities or (2) niche-based resource acquisition with competitive interactions increasing resource use efficiency. Here, we ask whether weeding, as employed in most experiments to date, might contribute to the positive diversity-productivity relationship reported for many grasslands. Using all 82 species from our local pool, we constructed 357 experimental grassland plots (2 × 4 m each), arranged as a completely randomized experiment in an arable field prepared to minimize existing seed bank. The plots were sown to vary species richness (1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35 or 40 species) and were maintained under both ambient conditions and experimental drought. A single monoculture plot was maintained for all 82 species, and each of the other eleven species richness levels was replicated 25 times. Plots were maintained strictly without weeding, and aboveground biomass was measured at 17, 19, 27 and 29 months after the start of this experiment. No single measure of biodiversity was significantly correlated with productivity consistently across all four sampling periods. Furthermore, there were only weak overall effects of six biodiversity variables (the species richness planted, observed, and sampled; Shannon diversity, effective species richness and evenness in the sampled area) on productivity under either precipitation treatment. Regression analysis identified no equation that used a consistent subset of the biodiversity measures as predictors. In view of these transient and insubstantial effects, results from previous experiments that employed weeding treatments are suspect as tests of the hypothesis that biodiversity has positive effects on productivity.

KEY WORDS: biodiversity; diversity-productivity; drought treatment; grassland; non-weeding treatment.

1. INTRODUCTION

The relationship between plant diversity and productivity (a basic measure of ecosystem properties), has become an important objective of ecological research during the last two decades and has been intimately associated with the high-profile discussions of biodiversity (e.g., Tilman et al. 1997, Hector et al. 1999, Tilman et al. 2001, van Ruijver and Berendse 2005, 2009, Li et al. 2010, Maron et al. 2011, Schnitzer et al. 2011). These discussions help frame the scientific basis for biodiversity conservation measures to reduce the unnaturally rapid extinction rates caused by human activities (Chapin et al. 2000, Mouquet et al. 2002). Many experimental and theoretical studies...
underscore the importance of biodiversity conservation (Schwartz et al. 2000, Hector et al. 2001), but among these, demonstrations that greater plant diversity is associated with greater productivity have generated considerable debate (Grime 1997, Huston et al. 2000, Kaiser 2000). Different patterns have been reported, including negative, neutral, U-shaped and hump-shaped relationships between diversity and productivity, although a positive relationship has been commonly reported (reviewed in Schläpfer and Schmid 1999, Mittelbach et al. 2001, Hooper et al. 2005). Several potential problems with previous studies, however, lead us to question the reliability of positive productivity-diversity relationships derived from manipulative field experiments.

Two general mechanisms are thought to drive positive relationships between biodiversity and productivity. First, the 'sampling (or selection) effect hypothesis' (Huston 1997, Loreau and Hector 2001, Wardle 2001, Roscher et al. 2009, van Ruijver and Berendse 2009) suggests that species-rich communities have a higher chance of containing a highly productive species that dominate the community, or species with strong ability to suppress colonization of other species. Second, under the 'complementarity effect hypothesis', high-diversity communities provide on average a greater variety of resource capture characteristics, leading to more complete use of available resources, increasing biomass production (Knops et al. 1999, Loreau and Hector 2001, Tilman et al. 2001, Roscher et al. 2009).

However, artificially assembled plant communities employed in experiments to test these hypotheses and document the positive diversity – productivity relationship generally need frequent weeding to maintain species richness at planned levels (e.g., Tilman et al. 1996, Hector et al. 1999, Pfisterer and Schmid 2002, Polley et al. 2003, Hooper and Dukes 2004, Roscher et al. 2005, van Ruijver and Berendse 2005, Marquard et al. 2009, Maron et al. 2011). More weeding is required in low diversity plots for a number of reasons, including the greater availability of potential new colonists for low diversity plots. Higher weeding disturbance, especially in monocultures, could affect the growth of sown species more negatively than in high-diversity plots (Huston et al. 2000, Wardle 2001) and thus affect the biodiversity-productivity relationship. Under such conditions the observed positive effects of biodiversity on productivity in manipulative field experiments is potentially overestimated because of the confounding effects of diversity - dependent weeding disturbance.

Biodiversity of a plant community may be characterized in different spatial and temporal scales, and thus patterns of diversity-productivity relationship may depend on the choice of diversity variables. For example, analyses that use diversity as originally sown or later realized will likely generate different conclusions, even though sown and realized diversity may be positively related (e.g., Tilman et al. 2001, Wang et al. 2007). Similarly, with respect to spatial scale, the diversity-productivity relationship may depend on whether whole community diversity that of particular samples is used. The diversity-productivity relationship may also be influenced by choice of diversity measure (e.g., species richness, Shannon index, effective species richness, evenness, etc.). These potential influences have not been well considered in previous studies of the diversity-productivity relationship.

In this paper we use empirical data from a previous experiment (Wang et al. 2007) to examine the relationship between biodiversity and un-weeded community productivity at two water levels (natural rain and to the experimental drought). In order to examine potential effects of diversity measure on the relationship we inspect the relationships achieved using six different measures.

2. STUDY AREA

This experiment was conducted on an arable field near Heishiding Nature Reserve, in Fengkai County, Guangdong Province, China (111°53’ E, 23°26’ N, 70 m a.s.l.) during 2004–2006. Mean annual air temperature was 21.5°C, and mean annual precipitation amounted to 1554.1 mm over the three-year course of the experiment. The topsoil of the site was sandy loam.
3. METHODS

3.1. Experimental design

In a rectangular field (about 70 × 100 m), burned completely and fenced in December 2003, we removed the upper 10–15 cm of soil by bulldozer to reduce the seed bank. In early spring 2004 we established 357 experimental plots (each 2 × 4 m size) separated from each other by a distance of 1 m. We seeded the plots to have 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35 or 40 plant species in a completely randomized design, using all 82 species (seeds were collected locally) from our local species pool. Each of the 82 species was seeded in monoculture without replication, but the other eleven species richness levels were replicated 25 times. At each level of diversity, replicate comprised random and independent combinations of species from the species pool. Plots were sown with a total 10 g seeds per m² divided evenly by weight among the species in mixtures. The species pool included 11 legumes, 18 grasses and 53 nonlegume forbs and semi-shrubs (see Wang et al. 2007 and Wang et al. 2010 for details of design and methods).

After 25 June 2005, we divided each of the 357 plots equally into two 2 × 2 m size subplots. One was held under ambient field conditions (control subplot), while a drought treatment was applied to the other (drought subplot). Drought treatments were applied in adjacent rows by covering the drought treatment subplots with a transparent bow-shaped PVC roof to keep out rain. Roofs were 5-m wide with the height ca 1.5 m at the edges and 2 m in the middle. A 20 × 20 cm (deep × wide) ditch was excavated around sheltered area to minimize water uptake by plants in the drought treatments. The roofs reduced light intensity by 15–18%. On 25 June 2006 we removed the PVC roofs and stopped the drought treatment. Over the 1-year drought interval, the unsheltered subplots received 1688.4 mm of natural rain. After establishment, all plots were left undisturbed, without weeding so that the communities developed naturally.

Our analysis is based on data of aboveground plant living biomass. Since most were annuals, with aboveground tissue being newly produced within a single growing season, aboveground living biomass may be used as an index of primary productivity. During the drought stress period aboveground living biomass in each subplot was measured on the following harvest dates: 25–31 August 2005, 25–29 October 2005 and 25–29 June 2006. After removal of the roofs, aboveground living biomass was measured a fourth time on 25–29 August 2006.

To obtain the measurements, we first recorded total species richness of each subplot by direct assessment by trained observers, and then sampled the aboveground living biomass in each subplot by harvesting a strip of 30 × 200 cm to the soil surface. This sample was sorted to species, dried and then weighed separately. At each biomass assessment, two parallel sampling strips were established in 8 plots > 0.5 m from the drainage ditch in both unsheltered and sheltered subplot. Location of paired sampling strips was moved for each harvest to avoid overlap.

3.2. Data analysis

We recorded six different measures of biodiversity for each subplot:

(1) The species richness planted or sown in a 2 × 4 m plot (SR_{planted});
(2) The number of species visually observed in a 2 × 2 m subplot (SR_{observed}).

Following measures of biodiversity based on each sampled strip (30 × 200 cm):

(3) Species richness/0.6 m² sampled area (SR_{sampled});
(4) Shannon diversity in the sampled area of 0.6 m² clip strip (H_{sampled} = ∑(Pi)log₂(Pi)), where P_i is the proportional biomass of species i;
(5) Effective species richness 2^H_{sampled}, where H is Shannon diversity index of the 0.6 m² clip strip;
(6) Pielou’s evenness index (J_{sampled}), where J_{sampled} = H_{sampled} / log₂(SR_{sampled}).

Except for SR_{planted}, the other five measures of biodiversity for each subplot were recorded at all four harvest dates.

We used linear regression analysis to assess how variation in aboveground biomass was related to variation in biodiversity. We modelled the behaviour of aboveground biomass as a response variable in terms of
SR_{planted}, SR_{observed}, SR_{sampled}, H_{sampled}, 2H_{sampled} and J_{sampled} entered as independent, predictor, explanatory or covariate variables.

Analyses were conducted as follows. We first used simple linear regression models to examine the singular effect of each of the six measures of biodiversity on aboveground biomass. Next, attempted to model aboveground biomass through multiple linear regression, using all six biodiversity. Finally, we used a forward stepwise selection procedure to combine significant ($P<0.05$) variables to identify the simplest model of the relationship. All analyses repeated for both treatment levels and at four harvest dates, using SPSS 16.0 for Windows.

4. RESULTS

Some of the sown plant species failed to establish, particularly in the high-diversity plots, while some low-diversity plots were invaded by other species. Nonetheless, sown and realized species richness was positively correlated for datasets representing both water levels, and at all four sampling times ($P<0.003$ for all).

Using 95% confidence intervals, we compared the slope of the relationship between species diversity (i.e., SR_{planted}, SR_{observed}, SR_{sampled}, H_{sampled}, 2H_{sampled} and J_{sampled}, respectively, see Methods) and aboveground biomass with zero for all four sampling times to visualize behaviour of the relationship over time (Fig. 1). Although all six measures of biodiversity had significant effects on aboveground biomass, effects were not consistent. We found that the effect of biodiversity on aboveground biomass varied between positive or negative and in magnitude over the course of the experiment, but pattern of results differed among measures of biodiversity. Furthermore, the pattern of results was not significantly influenced by drought treatment (Fig. 1).

Despite the prevalence of positive statistical correlations between diversity and biomass in our study, these were generally quite weak. The $r^2$ values over all six biodiversity measures and sampling periods ranged from 0.001 to 0.137 (mean = 0.033, SD = 0.036) under the ambient rainfall treatment, and from 0 to 0.126 (mean = 0.030, SD = 0.034) under the drought treatment (Table 1). Thus, biodiversity explained less than 13.7% of the variation in aboveground biomass in our experiment. The results suggest that none of the six measures of biodiversity provide reliable single predictors of aboveground biomass in these grassland plant communities.

Multiple regression analyses explained only a little more of the variation by including several driving variables. Values of $R^2$ ranged from 0.084 to 0.190 in control conditions, and from 0.046 to 0.159 under drought conditions (Table 2).

When we modelled aboveground biomass by forcing all six measures of biodiversity into the model (Table 3), no more than one standard regression coefficient in the multiple regression equation ever differed significantly from zero. Thus, one diversity variable was always sufficient to encompass any significant effect on aboveground biomass. When modelled using three somewhat complementary variables (SR_{planted}, SR_{observed} and SR_{sampled}), regression coefficients were significant

<table>
<thead>
<tr>
<th>Species diversity</th>
<th>Control</th>
<th>Drought</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r^2$ (range)</td>
<td>$r^2$ (mean ± SD)</td>
</tr>
<tr>
<td>SR_{planted}</td>
<td>0.007 ~ 0.056</td>
<td>0.028 ± 0.021</td>
</tr>
<tr>
<td>SR_{observed}</td>
<td>0.001 ~ 0.018</td>
<td>0.018 ± 0.023</td>
</tr>
<tr>
<td>SR_{sampled}</td>
<td>0.001 ~ 0.051</td>
<td>0.018 ± 0.024</td>
</tr>
<tr>
<td>H_{sampled}</td>
<td>0.004 ~ 0.097</td>
<td>0.040 ± 0.044</td>
</tr>
<tr>
<td>2H_{sampled}</td>
<td>0.002 ~ 0.072</td>
<td>0.033 ± 0.032</td>
</tr>
<tr>
<td>J_{sampled}</td>
<td>0.005 ~ 0.137</td>
<td>0.063 ± 0.059</td>
</tr>
</tbody>
</table>
Effects of species diversity on productivity

at only few sampling times for both control and drought conditions. When diversity was modelled in terms of three common indices \( H_{\text{sampled}} \), \( 2H_{\text{sampled}} \) and \( J_{\text{sampled}} \), the standard regression coefficients never significantly different from zero. Thus, no single measure of biodiversity had consistent predictive power for aboveground biomass.

When we reduced the complexity of the regression equation by eliminating one or more biodiversity variables by stepwise elimination, none of the six measures of biodiversity was consistently retained as significant variable under any treatment combination (Table 4).

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**Table 2.** Analyses, using multiple regression models, of the effects of \( SR_{\text{planted}} \), \( SR_{\text{observed}} \), \( SR_{\text{sampled}} \), \( H_{\text{sampled}} \), \( 2H_{\text{sampled}} \) and \( J_{\text{sampled}} \) (see Fig. 1 and text) on aboveground biomass. The squared multiple correlation coefficients \( R^2 \), \( F \) values and the levels of significance \( (P) \) were shown, for control and drought conditions, respectively, at each of the four sampling times. \( N = 357 \). Overall model df = 6 and error df = 350.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Harvest time</th>
<th>Control</th>
<th>Drought</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( R^2 )</td>
<td>( F ) values</td>
<td>( P )</td>
</tr>
<tr>
<td>Biomass</td>
<td>08/2005</td>
<td>0.190</td>
<td>13.61</td>
</tr>
<tr>
<td></td>
<td>10/2005</td>
<td>0.105</td>
<td>6.85</td>
</tr>
<tr>
<td></td>
<td>06/2006</td>
<td>0.084</td>
<td>5.33</td>
</tr>
<tr>
<td></td>
<td>08/2006</td>
<td>0.097</td>
<td>6.23</td>
</tr>
</tbody>
</table>

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**Table 3.** Multiple regressions for the dependence of aboveground biomass on the six biodiversity variables \( SR_{\text{planted}} \), \( SR_{\text{observed}} \), \( SR_{\text{sampled}} \), \( H_{\text{sampled}} \), \( 2H_{\text{sampled}} \) and \( J_{\text{sampled}} \) (see Fig. 1 and text). The standard regression coefficients and the levels of significance were shown, for control and drought conditions, respectively, at each of the four sampling times, where *: \( P \leq 0.05 \), **: \( P < 0.01 \), ***: \( P < 0.001 \). \( N = 357 \). Overall model df = 6 and error df = 350.

<table>
<thead>
<tr>
<th>Species diversity</th>
<th>Control</th>
<th>Drought</th>
</tr>
</thead>
<tbody>
<tr>
<td>( SR_{\text{planted}} )</td>
<td>0.20***</td>
<td>0.06</td>
</tr>
<tr>
<td>( SR_{\text{observed}} )</td>
<td>0.13</td>
<td>0.18</td>
</tr>
<tr>
<td>( SR_{\text{sampled}} )</td>
<td>-0.36</td>
<td>-0.19</td>
</tr>
<tr>
<td>( H_{\text{sampled}} )</td>
<td>-0.04</td>
<td>-0.10</td>
</tr>
<tr>
<td>( 2H_{\text{sampled}} )</td>
<td>-0.12</td>
<td>-0.10</td>
</tr>
</tbody>
</table>

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**Table 4.** Multiple regressions for the dependence of aboveground biomass on the biodiversity variables derived by stepwise selection from a model that initially contained the six biodiversity variables \( SR_{\text{planted}} \), \( SR_{\text{observed}} \), \( SR_{\text{sampled}} \), \( H_{\text{sampled}} \), \( 2H_{\text{sampled}} \) and \( J_{\text{sampled}} \) (see Fig. 1 and text). The partial correlations coefficients and the levels of significance were shown, for control and drought conditions, respectively, at each of the four sampling times, where *: \( P \leq 0.05 \), **: \( P < 0.01 \), ***: \( P < 0.001 \). \( N = 357 \). Overall model df = 6 and error df = 350.

<table>
<thead>
<tr>
<th>Species diversity</th>
<th>Control</th>
<th>Drought</th>
</tr>
</thead>
<tbody>
<tr>
<td>( SR_{\text{planted}} )</td>
<td>+0.23***</td>
<td>~</td>
</tr>
<tr>
<td>( SR_{\text{observed}} )</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>( SR_{\text{sampled}} )</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>( H_{\text{sampled}} )</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>( 2H_{\text{sampled}} )</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>( J_{\text{sampled}} )</td>
<td>-0.37***</td>
<td>-0.29***</td>
</tr>
</tbody>
</table>
Fig. 1. The slope (mean with 95% CI) relating community aboveground biomass to species diversity, are graphed against the months of experiment for two water levels, natural rain and experimental drought. The species diversity indices used are: (a) the species richness planted ($SR_{\text{planted}}$) in a 2 × 4 m plot; (b) the actual species richness visually observed ($SR_{\text{observed}}$) in a 2 × 2 m subplot; (c) species richness / 0.6 m² sampled area ($SR_{\text{sampled}}$); (d) Shannon diversity in the sampled area of 0.6 m² clip strip ($H_{\text{sampled}}$); (e) effective species richness $2^{H_{\text{sampled}}}$, where $H$ is Shannon diversity index; and (f) Pielou's evenness index $J_{\text{sampled}}$. Dashed reference line refers to zero, that is, no relationship between species diversity and community biomass.
5. DISCUSSION AND CONCLUSIONS

This study explored the dependence of aboveground biomass on biodiversity in field experiments at two different precipitation levels and using six different measures of species diversity. Unlike most previous studies that have found predictive relationships between biodiversity and productivity, however, weeding treatments were not applied and thus soil perturbation and soil nutrient loss were minimized. Under these conditions we did not find strong relationships between plant biodiversity and productivity of a grassland ecosystem.

None of six commonly used measures of biodiversity was consistently and significantly correlated with productivity, under either ambient rainfall or drought conditions. Even multiple regression models employing the best combinations of the biodiversity variables as drivers demonstrated little and only weak dependence of productivity on biodiversity. Moreover, no consistent subset of biodiversity variables gave a reasonable predictive equation. Overall, the effects of biodiversity on productivity were transient and insubstantial in our study, and thus add no evidence to support the biodiversity-productivity hypothesis.

Various researchers have sought mechanisms responsible for the positive diversity-productivity relationship, invoking sampling effect, positive species interactions and differential resource use to explain high productivity within species-rich plant communities (Huston 1997, Mulder et al. 2001, Cardinale et al. 2007). It has also been suggested that local ecological processes could reduce productivity in species-poor assemblages (e.g., the effect of diversity-dependent disease on plant productivity; Maron et al. 2011, Schnitzer et al. 2011). Until our study none have considered whether perturbations caused by weeding could be a major driver suggesting positive relationship between diversity and biodiversity in grasslands.

In general, low-diversity plant communities are more prone to invasion than high-diversity communities (Knops et al. 1999, Lepš et al. 2001, Pfisterer et al. 2004, Roscher et al. 2009), and the biomass of invading species is often negatively correlated with sown species richness (Roscher et al. 2009). Thus, the soil nutrient loss induced by removing biomass of invading species will be more severe in low-diversity plots than in high-diversity plots. In addition, soil perturbation caused by weeding may directly affect the growth of sown species. Combination of these two diversity-dependent processes might lead to greater reduction in biomass at low versus high diversity, contributing to an apparent positive relationship between biodiversity and productivity that is an artefact of weeding. As a consequence, the strong and consistently positive biodiversity-productivity relationship observed in many field experiments with hand-weeding to maintain composition of experimental communities and biodiversity gradients (e.g., Tilman et al. 2001, van Ruijver and Berendse 2009), may have been caused by differences between in weeding intensity among biodiversity treatments.

The slope of the relationship between sown species richness and community biomass at the first harvest was high and then descended steeply under both precipitation treatments (Fig. 1a). This suggests that ‘sampling effect’ or ‘selection probability effect’ likely played only a transient role in affecting the productivity of plant communities in the initial stage of the experiment.

Pfisterer et al. (2004) found that diversity-productivity relationships were positive when all unsown species were weeded out of experimental grassland communities, however, the positive relationship nearly disappeared when weeding ceased. A comparison study between weeding treatments with field experimental grasslands in “The Jena Experiment” in Germany (Roscher et al. 2009), had also shown that the positive relationship between total species richness (sown plus colonising species) and total aboveground biomass (sown plus colonising species) decayed in the 3rd year of the experiment, regardless of weeding treatment. Thus, the results reported here support those of Pfisterer et al. (2004) and Roscher et al. (2009) in showing that weeding treatment can strongly influence the diversity-productivity relationship in grassland ecosystems. Results to date are insufficient to support strong inference about whether biodiversity has positive effects on
biomass production. In particular, more rigorous experimental designs and analysis are needed to separate the potential effects of weeding on biomass production, from the effects of biodiversity on the variability of the biomass production.

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6. REFERENCES


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