

CO₂, nitrogen, and diversity differentially affect seed production of prairie plants

J. HILLERISLAMBERS,^{1,6} W. S. HARPOLE,² S. SCHNITZER,³ D. TILMAN,⁴ AND P. B. REICH⁵

¹Biology Department, University of Washington, Seattle, Washington 98195-1800 USA

²Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, Iowa 50011 USA

³Department of Biological Sciences, University of Wisconsin, Milwaukee, Wisconsin 53201 USA

⁴Ecology, Evolution and Behavior, University of Minnesota–Twin Cities, St. Paul, Minnesota 55108 USA

⁵Department of Forest Resources, University of Minnesota–Twin Cities, St. Paul, Minnesota 55108 USA

Abstract. Plant species composition and diversity is often influenced by early life history stages; thus, global change could dramatically affect plant community structure by altering seed production. Unfortunately, plant reproductive responses to global change are rarely studied in field settings, making it difficult to assess this possibility. To address this issue, we quantified the effects of elevated CO₂, nitrogen deposition, and declining diversity on inflorescence production and inflorescence mass of 11 perennial grassland species in central Minnesota, USA. We analyzed these data to ask whether (1) global change differentially affects seed production of co-occurring species; (2) seed production responses to global change are similar for species within the same functional group (defined by ecophysiology and growth form); and (3) seed production responses to global change match productivity responses. We found that, on average, allocation to seed production decreased under elevated CO₂, although individual species responses were rarely significant due to low power (CO₂ treatment df = 2). The effects of nitrogen deposition on seed production were similar within functional groups: C₄ grasses tended to increase while C₃ grasses tended to decrease allocation to seed production. Responses to nitrogen deposition were negatively correlated to productivity responses, suggesting a trade-off. Allocation to seed production of some species responded to a diversity gradient, but responses were uncorrelated to productivity responses and not similar within functional groups. Presumably, species richness has complex effects on the biotic and abiotic variables that influence seed production. In total, our results suggest that seed production of co-occurring species will be altered by global change, which may affect plant communities in unpredictable ways. Although functional groups could be used to generalize seed production responses to nitrogen deposition in Minnesota prairies, we caution against relying on them for predictive purposes without a mechanistic understanding of how resource availability and biotic interactions affect seed production.

Key words: diversity; elevated CO₂; fecundity; global change; grasslands; nitrogen deposition; prairies; recruitment; seed production.

INTRODUCTION

The effects of global change on seed production may affect plant community composition, because species differences at early life history stages can strongly influence community structure. For example, both theory and experimental studies suggest that low seed production limits interspecific competition, and thus, promotes diversity (Shmida and Ellner 1984, Hurtt and Pacala 1995, Tilman 1997). Secondary succession may also be driven by differences among species in their colonization ability, with better colonizers (those producing more seeds) being dominant during early succession, and better competitors arriving and dominating later in succession (Gleeson and Tilman 1990,

Fastie 1995, Lichter 2000). A trade-off between colonization and competitive ability can also promote diversity or control the relative abundance of species in late-successional communities (Tilman 1997, Turnbull et al. 1999). Taken together, these studies suggest that the differential effects of elevated CO₂, nitrogen deposition and the local loss of species on seed production of co-occurring species may affect community structure in unexpected ways (Stiling et al. 2004, Cleland et al. 2006).

Whether or not seed production of co-occurring species will be differentially affected by global change, and which global change factors have the strongest effects on seed production, is not well-known. Studies indicate that global change factors such as elevated CO₂, nitrogen deposition, or declining diversity differentially affect the productivity of co-occurring species (e.g., DeLucia et al. 1999, Reich et al. 2001, Polley et al. 2003, HilleRisLambers et al. 2004), but reproductive responses are rarely studied. Elevated CO₂ has been found to

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⁶ E-mail: jhrl@u.washington.edu

TABLE 1. Species, seasonality (i.e., time of seed production: 1, June; 2, August), functional group membership, and basis for hypothesized responses to global change factors.

Functional group and species	Abbreviation	Seasonality ($m = 1$ or 2)	Productivity limited by N?	Productivity stimulated by CO ₂ ?
C ₃ grasses				
<i>Agropyron repens</i>	Ar	1	Yes ^{1,2}	Yes ^{3,4}
<i>Bromus inermis</i>	Bi	1		
<i>Koeleria cristata</i>	Kc	1		
<i>Poa pratensis</i>	Pp	1		
C ₄ grasses				
<i>Andropogon gerardii</i>	Ag	2	No ^{1,2}	No ^{3,4}
<i>Bouteloua gracilis</i>	Bg	2		
<i>Schizachyrium scoparium</i>	Ss	2		
<i>Sorghastrum nutans</i>	Sn	2		
Forbs (excluding N fixers)				
<i>Solidago rigida</i>	Sr	2	Yes ⁵	Yes ⁴
Nitrogen-fixing forbs				
<i>Lespedeza capitata</i>	Lc	2	No ^{6,7,8}	Yes ³
<i>Lupinus perennis</i>	Lp	1		

Note: Sources are: 1, Tilman and Wedin (1991); 2, Wedin and Tilman (1993); 3, Poorter and Navas (2003); 4, Wand et al. (1999); 5, HilleRisLambers et al. (2004); 6, Suding et al. (2005); 7, Reich et al. (2003); 8, Ritchie and Tilman (1995).

strongly affect seed production of some species, but results are difficult to extrapolate to natural communities, because most studies focus on crop species (Jablonski et al. 2002) or examine the response of single species to elevated CO₂ (e.g., Huxman et al. 1999, LaDeau and Clark 2001). To our knowledge, fewer than 10 studies have examined the reproductive responses of co-occurring plant species to elevated CO₂ in the field (Navas et al. 1997, Grunzweig and Korner 2000, Thurig et al. 2003, Morgan et al. 2004, Stiling et al. 2004, Miyagi et al. 2007, Ramo et al. 2007, Williams et al. 2007); and only two studies have examined reproductive responses of co-occurring species to multiple global change factors in the field (Cleland et al. 2006, Ramo et al. 2007).

Determining how global change alters seed production of co-occurring members of a plant community would lend insight into the factors that constrain seed production, and may simplify efforts to forecast population or community dynamics under global change scenarios. For example, if species within functional groups respond similarly, or if seed production responses to global change correlate with productivity responses, it might be possible to extrapolate results from existing studies to predict the seed production responses of other species to global change. To address these issues, we determine the effects of elevated CO₂, nitrogen deposition and a gradient in species richness on the seed production of 11 co-occurring plant species in Minnesota. We used hierarchical Bayesian analyses to quantify the effects of the three global change factors on inflorescence production (per unit biomass), inflorescence mass, and the proportion of aboveground biomass that is seed. Using these results, we asked whether (1) global change differentially impacts seed production of co-occurring species, implying possible effects on

community dynamics; (2) seed production responses to global change are similar within four functional groups (Table 1); and (3) seed production responses to global change are similar to productivity responses (Table 1).

We hypothesized that global change would affect seed production similarly to productivity, because we expected that greater vegetative growth should indicate a greater availability of resources for seed production (Thurig et al. 2003). Thus, we predicted that seed production responses to elevated CO₂ and nitrogen deposition would be similar within functional groups (Reich et al. 2001, 2006, Poorter and Navas 2003). Specifically, we hypothesized that seed production of C₄ grasses would respond negatively and C₃ species, especially legumes, positively to elevated CO₂ (Polley et al. 2003, Morgan et al. 2004, Miyagi et al. 2007; but see Owensby et al. 1999). We also expected that seed production of N demanding species would increase (C₃ grasses and non-leguminous forbs), while seed production of species most adept at acquiring this limiting soil resource would decrease with nitrogen deposition (perennial C₄ grasses, legumes; Tilman 1984). Finally, we hypothesized that seed production responses to declining diversity would be similar to reproductive responses to nitrogen deposition, because declining diversity increases the availability of nitrogen (Tilman 1997).

MATERIALS AND METHODS

The Cedar Creek Natural History Area is a 2200-ha Long Term Ecological Research site in south-central Minnesota at the approximate presettlement prairie-forest border. Cedar Creek consists of hardwood forests, pine forests, abandoned agricultural fields, and oak savannas. Soils are derived from a sandy glacial outwash, and are extremely nitrogen poor (Tilman 1984, Tilman and Wedin 1991). The climate is conti-

mental, with cool winters (average temperature December to February is -8.45°C) and hot summers (average temperature June to August is 20.46°C). Based on climate data collected from 1982–2006, cumulative rainfall at Cedar Creek averages 81.5 cm each year, with most of the precipitation falling in summer (32.49 total cm precipitation between June and August) and the least in the winter (6.87 total cm precipitation). Climate in the year of data collection (2002) was slightly above average in terms of rainfall (cumulative rainfall 84.0 cm), warmer in the winter (-4.26°C) but slightly cooler in the spring (4.19°C vs. 6.88°C average).

The BioCON experiment was established in an abandoned agricultural field in 1997 (a description of the BioCON experiment is *available online*).⁷ Prior to the initiation of the global change treatments, the existing vegetation in the field was removed, and soils were treated with methyl bromide to kill seeds in the soil seed bank. In this ongoing experiment, factorial combinations of CO_2 treatments (ambient, 368 parts per million [ppm]; elevated, 560 ppm), nitrogen treatments (ambient, +4 g N annually), and species richness treatments (monocultures, four-, nine-, and 16-species plots) are applied to 366 4-m^2 plots equally divided across six rings. Although species richness, not diversity, was manipulated, we use “diversity” to refer to the treatment, as is the norm in manipulative biodiversity studies (e.g., Tilman 1997, Reich et al. 2001).

CO_2 treatments are applied to each ring using FACE technology (free air carbon dioxide enrichment; a more complete description of FACE is *available online*),⁸ with three rings at ambient atmospheric CO_2 concentrations and three rings at elevated CO_2 concentrations (see Plate 1). CO_2 treatments are imposed during daylight hours in the growing season, approximately mid-April to mid-October. Nitrogen and species richness treatments are applied at the plot level. Nitrogen deposition is mimicked by adding NH_4NO_3 three times annually. The diversity treatment was imposed when plots were established by seeding each 4-m^2 plot with 48 g of seed equally divided among the component species. The main experiment (analyzed here; see Reich et al. 2001, 2004 for more details) consists of 32 monoculture plots, 15 four-species plots, 15 nine-species plots, and 12 16-species plots. Species composition of four and nine species plots was randomly determined, but the 16-species plots all contain the same 16 perennial herbaceous species and monoculture plots are equally divided among species (with each species being represented by two monoculture plots per nitrogen and CO_2 treatment, randomly located across appropriate rings). Species composition of each plot is maintained by annual weeding of species not originally planted in the plot.

Focal species in the study are herbaceous perennial plants common to the Cedar Creek region and

representing four functional groups (Table 1). These include C_3 grasses (*Agropyron repens*, *Bromus inermis*, *Koeleria cristata*, *Poa pratensis*), C_4 bunchgrasses (*Andropogon gerardii*, *Bouteloua gracilis*, *Schizachyrium scoparium*, *Sorghastrum nutans*), a forb (*Solidago rigida*), and two nitrogen-fixing legumes (*Lespedeza capitata*, *Lupinus perennis*; see Plate 1). Five other species also planted in the experiment (three forbs and two legumes) were not sampled for this study, as most of these species were rarely present outside monoculture plots.

All data analyzed in this manuscript were collected in summer of 2002. We measured aboveground productivity in June and August in two different 1.5×0.1 m strips using electric clippers, and weighed the biomass after sorting it to species and drying it in a drying oven (at 60°C). Percent cover was assessed in a 0.5×1 m quadrat centered within each 4-m^2 plot in June and August. We counted inflorescences in the same 0.5×1.0 m permanent quadrat, at the time of seed dispersal for each species. We harvested two inflorescences with mature seeds from separate individuals of each species within each plot. Inflorescences were dried for five days at 40°C , after which seeds were removed from pods or seed heads and weighed. Biomass sampling efforts in previous years (performed in a separate clip strip each sampling period) occurred away from the 0.5×1.0 m permanent quadrat where percent cover was assessed and inflorescences counted. We did not harvest inflorescences from plants growing within 10 cm of the edge of the plot to avoid edge effects. Due to the time-intensive effort required, we did not collect inflorescences from the nine-species plots for four species (*Agropyron repens*, *Bromus inermis*, *Poa pratensis*, *Solidago rigida*).

ANALYSES

Our analyses involved three steps. First, we developed a statistical model that combines biomass, percent cover and inflorescence data to determine the effects of CO_2 , nitrogen, and diversity on biomass and inflorescence production. Next, we developed a statistical model to determine the effects of the global change factors on inflorescence mass. Finally, we combined coefficients describing global change effects on inflorescence production with coefficients describing global change effects on inflorescence mass to estimate global change effects on the allocation of aboveground biomass to seed production. We describe each of these steps here.

Statistical model 1: effects of global change on biomass and inflorescence production

We assume that aboveground biomass of species i in plot j , ring k , and time step l (b_{ijkl}) is lognormally distributed with mean \hat{b}_{ijkl} and standard deviation σ_{bi} :

$$\log(b_{ijkl}) \sim \mathcal{N}[\log(\hat{b}_{ijkl}), \sigma_{bi}^2] \quad b_{ijkl} > 0. \quad (1)$$

At the time of sampling, species were present in all plots in which they were planted, which means that observa-

⁷ (<http://www.biocon.umn.edu/>)

⁸ (<http://www.bnl.gov/face/>)

tions of zero (indicating no biomass of that species in a clip strip) arise from lack of detection rather than the extinction of the species from the plot. Observations of zero biomass are therefore treated as missing data in Eq. 1. However, these observations also provide information on biomass production, because they arise when the biomass of that species in the entire plot is low. Thus, we introduce a latent variable that we refer to as “biomass detection” and we model it as a Bernoulli process that is influenced by the amount of actual biomass in the plot (Appendix A).

Our expectation for biomass production (\hat{b}_{ijkl}) depends on parameters describing ring, species and time step-specific biomass production (θ_{bjkl}), the effects of nitrogen deposition (v_{bi}), and effects of species richness (δ_{b9i} , δ_{b4i} , δ_{b1i}). Nitrogen and species richness coefficients are multiplied by dummy vectors \mathbf{n}_{jk} , \mathbf{d}_{9jk} , \mathbf{d}_{4jk} , or \mathbf{d}_{1jk} ; which contain 1s and 0s indicating plots with nitrogen added and containing nine, four, or one species, respectively:

$$\log(\hat{b}_{ijkl}) = \theta_{bjkl} + v_i \mathbf{n}_{jk} + \delta_{b9i} \mathbf{d}_{9jk} + \delta_{b4i} \mathbf{d}_{4jk} + \delta_{b1i} \mathbf{d}_{1jk}. \quad (2)$$

Intercepts (α_{bil} , biomass production in high-diversity, ambient CO₂, and ambient nitrogen plots) and effects of elevated CO₂ on biomass production (χ_{bi}) are estimated from ring-specific parameters in ambient (rings two, four, and six) and elevated rings (rings one, three, and five):

$$\theta_{bi(j=2,4,6)l} \sim \mathcal{N}(\alpha_{bil}, \sigma_{bri}^2) \quad (3)$$

$$\theta_{bi(j=1,3,5)l} \sim \mathcal{N}(\alpha_{bil} + \chi_{bi}, \sigma_{bri}^2). \quad (4)$$

The parameter σ_{bri} describes the ring to ring variation in biomass production for species i . Essentially, this is a mixed effects model, with ring as a random effect in the estimation of α_{bil} and χ_{bi} . Biomass production at the two time steps are related through the parameter ϕ_i , which represents the difference in aboveground productivity between time step 1 and 2 (on a log scale):

$$\alpha_{bi2} = \alpha_{bi1} + \phi_i. \quad (5)$$

We assume that percent cover observations (l of two) of species i in ring j and plot k (p_{ijkl}) are normally distributed on the logit scale, with standard deviation σ_{pi} :

$$\text{logit}(p_{ijkl}) \sim \mathcal{N}[\text{logit}(\hat{p}_{ijkl}), \sigma_{pi}^2] \quad p_{ijkl} > 0. \quad (6)$$

Although our primary interest is in the relationship between biomass and inflorescence production, we included percent cover data in this statistical model because inflorescences were counted in the same area as percent cover. Thus, percent cover provides additional information on the abundance of each species in the percent cover quadrat where we counted inflorescences. As with biomass, we model percent cover detection as a

Bernoulli process depending on the amount of percent cover (Appendix B). We assume that percent cover depends on biomass in the same plot and two parameters (q_i and r_i):

$$\text{logit}(\hat{p}_{ijkl}) = q_i + \frac{1}{r_i(\hat{b}_{ijkl} - \bar{b}_i)}. \quad (7)$$

Exploratory analyses indicated that this functional form better fits the relationship between percent cover and biomass than a linear model in logit space. Subtracting the average biomass \bar{b}_i from \hat{b}_{ijkl} reduces the natural tendency for slope and intercept parameters to be correlated (slowing model convergence); this technique is called covariate centering.

Inflorescences of species i in plot j and ring k (f_{ijk}) arise through a Poisson process:

$$f_{ijk} \sim \text{Poisson}(\hat{f}_{ijk}). \quad (8)$$

Inflorescence production depends on the plot-specific biomass production of that species (at time step m , when the species in question is setting seed; Table 1) and parameters describing how that relationship is affected by rings, nitrogen, and diversity:

$$\hat{f}_{ijk} = \exp(\theta_{fik} + v_{fi} \mathbf{n}_{jk} + \delta_{f9i} \mathbf{d}_{9jk} + \delta_{f4i} \mathbf{d}_{4jk} + \delta_{f1i} \mathbf{d}_{1jk}) \hat{b}_{ijkm}. \quad (9)$$

Fitted parameters represent ring and species-specific effects (θ_{fik}), nitrogen deposition effects (v_{fi}), and species richness effects (δ_{f9i} , δ_{f4i} , δ_{f1i}) multiplied by dummy vectors \mathbf{n}_{jk} , \mathbf{d}_{9jk} , \mathbf{d}_{4jk} , or \mathbf{d}_{1jk} . Parameters describing inflorescence production in ambient conditions (α_{fi} : high diversity, no CO₂ or nitrogen added) and the effects of elevated CO₂ on biomass production (χ_{fi}) were estimated from ring-specific parameters which are normally distributed with standard deviation σ_{fri} (describing ring to ring variation in inflorescence production):

$$\theta_{fi(j=2,4,6)} \sim \mathcal{N}(\alpha_{fi}, \sigma_{fri}^2) \quad (10)$$

$$\theta_{fi(j=1,3,5)} \sim \mathcal{N}(\alpha_{fi} + \chi_{fi}, \sigma_{fri}^2). \quad (11)$$

We quantify the main effects of elevated CO₂, nitrogen deposition, and declining diversity on biomass and inflorescence production (Eqs. 2–4 and 9–11), but not the interactions between these global change factors, because we found extremely few significant interactions in exploratory analyses. Biomass production was more often affected by interactions between global change factors (as discussed in Reich et al. 2001, 2004); but parameters describing main effects from models with two-way and three-way interactions were strongly correlated with parameters presented here, and their direction or significance did not depend on the inclusion of interactions.

We were interested in determining whether average global change effects on inflorescence production and biomass production were significantly different from zero—in other words, whether the seed production of all species responded similarly to global change. To test this, we estimated parameters describing the average effects of global change treatments on all species (A_b , A_f , X_b , X_f , N_b , N_f , Δ_{b9} , Δ_{f9} , Δ_{b4} , Δ_{f4} , Δ_{b1} , Δ_{f1}), equivalent to designating species identity as a random effect in a mixed effects model. This necessitated the estimation of parameters describing the variance between species in global change coefficients (σ_{ba} , σ_{fa} , σ_{bc} , σ_{fc} , σ_{bn} , σ_{fn} , σ_{bd} , σ_{fd}). We estimated other across-species averages from species-specific parameters (e.g., slope and intercept parameters describing the relationship between percent cover and biomass), a common way to increase model efficiency and decrease model running time in hierarchical Bayesian statistics (see Appendix C for more details).

Statistical model 2: inflorescence mass

We assume that both samples (l) of inflorescence seed mass of species i in plot j and ring k are lognormally distributed with standard deviation σ_{wi} :

$$\log(w_{ijkl}) \sim \mathcal{N}[\log(\hat{w}_{ijk}), \sigma_{wi}^2]. \quad (12)$$

As with biomass and inflorescence production, we model inflorescence mass as a function of parameters describing ring and species-specific effects (θ_{wik}), the effects of nitrogen deposition (v_{wi}), and effects of species richness (δ_{w9i} , δ_{w4i} , δ_{w1i}) which are multiplied by dummy vectors \mathbf{n}_{jk} , \mathbf{d}_{9jk} , \mathbf{d}_{4jk} , or \mathbf{d}_{1jk} :

$$\log(\hat{w}_{ijk}) = \theta_{wik} + v_{wi}\mathbf{n}_{jk} + \delta_{w9i}\mathbf{d}_{9jk} + \delta_{w4i}\mathbf{d}_{4jk} + \delta_{w1i}\mathbf{d}_{1jk}. \quad (13)$$

As with biomass and inflorescence production, elevated CO₂ effects on total seed mass (χ_{wi}) are estimated from the appropriate ring-specific seed production parameters (θ_{wik}):

$$\theta_{wi(j=2,4,6)} \sim \mathcal{N}(\alpha_{wi}, \sigma_{wri}^2) \quad (14)$$

$$\theta_{wi(j=1,3,5)} \sim \mathcal{N}(\alpha_{wi} + \chi_{wi}, \sigma_{wri}^2). \quad (15)$$

We only quantify the main effects of global change treatments because preliminary analyses indicate that the interactions were rarely significant for this response metric.

We also quantified parameters describing the average effect of global change treatments on all 11 species (A_w , X_w , N_w , Δ_{9w} , Δ_{4w} , Δ_{1w}) from species-specific coefficients (α_{wi} , χ_{wi} , v_{wi} , δ_{w9i} , δ_{w4i} , δ_{w1i}). This is a mixed-effects model with species identity as a random effect, requiring the estimation of between-species variability in responses to global change (σ_{wa} , σ_{wc} , σ_{wn} , σ_{wd}). We also estimated other parameters describing average

effects over all species to increase model efficiency (Appendix C).

Estimating global change effects on the allocation of biomass to seed production

We estimated the impacts of global change treatments on allocation to seed production (the proportion of aboveground biomass in seed) by combining estimates of global change effects on inflorescence production per unit biomass (Eqs. 1–11) and estimates of global change effects on seed mass per inflorescence (Eqs. 12–15). We chose to integrate the results from our two models in this way because allocation to seed production is a metric of importance to plant life history and ecological processes (Bazzaz et al. 1987, Gleeson and Tilman 1990). Thus, we added species- and treatment-specific global change coefficients from inflorescence production models (Eq. 9) to coefficients from inflorescence mass models (Eq. 13). For example, to determine effects of elevated nitrogen on the proportion of biomass allocated to seed production for species i , we added the coefficient describing nitrogen deposition effects on inflorescence production per unit biomass (v_{fi} in units of inflorescences per gram biomass) to the coefficient describing nitrogen deposition effects on the mass of seeds per inflorescence (v_{wi} in units of grams per inflorescence). Adding coefficients estimated on a log scale (Eqs. 9 and 13) is equivalent to multiplying them. Credible intervals were determined by repeating this process with 5000 random samples from Markov chain Monte Carlo (MCMC) chains.

Model fitting

We used a hierarchical Bayesian approach to fit these models because classical statistics can not accommodate the multiple data sources in our first model (i.e. biomass, percent cover, and inflorescences; Ellison 2004, Clark 2005, HilleRisLambers et al. 2006). For consistency, we used this approach for both models, although our second model could have been fit using maximum likelihood methods (with identical results). We fit both statistical models numerically with Markov chain Monte Carlo simulation, using the software WinBUGs version 1.4 (*available online*).⁹ All parameters were given diffuse priors (Appendix C). We initialized four chains from dispersed values, discarding 10 000 samples as “burn-in,” and assessed convergence visually as well as with Gelman and Rubins scale reduction factor. All chains converged to the same parameter values, and Gelman and Rubins scale reduction factor indicated convergence. We thinned chains to reduce autocorrelation within chains to zero. An examination of the relationship between predicted and observed percent cover, biomass, inflorescence, and inflorescence mass data suggested reasonable model fits (Appendix D). Deviance information criterion (DIC) also indicated that models

⁹ (<http://www.mrc-bsu.cam.ac.uk/bugs>)

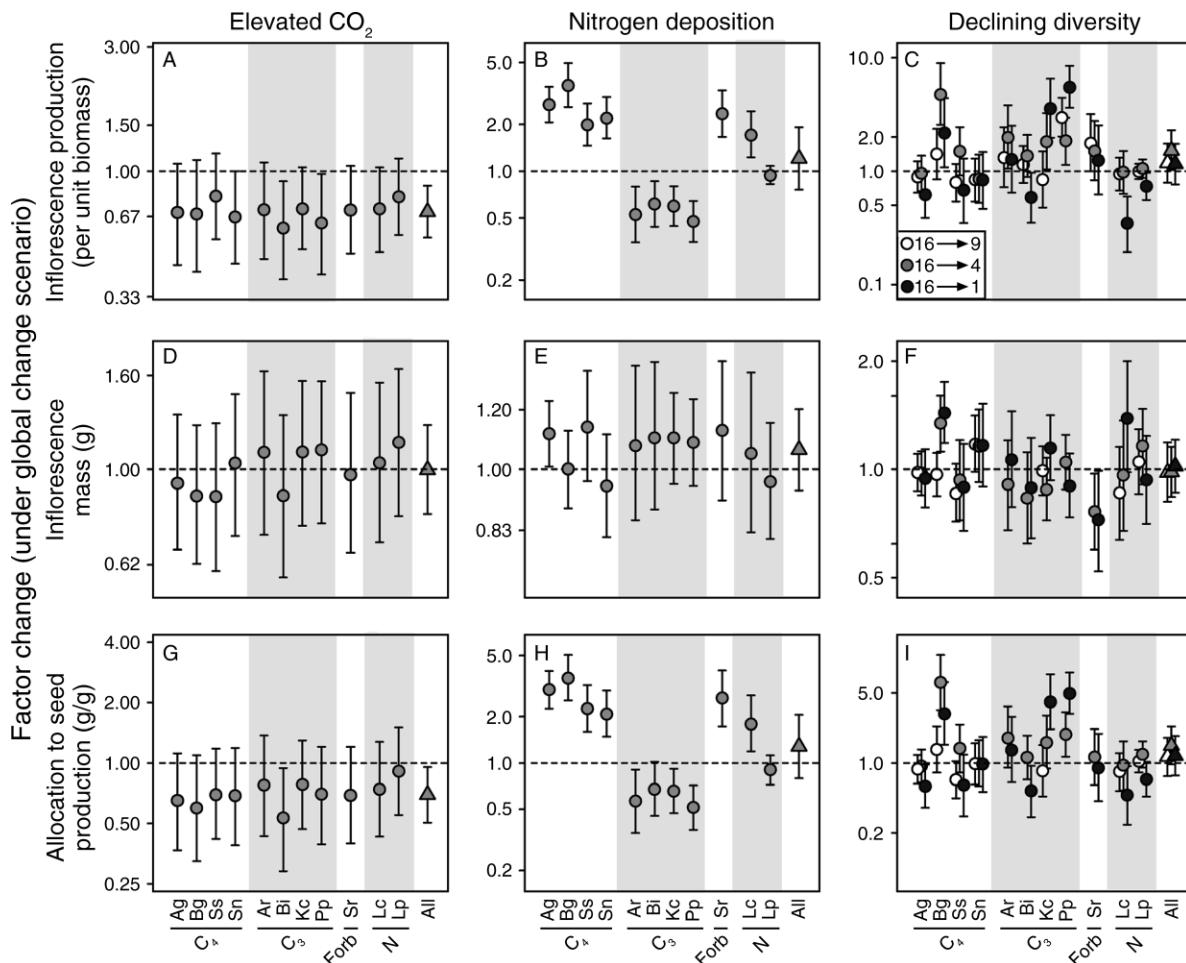


FIG. 1. Factor by which elevated CO₂ (first column), nitrogen deposition (second column), and declining diversity (third column) affect inflorescence production (top row), the mass of seeds produced per inflorescence (middle row), and the proportion of biomass allocated to seed production (bottom row; a function of inflorescence production and inflorescence mass). Factor change refers to a multiplicative change. For example, for panel A, each dot is the number by which one would multiply inflorescence production under ambient conditions (for the species in question) to get inflorescence production under elevated CO₂ conditions. Circles represent species-specific effects, with error bars representing 95% credible intervals. Species are arranged by functional group status: C₄ grasses, C₃ grasses, forbs, and nitrogen fixers (legumes). Functional groups are indicated by shading. Triangles represent average effects over all species, with error bars representing 95% credible intervals. The different shades of circles and triangles in the third column represent the effect of going from 16-species richness plots to 9-species richness plots (white symbols), from 16-species richness plots to 4-species richness plots (gray symbols), and from 16-species richness plots to monocultures (black symbols). Species abbreviations are in Table 1.

with global change effects better fit the data than a null model not including global change effects (Appendix D).

RESULTS

The inflorescence production of only two species was significantly affected by elevated CO₂ (*Bromus inermis* and *Poa pratensis*), although posterior means were all negative, resulting in a significantly negative effect of CO₂ over all species (Fig. 1). Nitrogen deposition effects on inflorescence production were positive for C₄ grasses, the forb, and one of the legumes (*Andropogon gerardii*, *Bouteloua gracilis*, *Schizachyrium scoparium*, *Sorghastrum nutans*, *Solidago rigida*, *Lespedeza capitata*) and negative for the C₃ grasses (*Agropyron repens*, *Bromus*

inermis, *Koeleria cristata*, *Poa pratensis*). Declining diversity had positive effects on four species (*Bouteloua gracilis*, *Agropyron repens*, *Koeleria cristata*, and *Poa pratensis*) and negative effects on four species (*Bromus inermis*, *Andropogon gerardii*, *Lespedeza capitata*, and *Lupinus perennis*), although results were not consistently significant across all diversity levels for these species. Elevated CO₂ did not significantly affect inflorescence mass for any species (Fig. 1). With the exception of one species (*Agropyron repens*), nitrogen deposition effects on inflorescence mass were also not significant (Fig. 1). Declining diversity positively affected the inflorescence mass of *Bouteloua gracilis*, and negatively affected the inflorescence mass of *Solidago rigida*.

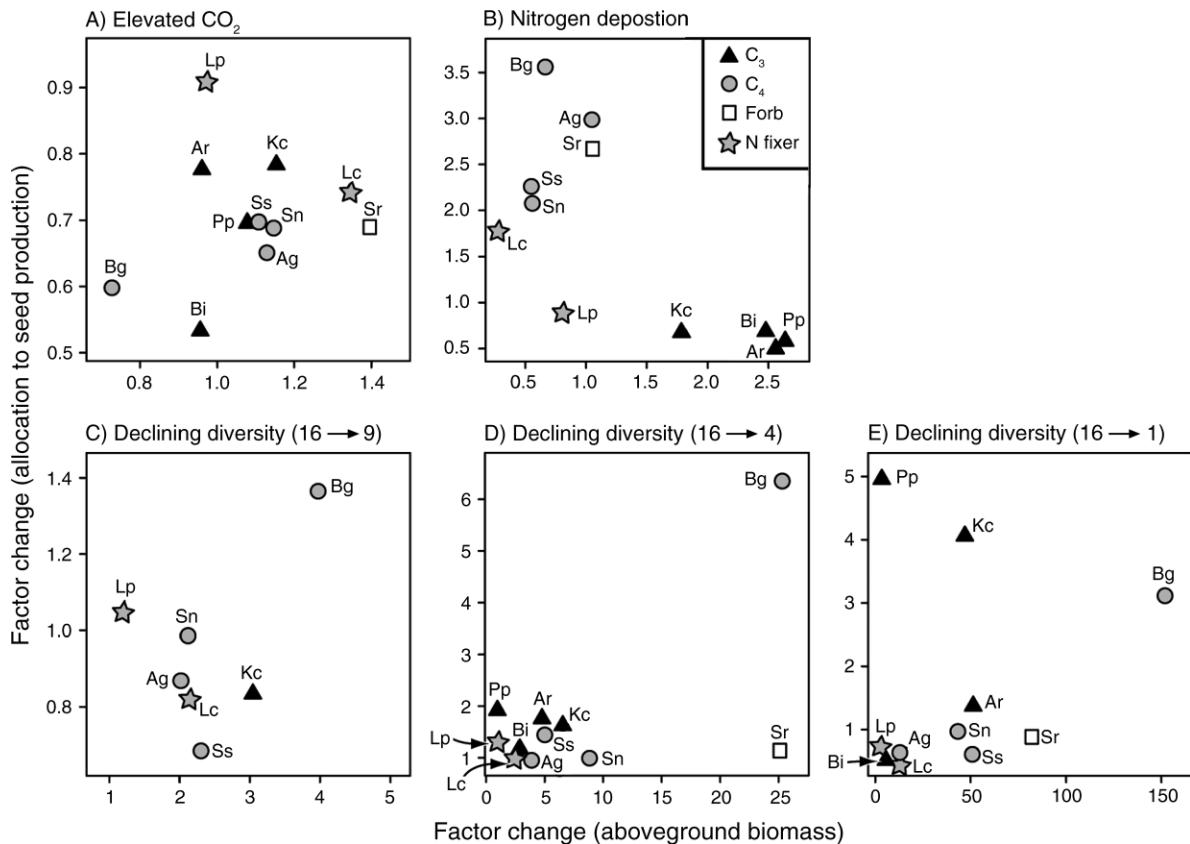


FIG. 2. The relationship between allocation to seed production responses (y -axis) and aboveground biomass (x -axis) responses to (A) elevated CO_2 and (B) nitrogen deposition. (C–E) Allocation to seed production responses vs. aboveground biomass responses to declining diversity for three diversity treatments. Each symbol represents one of the 11 species studied, with different symbols for each functional group (key in panel B). Species abbreviations are in Table 1.

Global change factors affected inflorescence production more often than inflorescence mass, and effect sizes on inflorescence production were also larger than those on inflorescence mass (Fig. 1). Effects of global change on the proportion of biomass allocated to seed production were thus primarily driven by effects on inflorescence production. Elevated CO_2 had negative effects on the allocation of biomass to seed production across all species, but significant effects on only one species (Fig. 1). Similar to inflorescence production, nitrogen deposition both increased and decreased the proportion of biomass allocated to seed production (Fig. 1). Allocation to seed production increased for three species (*Bouteloua gracilis*, *Koeleria cristata*, *Poa pratensis*) and decreased for two species (*Bromus inermis*, *Lepedeza capitata*) in low species richness plots (Fig. 1).

The effects of elevated CO_2 on productivity were not positively correlated with those on allocation to seed production ($r = 0.267$, $P = 0.427$, Fig. 2), and responses did not differ between the four functional groups. Nitrogen deposition effects on allocation to seed production were negatively correlated with effects on

productivity ($r = -0.727$, $P = 0.011$, Fig. 2B). For nitrogen deposition effects, allocation to reproduction could be generalized by functional groups, with C_4 grasses increasing and C_3 grasses decreasing allocation to seed production with increased nitrogen. The effect of declining diversity on productivity was not correlated to the effect of declining diversity on allocation to seed production ($r = 0.241$, $P = 0.603$, Fig. 2C; $r = 0.353$, $P = 0.285$, Fig. 2D; $r = 0.197$, $P = 0.562$, Fig. 2E); neither were the four functional groups predictive of the effects of declining diversity on reproductive responses (Fig. 2D).

DISCUSSION

Elevated CO_2 , nitrogen deposition, and declining diversity each affected seed production of at least one species, with the magnitude of effects varying between functional groups and response metrics (Fig. 1). Nitrogen deposition, for example, increased allocation to seed production of *Andropogon gerardii* by more than a factor of three, while *Bromus inermis* decreased its' allocation to seed production by 50% with elevated CO_2 (Fig. 1). Previous studies have also found such variable

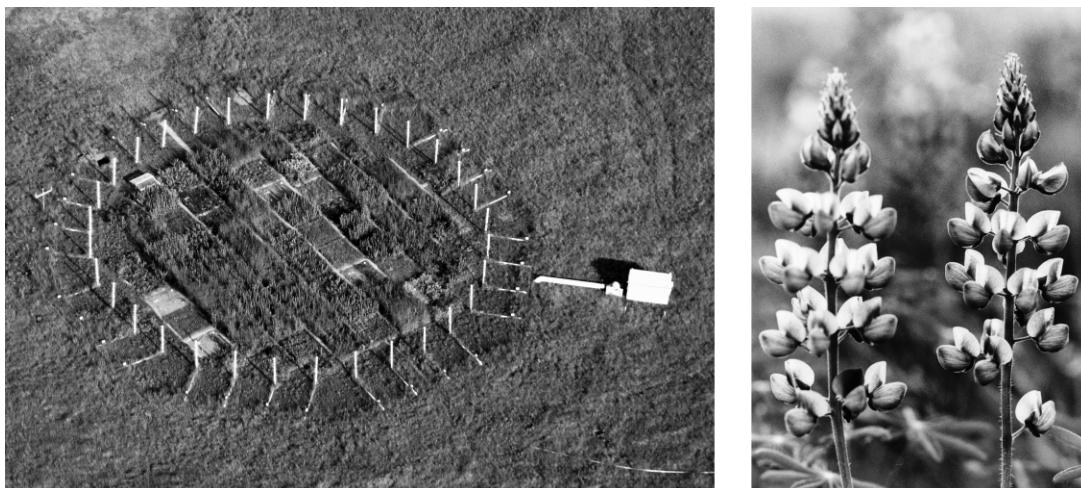


PLATE 1. (Left) An aerial picture of one of the six rings in which CO₂ treatments are imposed in this experiment. In elevated-CO₂ rings, the fan house (to the right of the ring in this picture) controls the amount of pure CO₂ that is mixed with air and blown into an underground pipe system connected to aboveground vertical emitter pipes surrounding the ring. The 66 4-m² plots inside the ring contain one, four, nine, or 16 species and are subjected to one of two nitrogen treatments (ambient or elevated). (Right) Flowering inflorescences of *Lupinus perennis*, one of the two legumes included in this experiment. Inflorescence production of this species was influenced by declining diversity. Photo credits: (left) D. Tilman; (right) J. HilleRisLambers.

and strong responses to global change (Jackson et al. 1995, Huxman et al. 1999, Grunzweig and Korner 2000, Smith et al. 2000, LaDeau and Clark 2001, Jablonski et al. 2002, Thurig et al. 2003). These changes could have implications for plant community dynamics. Secondary succession in these grasslands is largely driven by colonization ability (Gleeson and Tilman 1990). Thus, the order in which species arrive in abandoned agricultural fields may change because nitrogen deposition alters the seed production capabilities of these species relative to each other (Fig. 1). Plant community dynamics in late-successional communities may also be affected. Recruitment limitation, when areas suitable for the recruitment of a particular species never receive their propagules, is prevalent in these grasslands (Tilman 1997, Foster and Tilman 2003) and can promote diversity by limiting inter-specific interactions and slowing competitive exclusion (Shmida and Ellner 1984, Hurtt and Pacala 1995). An overall decrease in seed production with elevated CO₂ could therefore alter local (alpha) diversity (Fig. 1).

On average, elevated CO₂ negatively affected seed production, although individual species were mostly not significantly affected (Fig. 1). Presumably, individual species responses were not significant because the replication of CO₂ treatments is low ($df = 2$ for this treatment). Regardless, the absence of strong positive seed production responses to elevated CO₂ was surprising, because many studies have found strongly positive effects of elevated CO₂ on seed production (Ackerly and Bazzaz 1995, Farnsworth and Bazzaz 1995, Huxman et al. 1999, LaDeau and Clark 2001, Jablonski et al. 2002, Thurig et al. 2003; but see Grunzweig and Korner 2000, 2001, Ramo et al. 2007). However, many of these

previous studies were performed on annual plants and crop plants, both more likely to show positive responses in seed production to the addition of any limiting resource (Jablonski et al. 2002, Miyagi et al. 2007). Seed production is directly linked to population growth for annuals, and crop plants have been selected by humans to respond to increased resources with increased seed production (Jablonski et al. 2002). By contrast, a greater boost to population growth might be gained by these perennial plants when excess carbon resources are allocated to survival and growth rather than reproduction (Bazzaz et al. 1987). Perhaps seed production for these species was more limited by nitrogen than carbon or water (Miyagi et al. 2007), because seeds typically contain higher concentrations of nitrogen than vegetative biomass does (Bazzaz et al. 1987) and nitrogen is extremely limiting at Cedar Creek. Thus, increased photosynthates that were produced with elevated CO₂ might have been allocated to vegetative growth of these perennials, not to seed production. Finally, seed production might only respond positively to elevated CO₂ in years where water availability is low (rainfall was average in 2002), as seen with productivity responses (Owensby et al. 1999, Morgan et al. 2004). More research is needed to distinguish between these possibilities.

The effects of nitrogen deposition on allocation to seed production was negatively correlated to effects on productivity (Fig. 2B), with similar responses within functional groups (Fig. 1). The mechanistic reason for the productivity response to nitrogen deposition is generally accepted: in competition, the abundance of species adept at acquiring nitrogen (C₄ grasses, legumes), the most limiting soil resource in this habitat, are negatively affected by its' addition; while inferior

competitors for nitrogen benefit most from its addition (C_3 grasses, forbs; Tilman 1984, Wedin and Tilman 1993, Suding et al. 2005). However, the reason behind the reproductive allocation responses to nitrogen deposition, opposite that of productivity, is less clear. One possibility is that productivity responses to nitrogen deposition directly determine how seed production will respond to nitrogen deposition, because of an inherent trade-off between allocation to vegetative growth vs. reproduction (Fig. 2). Nitrogen is extremely limiting at Cedar Creek, so this possibility seems likely (Tilman 1984, Bazzaz et al. 1987, Tilman and Wedin 1991, Wedin and Tilman 1993, HillerisLambers et al. 2004, Harpole and Tilman 2006). It is puzzling that biomass responses to elevated CO_2 , which reduces resource limitation by water, were not similarly negatively correlated with allocation to reproduction responses to elevated CO_2 (Fig. 1), but perhaps such a trade-off would only have been obvious in an extremely low rainfall year when water is most limiting (Morgan et al. 2004).

Declining diversity both increased and decreased seed production of these species (Fig. 1). No obvious traits unite those species positively vs. negatively affected by declining diversity; presumably because species are not responding directly to a diversity gradient. Declining diversity could increase (1) soil mutualists (Burrows and Pfleger 2002), (2) host-specific pathogens and predators (Mitchell et al. 2002), (3) pollinator visitation, and (4) limiting resources such as nitrogen and water (Tilman et al. 1996). Species with increased seed production in lower diversity plots may therefore be responding to lower interspecific competition or higher densities of soil mutualists, which could increase their seed production; or to greater densities of pollinators (which could increase seed set). On the other hand, seed production of species that decline with diversity may be negatively affected by greater intraspecific competition (HillerisLambers et al. 2004) or higher pathogen loads (Mitchell et al. 2002). Each of the biotic and abiotic factors may differentially affect inflorescence production and seed mass of these 11 species, resulting in the idiosyncratic responses found here. Additional observations or experiments are needed to determine how biotic and abiotic forces combine to determine seed production in low-diversity communities.

The effects of elevated CO_2 and declining diversity on seed production may be difficult to generalize from existing studies examining the responses of productivity to these global change factors (Ackerly and Bazzaz 1995, Farnsworth and Bazzaz 1995). Seed production responses to these two global change factors were uncorrelated with productivity responses and could not be generalized by functional group membership (Fig. 2). This indicates that perennial plants change their allocation patterns in response to global change in ways that are not necessarily linked to how vegetative growth is affected (in contrast to annuals; Jablonski et al. 2002). For example, the

aboveground productivity of *Lespedeza capitata* and *Solidago rigida* increases by more than 30% with elevated CO_2 , implying that altered competitive interactions favor these species, yet the seed production of both these species decreased by more than 20% (Fig. 2). Functional groups defined by reproductive characteristics (e.g., seed size, pollination vector, breeding system) rather than ecophysiology may be more predictive of seed production responses to elevated CO_2 and declining diversity (Diaz and Cabido 1997, Lavorel and Garnier 2002), although we did not observe any obvious patterns with regards to these reproductive characteristics.

Our results also illustrate the importance of measuring multiple response variables to detect effects of experimentally manipulated global change factors. Global change effects on inflorescence mass were much smaller than effects on inflorescence number per unit biomass (Fig. 1). In fact, global change effects on individual seed mass were even smaller in magnitude and never significant (data not shown). Possibly, the number of seeds produced per inflorescence as well as the mass of those seeds did not show a response to global change treatments because of allometric constraints (but see Thuring et al. 2003). Had we chosen only to measure seed size or seed mass per inflorescence as a metric of global change effects on seed production, we might have (mistakenly) concluded that elevated CO_2 , nitrogen deposition and declining diversity are unlikely to alter plant community structure by affecting seed production.

In summary, we demonstrate that global change, primarily nitrogen deposition, can strongly impact the seed production of co-occurring perennial plants in Minnesota, which could have dramatic implications for community dynamics. However, we are far from being able to predict the ecological consequences of such responses. With the exception of studies on the seed production responses to elevated CO_2 , there are few studies performed in field settings with which to compare our results. Many studies manipulate one global change factor (primarily CO_2) and examine reproductive responses, without considering the effects of the multiple environmental changes plant communities will be exposed to (but see Cleland et al. 2006, Ramo et al. 2007, Williams et al. 2007). Moreover, although ecophysiological traits, like photosynthetic pathway or nitrogen-fixing ability, can be predictive of vegetative responses to global change factors, they are not always predictive of seed production responses (Fig. 2; Morgan et al. 2004). Our poor mechanistic understanding of the biotic and abiotic factors that determine how perennial species allocate resources to seed production further complicates generalization. Predicting how seed production will be affected by global change will therefore require additional empirical studies, as well as a better understanding of the causal mechanisms behind reproductive responses to limiting resource addition (e.g., CO_2 , nitrogen) or to changes in frequency- or diversity-dependent processes.

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APPENDIX A

Biomass detection model (*Ecological Archives* E090-127-A1).

APPENDIX B

Percent cover detection model (*Ecological Archives* E090-127-A2).

APPENDIX C

Bayesian model fitting (*Ecological Archives* E090-127-A3).

APPENDIX D

Model fit (*Ecological Archives* E090-127-A4).