

Complex facilitation and competition in a temperate grassland: loss of plant diversity and elevated CO₂ have divergent and opposite effects on oak establishment

Alexandra Wright · Stefan A. Schnitzer · Ian A. Dickie ·
Alex R. Gunderson · Gabriella A. Pinter · Scott A. Mangan ·
Peter B. Reich

Received: 4 January 2012 / Accepted: 16 July 2012 / Published online: 5 August 2012
© Springer-Verlag 2012

Abstract Encroachment of woody vegetation into grasslands is a widespread phenomenon that alters plant community composition and ecosystem function. Woody encroachment is often the result of fire suppression, but it may also be related to changes in resource availability associated with global environmental change. We tested the relative strength of three important global change factors (CO₂ enrichment, nitrogen deposition, and loss of herbaceous plant diversity) on the first 3 years of bur oak (*Quercus macrocarpa*) seedling performance in a field

experiment in central Minnesota, USA. We found that loss of plant diversity decreased initial oak survival but increased overall oak growth. Conversely, elevated CO₂ increased initial oak seedling survival and reduced overall growth, especially at low levels of diversity. Nitrogen deposition surprisingly had no net effect on survival or growth. The magnitude of these effects indicates that long-term woody encroachment trends may be most strongly associated with those few individuals that survive, but grow much larger in lower diversity patches. Further, while the CO₂ results and the species richness results appear to describe opposing trends, this is due only to the fact that the natural drivers are moving in opposite directions (decreasing species richness and increasing CO₂). Interestingly, the mechanisms that underlie both patterns are very similar, increased CO₂ and increased species richness both increase herbaceous biomass which (1) increases belowground competition for resources and (2) increases facilitation of early plant survival under a more diverse plant canopy; in other words, both competition and facilitation help determine community composition in these grasslands.

Communicated by Scott Collins.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-012-2420-y) contains supplementary material, which is available to authorized users.

A. Wright (✉) · S. A. Schnitzer · S. A. Mangan
Department of Biological Sciences,
University of Wisconsin–Milwaukee, Lapham Hall,
3209 N. Maryland Ave., Milwaukee, WI 53211, USA
e-mail: ajwright@uwm.edu

I. A. Dickie
Landcare Research, Lincoln, New Zealand

A. R. Gunderson
Biology Department, Duke University, Durham, NC, USA

G. A. Pinter
Department of Mathematical Sciences,
University of Wisconsin–Milwaukee, Milwaukee, USA

P. B. Reich
Department of Forest Resources,
University of Minnesota, St. Paul, MN, USA

P. B. Reich
Hawkesbury Institute for the Environment,
University of Western Sydney, Penrith, NSW 2751, Australia

Keywords Biodiversity · Global change · Ontogeny ·
Stress · Woody encroachment

Introduction

Encroachment of woody vegetation into grassland communities and the resultant conversion of these communities into closed canopy forests is an increasingly common phenomenon (Archer 1989; Van Auken 2000; Peterson and Reich 2001; Roques et al. 2001; Silva et al. 2001; Fensham et al. 2005), and grassland-dominated landscapes are

increasingly rare globally (Hoekstra et al. 2004). Species compositional shifts caused by woody encroachment into grasslands have important implications for community dynamics and ecosystem properties, such as carbon (C) storage and nitrogen (N) cycling (Post et al. 1982; Reich et al. 2001b; Jackson et al. 2002; McCulley et al. 2004; Knapp et al. 2008; McKinley and Blair 2008; Barger et al. 2011). While regional distributions of woody plants appear to be co-constrained by large-scale differences in precipitation and fire regimes (Staver et al. 2011), local success of woody plants in grasslands is strongly associated with a change in disturbance regime that favors woody plants over herbaceous species, such as fire suppression (Van Auken 2000; Roques et al. 2001; Silva et al. 2001) or increased grazing pressures by cattle (Archer et al. 1995; Brown and Archer 1999). However, recent evidence suggests that altered resource availability associated with global environmental changes [e.g., decreasing biodiversity and increasing atmospheric carbon dioxide (CO₂), and N deposition] may also drive woody encroachment into grasslands; the role of these factors and their interactions remain, however, poorly understood (Archer et al. 1995; Van Auken and Bush 1997; Polley et al. 2003; Davis et al. 1999; Dickie et al. 2007; Classen et al. 2010).

Increased global extinction rates and loss of biodiversity (e.g., Vitousek et al. 1997) may substantially alter grassland community composition. Specifically, loss of plant diversity at the patch scale may affect how plant species interact in positive (e.g., facilitation) and negative (e.g., competitive) ways; we outline these changes below.

Competition The relationship between diversity and productivity is complex and feeds back on itself at different spatial scales (Bengtsson et al. 2002). For example, macro-scale plant species richness patterns are a function of regional productivity gradients (Abrams 1995; Chase and Ryberg 2004; but see Adler et al. 2011). Conversely, at local scales, increasing levels of plant diversity drive increased production of biomass (Tilman et al. 2001; Reich et al. 2001a, 2012; van Ruijven and Berendse 2003; Roscher et al. 2005; Isbell et al. 2011; Zhang et al. 2012) because higher diversity communities contain a larger number of species with unique traits (Reich et al. 2012) and competition strategies. As increasing numbers of species co-occur in an assemblage, their complementary resource acquisition strategies use overall resource pools more completely. This complementary resource use results in greater community-level biomass (Tilman et al. 1997b) and, consequently, the community itself becomes less susceptible to colonization (Kennedy et al. 2002).

For the purposes of this study, we focus on the well-established positive relationship between local species richness and biomass production (Tilman et al. 2001; Reich

et al. 2001a; van Ruijven and Berendse 2003; Roscher et al. 2005; Isbell et al. 2011; Zhang et al. 2012). We predict that when applied to woody encroachment into grasslands, declining levels of herbaceous species diversity associated with global change can drive lower levels of biomass production (Schnitzer et al. 2011) and decreased competition for resources (Tilman et al. 1997a, b). Decreased plant diversity may therefore lead to increased susceptibility to woody encroachment (Naeem et al. 2000; Kennedy et al. 2002; Fargione and Tilman 2005).

Facilitation Loss of herbaceous species diversity may also alter facilitative interactions between plants. Survival rates of young plants tend to increase with increasing canopy cover, particularly in ecosystems that experience extreme abiotic conditions (Bertness and Callaway 1994; Miriti 2006; Cuesta et al. 2010; Bustamente-Sanchez et al. 2011; Farrer and Goldberg 2011). This facilitation effect is due to amelioration of the microclimate under higher density canopies (Callaway 1995; Callaway and Walker 1997; Cuesta et al. 2010; Bustamente-Sanchez et al. 2011). The strength of facilitation likely increases with increasing species diversity because higher diversity assemblages usually have increased canopy cover (Tilman et al. 2001). However, there is little empirical evidence to support a direct relationship between plant diversity and facilitation (but see Bruno et al. 2003 and Bulleri et al. 2008 for theoretical discussion).

Other global change factors, such as increased CO₂ and N deposition, also alter resource availability and can have interactive effects on woody encroachment into grasslands. For example, elevated atmospheric CO₂ appears to benefit woody seedlings grown alone in water-limited environments (Davis et al. 2007) and may help explain past woody range expansions (Kgope et al. 2009), particularly in the presence of fire (Bond and Midgley 2000). Elevated CO₂ increases plant water use efficiency (WUE), which should increase soil water availability (Polley et al. 2003; Reich 2009; Adair et al. 2011). Because woody plant establishment is often restricted by low soil water availability (Staver et al. 2011), an increase in soil moisture due to increased WUE may stimulate woody encroachment into grasslands. However, when soil water is limiting, increased soil water availability should also influence the growth of herbaceous species, although to our knowledge no study has assessed how CO₂ and herbaceous species richness simultaneously affect woody–herbaceous plant interactions. Finally, recent research has shown that past increases in atmospheric CO₂ concentrations may have strongly controlled woody encroachment into grasslands when atmospheric CO₂ was relatively low (180 ppm) but that CO₂ may not be such a strong driver of woody success under current conditions (approx. 370 ppm at the time of this experiment; Kgope et al. 2009).

N deposition often increases aboveground herbaceous productivity (Reich et al. 2001a, c) and reduces both light and soil water availability in herbaceous vegetation (Tilman 1987). The effect of N deposition may limit oak establishment due to increased competition for light and/or water. Indeed, these indirect effects of N addition reduce woody plant growth and survival in grasslands in central Minnesota (Davis et al. 1998, 1999), and this effect depends on herbaceous productivity (Dickie et al. 2007). To date, most studies have examined woody encroachment into grasslands by manipulating only one or two factors in isolation, and the interacting effects of these global change factors, while potentially substantial, are largely unknown.

We examined the simultaneous roles of herbaceous species richness, elevated CO₂, and N enrichment on bur oak seedling survival and growth in Minnesota, USA. Bur oaks (*Quercus macrocarpa*) and pin oaks (*Q. ellipsoidalis*) are common woody colonizers in grasslands in this region and are therefore appropriate candidates to address general trends in woody encroachment. We tested the following four hypotheses: (1) decreasing herbaceous species diversity decreases oak survival rates due to loss of the facilitative effects of a more diverse, higher canopy cover plant community; (2) decreasing herbaceous diversity increases resource availability and therefore promotes oak growth due to lack of competition from neighboring plants; (3) CO₂ enrichment increases oak performance (both survival and growth) due to increased soil water availability; (4) N deposition decreases oak performance by increasing herbaceous productivity and thus increasing competition for other limiting resources.

Materials and methods

Study site and experimental design

We conducted this study within the framework of the Biodiversity, CO₂, and N (BioCON) experiment at the Cedar Creek Ecosystem Science Reserve, located in central Minnesota. Soils at this site consist of nutrient-poor glacial outwash sand plain with low water- and nutrient-holding capacity (Reich et al. 2001a). Species richness levels in natural prairie communities at this field site range from approximately four species to 16 species per 0.5-m² plot, and aboveground biomass (AGB) ranges from approximately 50 to 150 g/m² (Knops 2006). Natural communities are dominated by *Schizachyrium* (C₄ grass, accounts for 69–76 % of all aboveground biomass in prairies). Several other species of C₃ grasses are also common (*Poa pratensis*, *Panicum oligosanthos*, and *Agrostis scabra*), and *Rumex acetosella* (forb) and *Andropogon gerardii* (C₄ grass) can be found in high abundances (Knops 2006).

Mean annual precipitation at Cedar Creek is 78 ± 7.5 cm [95 % confidence intervals (CI) for 1982–2009], while mean annual precipitation over the course of our study (2001–2004) was 79.9 ± 12.9 cm (95 % CI, no significant difference in rainfall from long-term average).

The BioCON experiment utilized six circular 20-m diameter plots; three are enriched to 560 μmol mol⁻¹ of CO₂, which is pumped from a ring of PVC tubes using a free air CO₂ enrichment system (FACE), while three control plots (hereafter referred to as “rings”) received approximately 370 μmol mol⁻¹ of CO₂, which was the ambient atmospheric CO₂ level at the time of the experiment. The level of 560 μmol mol⁻¹ of CO₂ was based on International Panel on Climate Change models for projected CO₂ concentrations by the year 2100. Nested within the rings are herbaceous species diversity and N treatments that subdivide the plots into 359 2 × 2-m square subplots. To manipulate species diversity, these plots were planted with 12 g m⁻² of seed of one, four, nine, or 16 species in 1997. Herbaceous species composition in each of these diversity treatments was randomly assigned from a pool of 16 species, representing four species from each of four functional groups (4 C₃ grasses, 4 C₄ grasses, 4 legumes, and 4 non-N fixing herbaceous plants). Species mixes were maintained with planted species only (although not reseeded) using hand weeding. Within each ring, 21–22 subplots were planted using one species, 20–21 were planted with four species, ten subplots with nine species, and eight subplots with 16 species (total of 59–61 subplots per ring, 6 rings). For each diversity level, half of the plots had either N added in dry granules annually (4 g N m⁻¹ year⁻¹ as NH₄NO₃) or no N added.

In each of the 359 plots, we sampled herbaceous biomass (above and belowground) each June and August from 2001 to 2004. Aboveground biomass was clipped in 10 × 100-cm strips at the soil surface and never sampled less than 15 cm from plot boundaries (to avoid edge effects). Belowground biomass was sampled to a depth of 100 cm using three 5-cm cores in the same area as the vegetation clip strips (see Reich et al. 2001c for more details). Biomass sampling was conducted in different areas of plots for every sampling date of this study. We recorded soil volumetric water content monthly between May and October 2001–2004 using time domain reflectometry and percentage light transmission below the canopy monthly between May and October 2001–2004 using a 1-m integrated photosynthetically active radiation sensor (Li-Cor Biosciences, Lincoln, NE). In June and August of 2001 and 2002, we measured available soil N (in the forms of nitrate and ammonium) in each plot by collecting four soil cores at 0–20 cm depth, extracting N using 1 M KCl, and analyzing the N content using an element analyzer (Costech Technologies model 4050; Valencia, CA)

according to Dijkstra et al. (2005). All measurements taken over the course of this experiment (above- and below-ground biomass, soil moisture, percentage light transmission, and soil N) were averaged across all sampling dates in order to obtain a single plot-level average over time.

Oak performance

In October 2001, we collected and germinated recently fallen acorns from multiple local adult bur oak trees at the Cedar Creek Ecosystem Science Reserve. We combined and homogenized the acorns and planted three germinated seeds in each of the 359 2×2 -m subplots. In June 2002, we recorded oak survival and the number of leaves per plant. In August 2002, we recorded plant survival, height, and the number of leaves per plant. In August 2004, we recorded survival, height, diameter, and the number of leaves per plant and then harvested all surviving individuals. We estimated aboveground oak biomass over the course of the study using an allometric relationship that we derived using the field measurements and aboveground dry mass from the final harvest [June 2002 AGB (in grams) = $-0.277 + (0.338 \times \text{leaves})$, $r^2 = 0.805$, $P < 2.2e-16$, $n = 227$; August 2002 AGB (in grams) = $-0.837 + (0.112 \times \text{height}) + (0.281 \times \text{leaves})$, $r^2 = 0.835$, $P < 2.2e-16$, $n = 227$].

Statistical analysis

Base models

We analyzed oak survival using a generalized linear mixed-effects model (GLMM) for repeated measures with a multinomial distribution (SAS PROC GLIMMIX; SAS Institute, Cary, NC). In this model, each 4-m^2 plot was the experimental unit, and the probability of survival was based on the number of oak seedlings surviving in each plot (0–3 possible survivors). This base model included N addition, species richness, CO_2 enrichment, and all first order interactions as fixed effects, and ring nested within CO_2 as a random effect (follows Reich et al. 2001a). The three sampling dates were treated as repeated measures, and each 4-m^2 plot was included as the subject. We analyzed growth (AGB of surviving seedlings per 4-m^2 plot) over time using a similarly structured mixed-effects model with a normal distribution (SAS PROC MIXED). Biomass was log-transformed to normalize residuals.

Covariate models

We evaluated the explanatory power of herbaceous biomass, percentage light transmission below the herbaceous vegetation, soil moisture, and soil N concentrations on oak

performance by including these measures as covariates. For both growth and survival, we constructed separate models, including all covariates in isolation as well as all combinations of covariates. We compared these covariate models to the base models described above using Akaike Information Criteria (AIC).

We present AIC scores for all GLMM's in the Electronic Supplementary Material (ESM) and only report on the best-fit model throughout the “Results” and “Discussion”. We use frequentist statistical tools (P values) to better interpret the contribution of each experimental main effect (N, CO_2 , and species richness), as well as interactions between main effects and covariates in this best-fit GLMM. We use this mixed statistical approach as it best utilizes the statistical tools available while still making the results interpretable to the broadest possible audience (Bolker et al. 2009).

Results

There was only one significant pair-wise interaction between main effects in our analyses ($\text{CO}_2 \times$ species richness effect on oak growth). We therefore present the results separately by treatments and discuss the $\text{CO}_2 \times$ species richness interaction at the end of the CO_2 results. There were no significant effects of N treatment on either survival (Table 1) or growth (Table 2), so no further results regarding this treatment are presented.

Table 1 The effects of species richness, carbon dioxide addition, nitrogen addition, and first and second order interactions on oak survival over time

Effect	df^a	F	P
Species richness	3, 342	3.04	0.0293*
Carbon dioxide (CO_2)	1, 4	5.43	0.0803
Nitrogen (N)	1, 342	0.19	0.6623
Species richness \times CO_2	3, 342	1.18	0.3192
Species richness \times N	3, 342	2.45	0.0637
$\text{CO}_2 \times$ N	3, 342	0.12	0.7330
Richness \times $\text{CO}_2 \times$ N	3, 342	0.49	0.6877
Time	2, 342	73.69	<0.0001*
Time \times species richness	6, 342	0.23	0.9681
Time \times CO_2	2, 342	8.56	0.0002*
Time \times N	6, 342	0.14	0.8703

* Significant results at $P < 0.05$

This generalized linear mixed model (GLMM) was fit with a multinomial error distribution

^a Denominator degrees of freedom (df) = 4 for the main effect of CO_2 and reflects the inclusion of $\text{CO}_2 \times$ ring as a random effect

Table 2 The base model (without covariates) and the best-fit model (with covariates) examining the effects of species richness, CO₂ addition, and N addition on oak growth over time

Effect	df ^a	Without covariates		With covariates	
		F	P	F	P
Species richness	3, 205	8.60	<0.0001*	1.85	0.1401
CO ₂	1, 4	2.15	0.2168	0.28	0.6226
N	1, 205	0.35	0.5559	0.25	0.6169
Species richness × CO ₂	3, 205	2.83	0.0395*	2.57	0.0556
Species richness × N	3, 205	0.77	0.5118	1.25	0.2913
CO ₂ × N	1, 205	0.10	0.7478	0.51	0.4755
Richness × CO ₂ × N	3, 205	0.90	0.4433	2.09	0.1032
Time	2, 205	39.98	<0.0001*	40.59	<0.0001*
Time × species richness	6, 205	8.28	<0.0001*	7.98	<0.0001*
Time × CO ₂	2, 205	3.24	0.0412*	2.82	0.0617
Time × N	2, 205	0.52	0.5961	0.54	0.5808
Nitrate	1, 205	–	–	69.45	<0.0001*
Moisture	1, 205	–	–	1.35	0.2468

* Significant results at $P < 0.05$

^a Denominator degrees of freedom are shown for the model including covariates. For the model not including covariates, denominator degrees of freedom for all effects other than CO₂ = 207. In both models, denominator $df = 4$ for the main effect of CO₂ and reflects the inclusion of CO₂ × ring as a random effect

Species richness

The best main-effect predictor of both oak survival and growth was herbaceous species richness (Tables 1, 2, respectively). Oak survival was lowest (34 %) in the monoculture plots and highest (54 and 49 %) in the most species rich plots (9- and 16-species, respectively) (Fig. 1; Table 1). These responses were evident within 8 months of planting the oaks (by June 2002) and did not change significantly between June 2002 and the harvest date in August 2004.

The surviving oak seedlings grew equally well across species richness levels during the first 8 months of the experiment, at which point seedlings in higher diversity treatments nearly stopped growing, while seedlings in single-species plots continued to grow (Table 2). By the final sampling date, oak growth (measured as total aboveground biomass per plant) was more than fourfold greater in herbaceous monoculture plots than in high species richness plots (averaged across CO₂ treatments; Fig. 2).

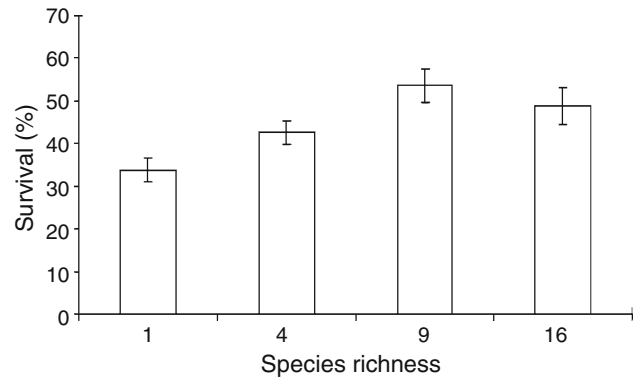


Fig. 1 Proportion of oaks surviving in one-, four-, nine-, and 16-species plots. Bars: mean ± standard error (SE) of the raw proportion survival measurement. Displayed results are averaged across sampling dates. Tukey tests for multiple comparisons between diversity levels were not possible using the generalized linear mixed model (GLMM) with a multinomial distribution in the SAS program; for this reason letters indicating significance between groups are not displayed

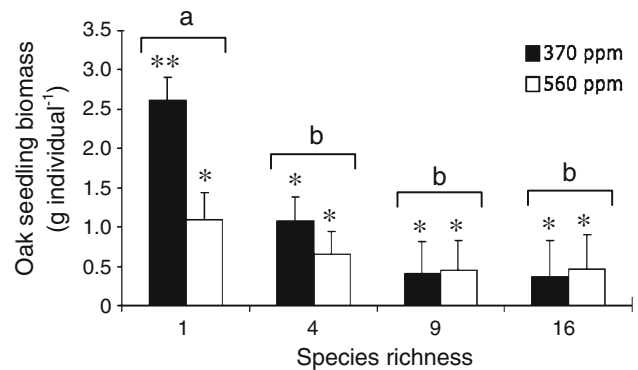


Fig. 2 Final aboveground biomass of oak seedlings in relation to planted species richness at two carbon dioxide (CO₂) levels. Decreased oak growth due to herbaceous diversity was determined using Tukey–Kramer multiple comparisons and is denoted using letters (*a* vs. *b*). Due to significant interactions between CO₂ enrichment and species richness, differences in oak growth at different levels of CO₂ enrichment were also determined using Tukey–Kramer multiple comparisons and are denoted using asterisks (double asterisks vs. asterisks)

Elevated CO₂

Atmospheric CO₂ enrichment had an initial positive effect on oak survival in June 2002, but the strength of the effect was weak by August 2004, resulting in a significant CO₂ enrichment by time interaction on oak survival ($F_{2,342} = 8.56, P = 0.0002$) (Table 1; Fig. 3). There were no interactions between CO₂ and any of the other main effects on oak survival.

CO₂ enrichment had no effect on oak growth rates averaged across sampling dates (Table 2). However, there was a significant CO₂ enrichment by time interaction ($F_{2,205} = 3.24, P = 0.0412$) because seedlings grown in

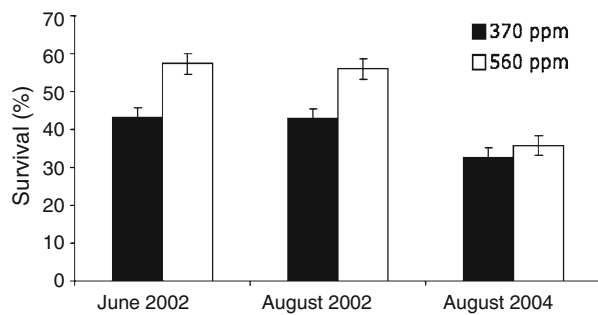


Fig. 3 Oak survival at two CO₂ levels, averaged over the three sampling dates. Bars: mean \pm SE of the raw proportion survival measurement. Tukey tests for multiple comparisons between different levels of CO₂ and time were not possible using the GLMM with a multinomial distribution in the SAS program; for this reason letters indicating significance between groups are not displayed

CO₂ enriched plots grew less than in ambient plots in the latter part of the study (Table 2). There was also a significant species richness \times CO₂ enrichment interaction, driven mostly by the significantly greater growth of oaks in herbaceous monoculture plots under ambient CO₂ conditions (Table 2; Fig. 2). Further, the two-way interactions between species richness and time, CO₂ and time, and species richness and CO₂ indicate that the significantly greater growth in the monoculture plots (one-species) by August 2004 was almost exclusively due to older oaks growing in ambient CO₂ conditions (Fig. 2).

Covariate models

Oak survival The best-fit and most parsimonious model for oak survival was the base model that included only the manipulated factors, with no covariates included (AIC = 18723.08; ESM 1). This finding indicates that none of the variables measured in the experiment either added additional explanatory power beyond that already included by species richness and CO₂ enrichment or better explain survival responses if these measures co-vary with treatments.

Oak growth The best-fit and most parsimonious model for oak growth included soil moisture and extractable soil nitrate as covariates (AIC = -742.6; ESM 1). Soil nitrate explained the largest amount of growth variance, and the combination of reduced soil moisture and reduced soil nitrate explained the reduced oak growth found in high diversity plots, enriched CO₂ plots, and the CO₂ \times time interaction (Table 2).

Discussion

Our results support the hypotheses that loss of plant diversity and atmospheric CO₂ enrichment influence

woody encroachment into grassland ecosystems. Importantly, the CO₂ results and species richness results appear to describe opposing trends because the drivers are moving in opposite directions in the natural world (decreasing species richness and increasing CO₂). However, the mechanisms that underlie both patterns are very similar, with increased CO₂ and increased species richness both increasing herbaceous biomass which (1) increases belowground competition for resources and (2) increases facilitation of early plant survival under a more diverse plant canopy.

Hypotheses 1 and 2: decreased herbaceous species diversity limits early oak survival but promotes oak growth later in development

Our findings demonstrate that herbaceous species diversity facilitates early oak seedling survival. We suggest that this diversity effect is due to the amelioration of plant stress under more diverse herbaceous canopies where temperature, humidity, and shallow soil moisture may be buffered by increased herbaceous cover. For the purposes of our experiment, we measured herbaceous biomass, and found that it did not directly explain a significant proportion of the growth response or the survival response (ESM 1). Although we did not measure the micro-climate variables directly related to facilitation in 2001–2004, we did measure some of them in 2010 in these same plots to better understand our results. This more recent work demonstrated that increasing herbaceous diversity slightly increased surface soil moisture (0–6 cm) in 16-species plots compared with one-species plots and that higher diversity plots were, on average, 1.7 °C cooler than one-species plots. This interpretation (of amelioration of surface stress by higher diversity communities) is further corroborated by results from 2006 in BioCON, where during a dry summer, soil moisture at a depth of 0–17 cm was slightly higher in diverse plots than monocultures, whereas in deeper horizons, increasing diversity reduced soil moisture (Adair et al. 2011). Further work should be conducted in this area to show a direct causal relationship between micro-climate variables and plant facilitation at this site.

Positive facilitative relationships between aboveground cover and seedling survival have been documented in grasslands (Dickie et al. 2007), shrublands (Cuesta et al. 2010), and temperate forest gaps (Montgomery et al. 2010), although evidence for a relationship between herbaceous cover and shrub invasion in southwestern arid grasslands of the USA is varied (Van Auken and Bush 1997; Brown and Archer 1999; Van Auken 2000). In those examples where herbaceous biomass has been found to increase seedling survival, aboveground cover protects small seedlings from

some type of environmental severity (e.g., extreme temperatures, increased surface soil drying, and increased rates of evapotranspiration). Aboveground cover may be particularly important for smaller/younger seedlings, which tend to have less well-developed root systems and less non-structural C reserves to survive short periods of stress (Niinemets 2010). As plants grow in size, their root systems become more developed, and they become less susceptible to short periods of extreme abiotic conditions. Indeed, we found a facilitative effect of herbaceous diversity for small oak survival, but following initial establishment, loss of plant diversity did not further decrease the survival of larger oak seedlings. We predict that this was likely because larger seedlings were less susceptible to environmental stressors.

Surviving oaks in low-diversity plots grew rapidly after the first year, which was due to a greater availability of soil N and soil moisture in the absence of strong competition from neighbors (Table 2). Indeed, soil moisture is one of the most important factors determining regional range limits for woody species (Staver et al. 2011; but see Brown and Archer 1999). In contrast, oaks in higher diversity plots grew very little after the first 8 months because of intense competition for soil resources from herbaceous plants (Table 2). We propose that the increase in competition intensity with oak age is due to greater absolute resource requirements as oaks grow (and decreasing influence of resources from acorns; Ovington and MacRae 1960). Increased resource requirements as plants grow should result in increased growth limitations, which was evident for the oldest plants growing in the highest diversity plots. Soil N is the most limiting resource in grasslands at this field site (Tilman 1987), and limited availability of extractable soil nitrogen (nitrate) was the best covariate predictor of oak growth at each individual time point (Table 2). This resulted in little difference in oak growth between diversity levels at the beginning of the study due to little need for N, but significantly less growth of oaks in high-diversity plots over time.

This fits well with evidence that herbaceous species richness is positively related to biomass production in grasslands (Tilman et al. 2001; Reich et al. 2001a; van Ruijven and Berendse 2003; Roscher et al. 2005; Isbell et al. 2011) and that this often leads to decreased availability of resources at higher levels of plant diversity (Tilman et al. 1997b; Dijkstra et al. 2005; Reich 2009). Further, work on woody encroachment patterns in the semi-arid southwestern USA demonstrates a similar pattern between woody growth limitation and belowground competition for resources. While woody growth does not appear to be significantly affected by aboveground or overall competition in these systems (usually attributed to grazing, not diversity; Brown and Archer 1989; Van Auken

and Bush 1997; Brown and Archer 1999), there is strong evidence that woody seedlings compete heavily with herbaceous species for belowground resources (Van Auken and Bush 1997) and that this may affect woody encroachment patterns.

Hypotheses 3 and 4: CO₂ enrichment increases oak performance due to increased soil water availability and N deposition decreases oak performance due to increased herbaceous productivity and competition for other limiting resources

Contrary to our original hypothesis, our data demonstrate that the effect of CO₂ enrichment on woody encroachment is not consistently positive, but instead also depends on oak ontogenetic stage. Atmospheric CO₂ enrichment had a positive effect on oak survival at the beginning of the study and a negative effect on growth of surviving oaks, especially at low levels of plant diversity. The positive effect of CO₂ enrichment on early oak survival may have been the result of higher herbaceous biomass in high CO₂ plots (12 % higher than in ambient CO₂ plots; Reich et al. 2001a) and amelioration of surface soil moisture in high CO₂ plots (Adair et al. 2011). Although there was no direct effect of herbaceous biomass on oak survival or growth (ESM 1), increased herbaceous cover in elevated CO₂ plots may have reduced abiotic stress for the germinating oak acorns under the denser herbaceous canopy. This pattern also emerged early on in oak development when oaks were smaller and likely more vulnerable to abiotic stressors, such as heat and drought (Niinemets 2010).

Subsequently, the positive CO₂ effect for survival transitioned to competition for resources as the oaks grew. CO₂ enrichment decreased longer term oak growth rates because older seedlings in CO₂-enriched plots experienced more intense competition for resources, which limited oak growth over time. The enhanced oak growth effect was most evident in ambient CO₂-monoculture plots, where competition intensity was lowest due to low competition from both low herbaceous diversity and a lack of CO₂ fertilization (which in combination resulted in lower herbaceous biomass than in plots with higher diversity or elevated CO₂).

The negative effect of CO₂ enrichment on oak growth is surprising and important in the context of recent work on the singular effect of CO₂ enrichment on woody encroachment (Bond and Midgley 2000; Polley et al. 2003; Davis et al. 2007; Staver et al. 2011). These recent studies suggest that CO₂ enrichment should have positive effects on overall trends in woody encroachment (Davis et al. 2007) due to enhanced WUE (Polley et al. 2003) and subsequent access to limiting soil moisture (Staver et al. 2011), as well as to increased total C availability for woody

species that need to invest large amounts of energy towards re-sprouting after fire (Bond and Midgley 2000). Our results suggest that as oaks encroach into grasslands, herbaceous species may respond quickly to increased CO₂ enrichment and grow more due to the CO₂ fertilization effect. When oaks arrive into higher biomass grasslands following increases in levels of atmospheric CO₂, they may be more limited by competition from greater herbaceous biomass than they are facilitated by CO₂ enrichment (e.g., Davis et al. 2007). We predict that while possibly this has strong implications for how we interpret future trends of woody encroachment in grasslands, past trends in woody encroachment may have been more strongly controlled by increasing levels of atmospheric CO₂ due to the greater response potential at lower levels of CO₂ (Kgope et al. 2009).

Also contrary to our original hypothesis, N addition had no detectable effect on oak survival or growth over time. The reason for this is unclear, but may be in part due to the lower concentrations of N applied compared with similar studies at this site (Davis et al. 1999) and the impact of these on the multiple resource factors that are important (light, water, and N). For example, the BioCON N addition strongly increased soil N pools, but also increased competition for those pools (Reich et al. 2001a; Reich 2009), while also slightly decreasing soil water supply (Reich 2009; Adair et al. 2011) and increasing light transmission (through a compositional shift towards vertical grasses; Reich 2009). It is possible that in terms of impacts on oak performance, increased root competition (Davis et al. 1998, 1999; Reich 2009) and lower soil water content (Reich 2009; Adair et al. 2011) offset possible positive impacts of greater soil N pools and higher light availability (Reich 2009).

Synthesis

Woody encroachment into grassland communities is a widespread phenomenon that drives changes in community and ecosystem-level processes. Our findings demonstrate that the simultaneous loss of herbaceous plant diversity and increasing atmospheric CO₂ concentrations associated with global environmental change will affect oak encroachment into grasslands. Due to the divergent trajectories of species richness and atmospheric CO₂ concentrations (decreasing species richness and increasing CO₂ concentrations), these results indicate that woody encroachment will be affected in divergent ways and that those divergent effects will themselves vary depending upon the oak life stage and process in question.

In this experiment, survival was 15–20 % lower in herbaceous monoculture than in high-diversity plots. Thus,

if local plant diversity is driven to a low level, oak establishment may become increasingly rare. However, atmospheric CO₂ enrichment may counteract this trend; in our experiment, a CO₂ doubling buffered the survival reduction in low-diversity plots and increased initial oak survival by approximately 14 %. The magnitude of species loss paired with the degree of atmospheric CO₂ enrichment will determine the actual outcome of these trends for early oak establishment.

For oaks that establish, however, decreased plant diversity may prove beneficial. We found that surviving oaks grew twice as much in one-species plots than they did in 16-species plots. Oak growth was only modestly counteracted by a CO₂ doubling (17 % growth reduction in lower diversity plots). Further, the actual conversion of these grasslands into closed canopy forests will depend heavily on oak growth: past research has shown that woody encroachment rates are strongly controlled by the ability of oaks to grow into larger size classes and eventually escape from under the herbaceous canopy (Bond and Midgley 2000). The strong growth advantage of oaks growing in lower diversity plots, regardless of CO₂ concentrations, suggests that loss of species richness may have an overall positive effect on woody encroachment in lower diversity grassland patches.

In a broader theoretical context, our findings support the notion that both competition and facilitation are operating in these plant communities. Young plants are particularly sensitive to severe environmental conditions and thus benefit from the facilitative effects of higher total community biomass, which can ameliorate harsh environmental conditions. As plants grow and become less susceptible to environmental stress in terms of survival, however, the positive effects of facilitation diminish and resource competition becomes a stronger determinant of plant growth and, hence, longer term plant performance. Thus, overall community composition in these grasslands may be structured by a balance that promotes the establishment of colonizers in areas of high plant diversity through facilitation, but this higher diversity later limits overall growth through increased interspecific competition.

Acknowledgments This research was supported by the Department of Energy Program for Ecological Research Grant DE-FG02-96ER62291, the National Science Foundation Long-term Ecological Research Grant DEB-0080382, the NSF Long-term Research in Environmental Biology DEB-0716587, the University of Minnesota, the University of Wisconsin–Milwaukee (UWM) AOP program, the UWM Department of Biological Sciences, and the NSF Graduate Research Fellowship Program. IAD was additionally supported by the New Zealand Ministry of Science and Innovation (Ecosystem Resilience OBI). We thank Sarah Christman and all other Cedar Creek interns for field assistance, and Joe Mascaro and Ramesh Laungani for helpful comments during the writing process. The authors declare

that they have no conflict of interest and that all experiments were conducted in compliance with U.S. law.

References

- Abrams PA (1995) Monotonic or unimodal diversity-productivity gradients: what does competition theory predict? *Ecology* 76:2019–2027
- Adair CE, Reich PB, Trost JJ, Hobbie SE (2011) Elevated CO₂ stimulates grassland soil respiration by increasing carbon inputs rather than by enhancing soil moisture. *Glob Change Biol* 17:3546–3563
- Adler PB, Seabloom EW, Borer ET, Hillebrand H, Hautier Y, Hector A, Harpole WS, O'halloran LR, Grace JB, Anderson TM, Bakker JD, Biederman LA, Brown CS, Buckley YM, Calabrese LB, Chu C-J, Cleland EE, Collins SL, Cottingham KL, Crawley MJ, Damschen EI, Davies KF, Decrappeo NM, Fay PA, Firn J, Frater P, Gasarch EI, Gruner DS, Hagenah N, Hille Ris Lambers J, Humphries H, Jin VL, Kay AD, Kirkman KP, Klein JA, Knops JMH, La Pierre KJ, Lambrinos JG, Li W, Macdougall AS, McCulley RL, Melbourne BA, Mitchell CE, Moore JL, Morgan JW, Mortensen B, Orrock JL, Prober SM, Pyke DA, Risch AC, Schuetz M, Smith MD, Stevens CJ, Sullivan LL, Wang G, Wragg PD, Wright JP, Yang LH (2011) Productivity is a poor predictor of plant species richness. *Science* 333:1750–1753. doi:10.1126/science.1204498
- Archer S (1989) Have southern Texas savannas been converted to woodlands in recent history? *Am Nat* 134:545–561
- Archer S, Schimel D, Holland E (1995) Mechanisms of shrubland expansion: land use, climate or CO₂? *Climatic Change* 29:91–99
- Barger NN, Archer SR, Campbell JL, Huang C-Y, Morton JA, Knapp AK (2011) Woody plant proliferation in North American drylands: a synthesis of impacts on ecosystem carbon balance. *J Geophys Res* 116:G00K07. doi:10.1029/2010JG001506
- Bengtsson J, Engelhardt K, Giller P, Hobbie S, Lawrence D, Levine J, Vila M, Wolters V (2002) Slippin' and slidin' between the scales: the scaling components of biodiversity–ecosystem functioning relations. In: Loreau M, Naeem S, Inchausti P (eds) *Biodiversity and ecosystem functioning synthesis and perspectives*. Oxford University Press, New York, pp 209–220
- Bertness M, Callaway R (1994) Positive interactions in communities. *Trends Ecol Evol* 9:191–193
- Bolker B, Brooks M, Clark C, Geange S, Poulsen J, Stevens M, White J (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135
- Bond WJ, Midgley GF (2000) A proposed CO₂-controlled mechanism of woody plant invasion in grasslands and savannas. *Glob Change Biol* 6:865–869
- Brown J, Archer S (1989) Woody plant invasion of grasslands: establishment of honey mesquite (*Prosopis glandulosa* var. *glandulosa*) on sites differing in herbaceous biomass and grazing history. *Oecologia* 80:19–26
- Brown J, Archer S (1999) Shrub invasion of grassland: recruitment is continuous and not regulated by herbaceous biomass or density. *Ecology* 80:2385–2396
- Bruno J, Stachowicz J, Bertness M (2003) Inclusion of facilitation into ecological theory. *Trends Ecol Evol* 18:119–125
- Bulleri F, Bruno J, Benedetti-Cecchi L (2008) Beyond competition: incorporating positive interactions between species to predict ecosystem invasibility. *PLoS Biol* 6:e162
- Bustamante-Sanchez MA, Armesto JJ, Halpern CB (2011) Biotic and abiotic controls on tree colonization in three early successional communities of Chiloé Island, Chile. *J Ecol* 99(1):228–299
- Callaway R (1995) Positive interactions among plants. *Bot Rev* 61:306–349
- Callaway R, Walker L (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* 78:1958–1965
- Chase JM, Ryberg WA (2004) Connectivity, scale-dependence, and the productivity-diversity relationship. *Ecol Lett* 7:676–683. doi:10.1111/j.1461-0248.2004.00622.x
- Classen A, Norby R, Company C, Sides K (2010) Climate change alters seedling emergence and establishment in an old-field ecosystem. *PLoS One* e13476:1–8
- Cuesta B, Villar-Salvador P, Puértolas J, Rey Benayas JM, Michalet R (2010) Facilitation of *Quercus ilex* in Mediterranean shrubland is explained by both direct and indirect interactions mediated by herbs. *J Ecol* 98:687–696. doi:10.1111/j.1365-2745.2010.01655.x
- Davis M, Wragg K, Reich P (1998) Competition between tree seedlings and herbaceous vegetation: support for a theory of resource supply and demand. *J Ecol* 86:652–661
- Davis M, Wragg K, Reich P, Tjoelker M, Schaeffer T, Muermann C (1999) Survival, growth, and photosynthesis of tree seedlings competing with herbaceous vegetation along a water-light-nitrogen gradient. *Plant Ecol* 145:341–350
- Davis MA, Reich PB, Knoll MJB, Dooley L, Hundtoft M, Attleson I (2007) Elevated atmospheric CO₂: a nurse plant substitute for oak seedlings establishing in old fields. *Glob Change Biol* 13:2308–2316. doi:10.1111/j.1365-2486.2007.01444.x
- Dickie IA, Schnitzer SA, Reich PB, Hobbie SE (2007) Is oak establishment in old-fields and savanna openings context dependent? *J Ecol* 95:309–320. doi:10.1111/j.1365-2745.2006.01202.x
- Dijkstra F, Hobbie S, Reich P, Knops J (2005) Divergent effects of elevated CO₂, N fertilization, and plant diversity on soil C and N dynamics in a grassland field experiment. *Plant Soil* 272:41–52
- Fargione JE, Tilman D (2005) Diversity decreases invasion via both sampling and complementarity effects. *Ecol Lett* 8:604–611. doi:10.1111/j.1461-0248.2005.00753.x
- Farrer EC, Goldberg DE (2011) Patterns and mechanisms of conspecific and heterospecific interactions in a dry perennial grassland. *J Ecol* 99(1):265–276
- Fensham R, Fairfax R, Archer S (2005) Rainfall, land use and woody vegetation cover change in semi-arid Australian savanna. *Ecology* 93:596–606
- Hoekstra JM, Boucher TM, Ricketts TH, Roberts C (2004) Confronting a biome crisis: global disparities of habitat loss and protection. *Ecol Lett* 8:23–29. doi:10.1111/j.1461-0248.2004.00686.x
- Isbell F, Calcagno V, Hector A, Connolly J, Harpole WS, Reich PB, Scherer-Lorenzen M, Schmid B, Tilman D, Van Ruijven J, Weigelt A, Wilsey BJ, Zavaleta ES, Loreau M (2011) High plant diversity is needed to maintain ecosystem services. *Nature* 477:199–202. doi:10.1038/nature10282
- Jackson R, Banner J, Jobbágy E, Pockman W, Wall D (2002) Ecosystem carbon loss with woody plant invasion of grasslands. *Nature* 418:623–626
- Kennedy T, Naeem S, Howe K, Knops J, Tilman D, Reich P (2002) Biodiversity as a barrier to ecological invasion. *Nature* 417:636–638
- Kgope BS, Bond WJ, Midgley GF (2009) Growth responses of African savanna trees implicate atmospheric [CO₂] as a driver of past and current changes in savanna tree cover. *Aust Ecol* 35:451–463. doi:10.1111/j.1442-9993.2009.02046.x
- Knapp AK, Briggs JM, Collins SL, Archer SR, Bret-Harte MS, Ewers BE, Peters DP, Young DR, Shaver GR, Pendall E, Cleary MB (2008) Shrub encroachment in North American grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. *Glob Change Biol* 14:615–623. doi:10.1111/j.1365-2486.2007.01512.x

- Knops JMH (2006) Fire does not alter vegetation in infertile prairie. *Oecologia* 150:477–483. doi:[10.1007/s00442-006-0535-8](https://doi.org/10.1007/s00442-006-0535-8)
- McCulley R, Archer S, Boutton T, Hons F, Zuberer D (2004) Soil respiration and nutrient cycling in wooded communities developing in grassland. *Ecology* 85:2804–2817
- McKinley D, Blair J (2008) Woody plant encroachment by *Juniperus virginiana* in a mesic native grassland promotes rapid carbon and nitrogen accrual. *Ecosystems* 11(3):454–468. doi:[10.1007/s10021-008-9133-4](https://doi.org/10.1007/s10021-008-9133-4)
- Miriti MN (2006) Ontogenetic shift from facilitation to competition in a desert shrub. *J Ecol* 94:973–979. doi:[10.1111/j.1365-2745.2006.01138.x](https://doi.org/10.1111/j.1365-2745.2006.01138.x)
- Montgomery RA, Reich PB, Palik BJ (2010) Untangling positive and negative biotic interactions: views from above and below ground in a forest ecosystem. *Ecology* 91:3641–3655
- Naeem S, Knops J, Tilman D, Howe K, Kennedy T, Gale S (2000) Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. *Oikos*:91:97–108
- Niinemets Å (2010) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. *For Ecol Manage* 260:1623–1639. doi:[10.1016/j.foreco.2010.07.054](https://doi.org/10.1016/j.foreco.2010.07.054)
- Ovington J, MacRae C (1960) The growth of seedlings of *Quercus petraea*. *J Ecol* 48:549–555
- Peterson D, Reich P (2001) Prescribed fire in oak savanna: fire frequency effects on stand structure and dynamics. *Ecol Appl* 11:914–927
- Polley WH, Johnson H, Tischler C (2003) Woody invasion of grasslands: evidence that CO₂ enrichment indirectly promotes establishment of *Prosopis glandulosa*. *Plant Ecol* 164:85–94
- Post W, Emanuel W, Zinke P, Stangenberger A (1982) Soil carbon pools and world life zones. *Nature* 298:156–159
- Reich PB (2009) Elevated CO₂ reduces losses of plant diversity caused by nitrogen deposition. *Science* 326:1399–1402. doi:[10.1126/science.1178820](https://doi.org/10.1126/science.1178820)
- Reich P, Knops J, Tilman D, Craine J, Ellsworth D, Tjoelker M, Lee T, Wedin D, Naeem S, Bahauddin D (2001a) Plant diversity enhances ecosystem responses to elevated CO₂ and nitrogen deposition. *Nature* 410:809–810
- Reich P, Peterson D, Wedin D, Wragge K (2001b) Fire and vegetation effects on productivity and nitrogen cycling across a forest–grassland continuum. *Ecology* 82:1703–1719
- Reich P, Tilman D, Craine J, Ellsworth D, Tjoelker M, Knops J, Wedin D, Naeem S, Bahauddin D, Goth J (2001c) Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO₂ and N availability regimes? A field test with 16 grassland species. *New Phytol* 150:435–448
- Reich PB, Frelich LE, Voldseth RA, Bakken P, Adair EC (2012) Understorey diversity in southern boreal forests is regulated by productivity and its indirect impacts on resource availability and heterogeneity. *J Ecol* 100:539–545. doi:[10.1111/j.1365-2745.2011.01922.x](https://doi.org/10.1111/j.1365-2745.2011.01922.x)
- Roques K, O’connor T, Watkinson A (2001) Dynamics of shrub encroachment in an African savanna: relative influences of fire, herbivory, rainfall and density dependence. *J Appl Ecol* 38:268–280
- 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. doi:[10.1111/j.1461-0248.2005.00736.x](https://doi.org/10.1111/j.1461-0248.2005.00736.x)
- Schnitzer S, Klironomos J, HilleRisLambers J, Kinkel L, Reich P, Xiao K, Rillig M, Sikes B, Callaway R, Mangan S (2011) Soil microbes drive the classic plant diversity–productivity pattern. *Ecology* 92:296–303
- Silva J, Zambrano A, Fariñas M (2001) Increase in the woody component of seasonal savannas under different fire regimes in Calabozo, Venezuela. *J Biogeogr* 28:977–983
- Staver AC, Archibald S, Levin SA (2011) The global extent and determinants of savanna and forest as alternative biome states. *Science* 334:230–232. doi:[10.1126/science.1210465](https://doi.org/10.1126/science.1210465)
- Tilman D (1987) Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecol Monogr* 57:189–214
- Tilman D, Knops J, Wedin D, Reich P, Ritchie M, Siemann E (1997a) The influence of functional diversity and composition on ecosystem processes. *Science* 277:1300
- Tilman D, Lehman C, Thomson K (1997b) Plant diversity and ecosystem productivity: theoretical considerations. *Proc Natl Acad Sci USA* 94:1857–1861
- Tilman D, Reich P, Knops J, Wedin D, Mielke T, Lehman C (2001) Diversity and productivity in a long-term grassland experiment. *Science* 294:843–845
- Van Auken O (2000) Shrub invasions of North American semiarid grasslands. *Annu Rev Ecol Syst* 31:197–215
- Van Auken O, Bush J (1997) Growth of *Prosopis glandulosa* in response to changes in aboveground and belowground interference. *Ecology* 78:1222–1229
- 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 P, Mooney H, Lubchenco J, Melillo J (1997) Human domination of earth’s ecosystems. *Science* 277:494
- Zhang Y, Chen HYH, Reich PB (2012) Forest productivity increases with evenness, species richness and trait variation: a global meta-analysis. *J Ecology*. doi:[10.1111/j.1365-2745.2011.01944.x](https://doi.org/10.1111/j.1365-2745.2011.01944.x)