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LETTER

Fertilization decreases plant biodiversity even when light is not limiting

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Abstract

Many researchers hypothesize that plant richness declines at high soil fertility (and high productivity) due to light limitation. We tested this hypothesis in an old-field by independently manipulating fertilization and light levels via shade cloth (decreased light), vegetation tie-backs (increased light) and vegetation clipping (increased light). Droughts occurred during two of the four years of the study, and we found that higher light levels were generally associated with decreased plant richness in drought years but increased plant richness in wet years. Most importantly, fertilization decreased richness whether light availability limited richness (wet years) or did not limit richness (drought years), and the effects of fertilization and light manipulation treatments were additive. These results suggest that effects of fertilization on plant richness are at least partly independent of light levels and that competition for resources other than light plays a substantial role in the decline of plant richness after fertilization.

Keywords

Drought, eutrophication, light manipulation, old-field, plant species richness, productivity–biodiversity relationship, seed sowing, shade, species pool, temporal climate variability.

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INTRODUCTION

Biodiversity is central to the study of biology (Darwin 1872; Howard & Berlocher 1998), and research on the effects of human activities on biodiversity is becoming increasingly important as extinction levels increase (Ehrlich & Wilson 1991). Many factors are increasing extinction levels, but increased nitrogen deposition is predicted to be the third most important factor driving terrestrial extinctions (Vitousek *et al.* 1997; Sala *et al.* 2000; Clark & Tilman 2008).

Increased nutrient availability increases plant productivity, and species richness often is observed to increase from extremely low to moderate productivity but then decrease from moderate to high productivity (Rosenzweig & Abramsky 1993). This unimodal (hump-shaped) relationship is not universal (Mittelbach *et al.* 2001) and is primarily observed at particular spatial scales (Waide *et al.* 1999; Gross *et al.* 2000; Chase & Leibold 2002). However, plant species richness at small scales almost universally declines from moderate to high soil fertility (DiTommaso & Aarssen 1989). The increase in species richness from extremely low to moderate productivity is understood to be caused by a relaxation of environmental stress, but the reasons for the decrease in species richness from moderate to high productivity remain controversial (Rosenzweig & Abramsky 1993).

While many researchers hypothesize that intense competition for light is the primary factor responsible for declines in plant species richness at high soil fertility and high productivity (Newman 1973; Tilman 1988; Goldberg & Miller 1990), others suspect that these declines result from both light competition and belowground competition (Grime 1973; Grace 1990). The few community-level studies addressing the role of light limitation after fertilization have found contrasting results. A highly controlled glasshouse study showed that adding light to understory plants completely removed the negative effects of fertilization on plant species richness (Hautier *et al.* 2009). However, a fertilization and light manipulation study in the field showed that at least some of the decrease in plant species richness due to fertilization was independent of light levels (fig. 2B in Stevens & Carson 2002), and other field studies showed that shade cloth had no significant effect on plant richness (Rajaniemi 2002) and that greater belowground competition was the main factor responsible for declines in plant diversity after fertilization (Rajaniemi *et al.* 2003). These contrasting results likely reflect differences between studies with respect to the particular experimental methods used, the plant species used or the environmental conditions.

Our study was able to integrate many differences between previous studies. We independently manipulated light levels in an old-field community using three complementary experimental methods: shade cloth (decrease in light), vegetation tie-backs (increase in light) and vegetation clipping (increase in light). Fertilization was also independently manipulated, which can be thought of as another light manipulation treatment (decrease in light). In addition, we incorporated many different species in our study by experimentally increasing the size of the species pool via experimental seed sowing. These different species might be differentially affected by resource manipulations if fertilization and light levels affect extinction of existing species differently than the colonization of new species from the species pool (Tilman 1993; Dickson & Foster 2008; Hautier *et al.* 2009). Finally, we evaluated community responses to our manipulations across a range of different environmental conditions by measuring responses over a 4-year period encompassing both wet and dry years. Treatments may have different effects under different environmental conditions (Bakker *et al.* 2003; Vaughn & Young 2010), and studies conducted under different types of environmental conditions are especially important as climate change increases temporal variation in environmental conditions (Knapp *et al.* 2008).

Isolating the effect of light limitation on plant species richness from other factors associated with fertilization and increased productivity is a challenge because no single experimental method of manipulating light in the plant community is without problems. Light may be manipulated in herbaceous plant communities by using shade cloth to reduce light penetration (Rajaniemi 2002; Stevens & Carson 2002) or by clipping vegetation or tying back the canopy to increase light penetration (Wilson & Tilman 1993; Lamb *et al.* 2009). Each of these three approaches has advantages, but each also has shortcomings and the potential to introduce artefacts. In this study, we used all three of these approaches to experimentally manipulate light availability in the presence and absence of fertilization. Incorporating these complementary methods into a single experimental design provides a much more robust test of the effects of light limitation than using any one approach alone.

Here, we present results from a 4-year experiment conducted in an old-field plant community in Kansas. We tested the hypothesis that fertilization and increased productivity decrease plant species richness by leading to light limitation. In accord with previous predictions (Newman 1973; Tilman 1988), we predicted that experimentally decreasing light levels via shade cloth would cause a smaller decrease in richness when light levels were already low in fertilized plots than when light levels were higher in unfertilized plots. Similarly, we predicted that increasing light levels via clipping and vegetation tie-backs would cause a larger increase in richness in fertilized than non-fertilized plots. We also examined whether the experimental treatments affected existing and sown species differently, and we examined how the effects of treatments varied between wet years and drought years.

MATERIAL AND METHODS

The study was performed at the University of Kansas Field Station and Ecological Reserves in northeastern Kansas (95°19′07.4 W, 39°05′39.9 N). The study site was a 2-ha old-field abandoned from agriculture in 1970 consisting of Grundy Silty Clay Loam and Pawnee Clay Loam soil types with < 7% slope. No management has occurred except for mowing the field in 1986 and 1990. The field was dominated by *Solidago canadensis* (native perennial forb of clonal growth form) and was moderately productive (462 g m^{-2} of live aboveground biomass in 2004 control plots; see Figure S1) relative to other herbaceous sites (Gross *et al.* 2000).

In 2001, sixty-four 2 × 2 m plots were established across the field. To account for spatial heterogeneity across the field site, the 64 plots were blocked by spatial proximity resulting in eight experimental blocks. We applied eight unique treatment combinations to each block, conforming to a 4-factor incomplete factorial design with the following experimental manipulations: fertilization (no N fertilizer; 16 g N m^{-2} $year^{-1}$), clipping of the vegetation (not clipped; clipped), shade cloth (no shade cloth; shade cloth added) and vegetation tie-backs (no tie-backs; vegetation canopy tied back). In this design, fertilization and clipping were fully crossed as 2 × 2 factorial. However, the design is an incomplete factorial because tie-backs were applied only in non-clipped plots and shade cloth was applied only in clipped plots (see Figure S2). We incorporated an incomplete factorial design because not enough vegetation was present in clipped plots to successfully implement tie-backs for part of the growing season.

In April 2001, two 0.5 × 0.5 m subplots were established in opposite corners of each plot, such that each subplot was at least 0.3 m from the edge of each plot. We only examined resident species during 2001 and 2002, but we randomly chose one of the two subplots in each plot and sowed 45 locally growing plant species in March 2003 and 2004 at the rate of 400 seeds m^{-2} $year^{-1}$ for each species (see Table S1). Our seeding rate was at the high end of natural seed rain in prairies, but still lower than has been recorded for at least 20% of the prairie species sampled by Rabinowitz & Rapp (1980).

We hand broadcast 29–3–4 N–P–K Scotts Turf Builder fertilizer evenly over the entire plot area in the fertilized treatment. Half the fertilizer was added in April and the other half was added in July of each year. A total of 16 g N m^{-2} $year^{-1}$ was used because this is the maximal amount used by nearby landowners to fertilize grazing fields. The same level of fertilization caused a decrease in soil pH from 6.0 to 5.7 after 4 years in an experiment located < 500 m from our study (Foster *et al.* 2010). This level of pH change is unlikely to strongly affect vegetation (Troeh & Thompson 2005), but to be cautious we equalized pH by adding 10.2 g m^{-2} of powdered lime with 60% effective calcium carbonate to the soil surface of fertilized plots in late April 2004 (Whitney & Lamond 1993). The clipped plots were hand cut with hedge shears to 6 cm height in early June of each year and all clipped vegetation (but not ground litter) was removed from the plot. Shade cloth captured 86% of light and covered the entire plot and the sides of a 61-cm height PVC frame. Vegetation tie-backs were implemented by cutting 0.5 × 0.5 m holes into plastic netting, and then placing the hole over each subplot and pulling the vegetation underneath the netting surrounding the subplot (see Figure S3). In this way, the vegetation canopy inside the subplot was prevented from shading the subplot, even though vegetation outside the subplot still cast shade into the subplot. Shade cloth and tie-backs were added in June immediately after clipping and were removed each year at the end of the growing season.

We utilized clipping, shade cloth and vegetation tie-backs because each treatment alters light levels independently of fertilization, but each treatment is also likely to alter more than just light levels. For example, pulling vegetation underneath tie-backs could potentially damage individual plants as well as increasing light levels. If the changes in light penetration due to each light manipulation treatment affect plant species richness the same way, then we have good evidence that plant richness is being affected by alterations in light levels rather than confounding factors.

Environmental conditions changed over the course of the experiment, which created an ideal situation to test the effects of treatments in wet and drought years. There were abnormally dry to severe drought conditions July–September 2002, abnormally dry to extreme drought conditions July–September 2003 and no drought conditions during the 2001 and 2004 growing seasons (see Figure S4; NDMC *et al.* 2011).

We identified every plant species within each subplot September 2001–2004, and we estimated the per cent groundcover of each species. We used cardboard cutouts of known cover amounts to calibrate our estimates. Light levels were measured from all subplots in August or September of 2001–2004, and plant biomass was collected at the conclusion of the experiment (5–12 September 2004; more information in Table S2). Immediately outside subplots, we measured the following: soil ammonium and nitrate availability 20–26 July 2004 using cation and anion membrane resin strips; soil moisture levels 12 June 2003 and 23 June 2004 using a soil moisture probe; and soil temperature July–August 2004 using soil temperature data loggers (more information in Table S2). We measured soil nitrogen, soil moisture and soil temperature outside subplots because the measurements required disturbing the soil, and we did not want to disturb soil in the location where light levels, species richness and plant groundcover were being sampled.

Statistical analyses

We performed all statistical analyses using PROC MIXED in SAS 9.1.3 with Service Pack 4 (Cary, NC, USA). We examined the potential interactions between fertilization and the individual light manipulation treatments (shade cloth, vegetation tie-backs and clipping) on species richness and light penetration. Therefore, we used the following treatment subsets to perform fully factorial analyses of variance (ANOVAs; see Figure S2): (1) we used only data from clipped plots to analyse the interaction between fertilization and shade cloth; (2) we used only data from non-clipped plots to analyse the interaction between fertilization and vegetation tie-backs and (3) we used only data from plots without shade cloth or vegetation tie-backs to analyse the interaction between fertilization and clipping. We examined whether the effects of these treatments varied across wet and drought years by performing repeated-measures ANOVAs. We used non-sown richness and sown richness separately as response variables to simplify results and focus on the interactions between fertilization, light manipulation treatments and year (more information in Table S2). In the supporting information, we also show results from more complicated analyses where we analyse non-sown and sown species together in split-plot, repeated-measures ANOVAs (Table S3; Figures S5 and S6).

We examined whether the relationship between measured light levels and plant species richness changed in drought vs. wet years or changed due to fertilization. We performed repeated-measures analysis of covariance (RM-ANCOVA, also referred to as heterogeneity of slopes) with fertilization and year as the categorical variables and light penetration as the continuous variable. Measured light penetration was affected by the light manipulation treatments (shade cloth, vegetation tie-backs and clipping), so using light penetration as a predictor variable incorporated the effects of these treatments on light penetration. We performed two RM-ANCOVAs, one with the response variable of non-sown species richness 2001–2004 and the other with sown species richness 2003–2004. In the ANCOVAs, we used the 'estimate' function in SAS to estimate the significance of the relationship between light levels and species richness in different years.

All analyses over multiple years were repeated measures using the covariance structure with the lowest Akaike Information Criterion: compound symmetry for all analyses, except for the repeated-measure ANOVA testing the interaction between fertilization and vegetation tie-backs on light penetration where first-order ante dependence covariance structure was used (Littell *et al.* 2002). First-order ante dependence with the Kenward–Roger method led to uneven numbers in the F-value denominator degrees of freedom.

We used the 'slice' function to analyse treatment effects in individual years (Littell *et al.* 2002). This is similar to performing a contrast except slice uses an error term calculated across all years.

The blocking term was added to all analyses (except soil temperature) to remove any spatial variation and the effects of blocking are not reported. The blocking term was not added to the soil temperature analysis because several temperature data loggers were lost, which created an unbalanced analysis (see Table S2). Both blocking and year were analysed as random effects and all other treatments were analysed as fixed effects.

RESULTS

Effects of treatments on light penetration

We found that both fertilization and light manipulation treatments affected light penetration (Table 1; Fig. 1). Fertilization decreased light penetration in all years (all slice effects: $P < 0.05$), but decreased light penetration less in drought years than wet years (year × fertilizer interaction; Fig. 1d–f). Shade cloth decreased light penetration in all years (all slice effects: $P < 0.05$), but decreased light penetration more in drought years, when light penetration was high, than in wet years, when

Table 1 ANOVA analyses of light penetration and species richness (significant effects are in bold)

	Light penetration		Non-sown richness		Sown richness	
Shade (Sh)	$\mathbf{F_{1,21} = 485.04}$	$\mathbf{P < 0.001}$	$F_{1,21} = 1.08$	$P = 0.312$	$F_{1,21} = 0.28$	$P = 0.602$
Fertilizer (F)	$\mathbf{F_{1,21} = 25.81}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,21} = 22.08}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,21} = 21.03}$	$\mathbf{P < 0.001}$
Sh × F	$F_{1,21} = 1.79$	$P = 0.195$	$F_{1,21} = 0.47$	$P = 0.500$	$F_{1,21} = 0.12$	$P = 0.728$
Year (Y)	$\mathbf{F_{3,84} = 45.49}$	$\mathbf{P < 0.001}$	$\mathbf{F_{3,84} = 55.53}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,28} = 158.33}$	$\mathbf{P < 0.001}$
Y × Sh	$\mathbf{F_{3,84} = 4.57}$	$\mathbf{P = 0.005}$	$\mathbf{F_{3,84} = 9.85}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,28} = 19.01}$	$\mathbf{P < 0.001}$
Y × F	$\mathbf{F_{3,84} = 3.13}$	$\mathbf{P = 0.030}$	$F_{3,84} = 0.06$	$P = 0.982$	$\mathbf{F_{1,28} = 11.31}$	$\mathbf{P = 0.002}$
Y × Sh × F	$F_{3,84} = 1.58$	$P = 0.201$	$F_{3,84} = 2.01$	$P = 0.119$	$F_{1,28} = 0.14$	$P = 0.711$
Tie back (T)	$\mathbf{F_{1,32.8} = 17.52}$	$\mathbf{P < 0.001}$	$F_{1,21} = 1.23$	$P = 0.281$	$F_{1,21} = 1.06$	$P = 0.316$
Fertilizer (F)	$\mathbf{F_{1,32.8} = 72.63}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,21} = 19.62}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,21} = 21.62}$	$\mathbf{P < 0.001}$
T × F	$F_{1,32.8} = 0.27$	$P = 0.604$	$F_{1,21} = 1.64$	$P = 0.215$	$F_{1,21} = 0.54$	$P = 0.471$
Year (Y)	$\mathbf{F_{3,36.2} = 38.21}$	$\mathbf{P < 0.001}$	$\mathbf{F_{3,84} = 23.25}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,28} = 128.14}$	$\mathbf{P < 0.001}$
Y × T	$\mathbf{F_{3,36.2} = 3.16}$	$\mathbf{P = 0.036}$	$F_{3,84} = 2.21$	$P = 0.093$	$F_{1,28} = 0.60$	$P = 0.446$
Y × F	$\mathbf{F_{3,36.2} = 3.39}$	$\mathbf{P = 0.028}$	$F_{3,84} = 2.13$	$P = 0.102$	$\mathbf{F_{1,28} = 6.25}$	$\mathbf{P = 0.019}$
Y × T × F	$F_{3,36.2} = 1.52$	$P = 0.226$	$F_{3,84} = 0.40$	$P = 0.752$	$F_{1,28} = 1.09$	$P = 0.305$
Clipping (C)	$F_{1,21} = 1.92$	$P = 0.180$	$F_{1,21} = 3.69$	$P = 0.068$	$F_{1,21} = 0.56$	$P = 0.463$
Fertilizer (F)	$\mathbf{F_{1,21} = 42.31}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,21} = 27.05}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,21} = 15.90}$	$\mathbf{P < 0.001}$
C × F	$F_{1,21} = 0.40$	$P = 0.536$	$F_{1,21} = 2.50$	$P = 0.129$	$F_{1,21} = 0.44$	$P = 0.514$
Year (Y)	$\mathbf{F_{3,84} = 59.72}$	$\mathbf{P < 0.001}$	$\mathbf{F_{3,84} = 29.45}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,28} = 135.28}$	$\mathbf{P < 0.001}$
Y × C	$F_{3,84} = 1.13$	$P = 0.344$	$\mathbf{F_{3,84} = 6.80}$	$\mathbf{P < 0.001}$	$F_{1,28} = 0.85$	$P = 0.364$
Y × F	$\mathbf{F_{3,84} = 4.78}$	$\mathbf{P = 0.004}$	$F_{3,84} = 1.25$	$P = 0.296$	$\mathbf{F_{1,28} = 4.51}$	$\mathbf{P = 0.043}$
Y × C × F	$F_{3,84} = 1.18$	$P = 0.322$	$F_{3,84} = 1.49$	$P = 0.224$	$F_{1,28} = 0.42$	$P = 0.523$

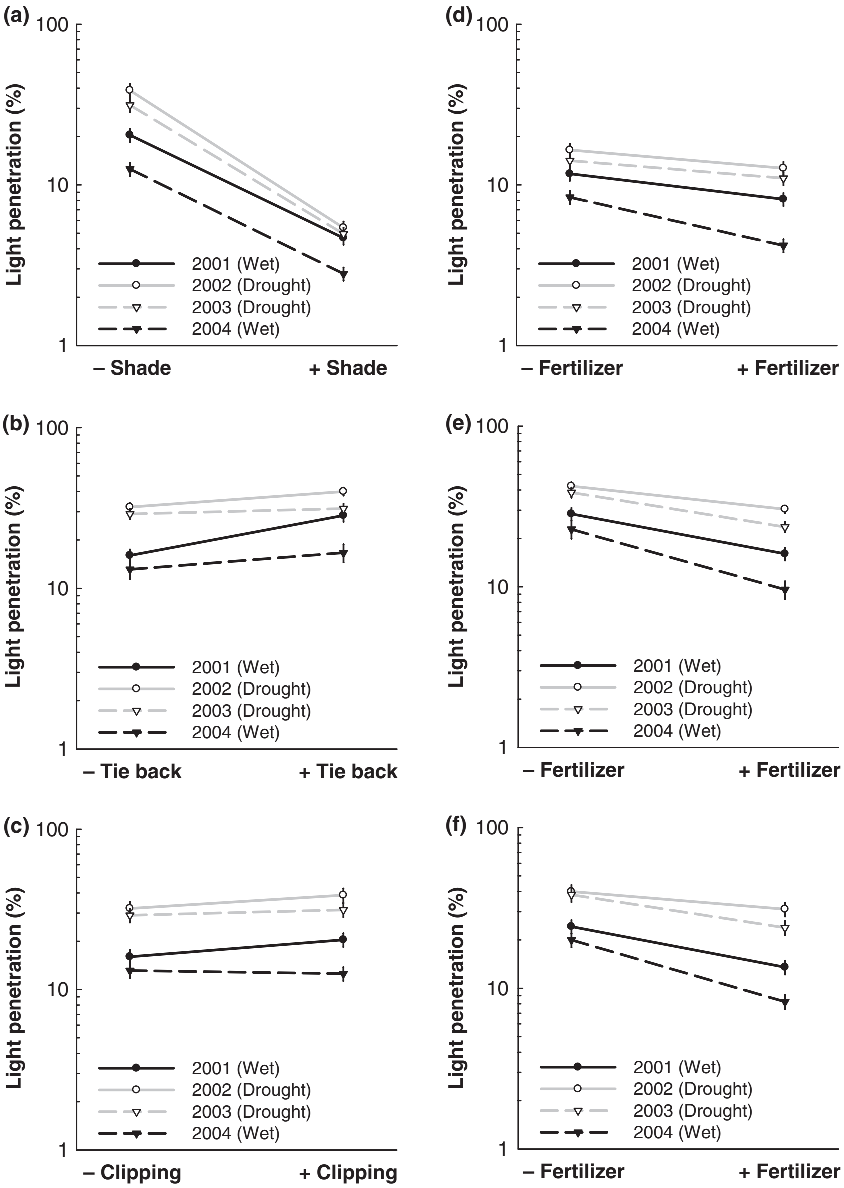

Figure 1 The response of light penetration to shade cloth (a) and fertilization (d), vegetation tie-backs (b) and fertilization (e), and clipping (c) and fertilization (f) in wet and drought years (lines are shown to visualize change from the absence to the presence of the treatment; ±1 standard error).

light penetration was already low (year × shade interaction; Fig. 1a). Tie-backs increased light penetration in 2001 (slice: $F_{1,20.3} = 22.25$; $P < 0.001$) and 2002 (slice: $F_{1,18.1} = 8.35$; $P = 0.010$), but did not significantly affect light penetration in 2003 or 2004 (year × tie-back interaction; Fig. 1b). Shade cloth had a greater effect on light penetration than did vegetation tie-backs (Fig. 1a,b). Although light penetration must have increased immediately after clipping, enough biomass grew back to prevent the clipping treatment from significantly affecting late growing season light penetration (Fig. 1c).

Effects of shade cloth on species richness (as evaluated within clipped plots)

Shade cloth had opposite effects on species richness in wet and drought years. Shade cloth decreased non-sown species richness in the wet year of 2001 (slice: $F_{1,70.8} = 16.36$; $P < 0.001$), increased non-sown richness in the drought year of 2002 (slice: $F_{1,70.8} = 5.14$; $P = 0.027$) and did not significantly affect non-sown richness in 2003 and 2004 (year × shade interaction; Table 1; Fig. 2a). Shade cloth decreased sown species richness in the wet year of 2004 (slice: $F_{1,39.8} = 8.71$; $P = 0.005$) and increased sown richness in the drought year of 2003 (slice: $F_{1,39.8} = 4.36$; $P = 0.043$) (year × shade interaction; Table 1; Fig. 3a).

Fertilization decreased non-sown species richness from 10.3 to 8.1 on average (fertilizer main effect; Table 1). Fertilization decreased sown species richness in the wet year 2004 (slice: $F_{1,39.8} = 32.34$; $P < 0.001$), but sown richness was already low in a drought year and was not decreased much further by fertilization (2003 slice: $F_{1,39.8} = 3.24$; $P = 0.080$; year × fertilizer interaction; Table 1; Fig. 3d).

Effects of vegetation tie-backs on species richness (as evaluated within non-clipped plots)

Tie-backs increased non-sown species richness in the wet year of 2004 (slice: $F_{1,60.6} = 4.73$; $P = 0.034$), but did not significantly affect non-sown richness in other years (trend towards a year × tie-back interaction; Table 1; Fig. 2b). Tie-backs did not significantly affect sown species richness in either 2003 or 2004 (Table 1; Fig. 3b).

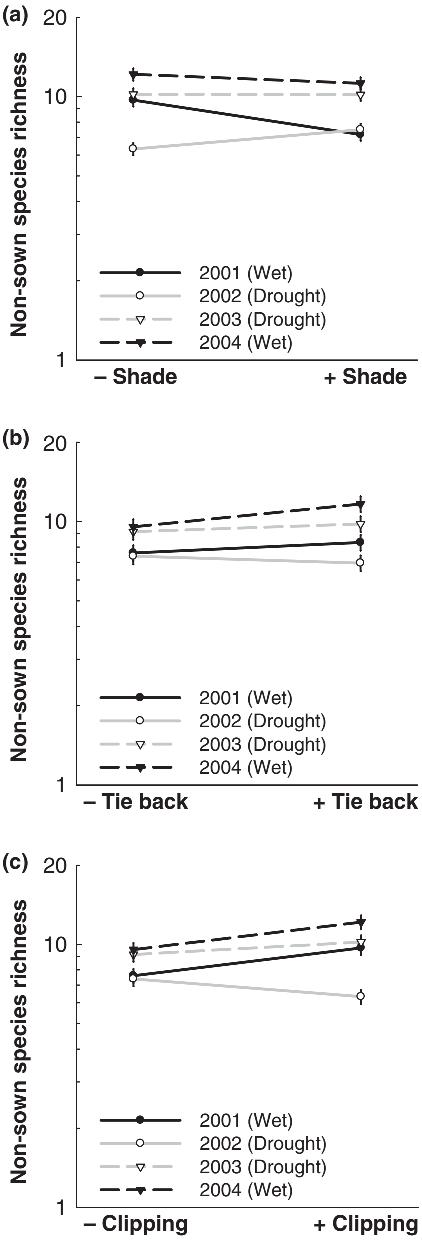

Figure 2 The response of non-sown plant species richness to shade cloth (a), vegetation tie-backs (b) and clipping (c) in wet and drought years (lines are shown to visualize change from the absence to the presence of the treatment; ±1 standard error).

Fertilization decreased non-sown species richness from 10.1 to 7.5 on average (fertilizer main effect; Table 1). Fertilization decreased sown species richness in the wet year 2004 (slice: $F_{1,48.8} = 24.97$; $P < 0.001$), but sown richness was already low in the drought year 2003 and was not significantly decreased by fertilization (year × fertilizer interaction; Table 1; Fig. 3e).

Effects of clipping on species richness (as evaluated in the absence of shade cloth and tie-backs)

Clipping increased non-sown species richness in the wet years of 2001 (slice: $F_{1,74.3} = 8.34$; $P = 0.005$) and 2004 (slice: $F_{1,74.3} = 8.14$; $P = 0.006$), showed a trend towards a decrease in non-sown richness in the drought year of 2002 (slice: $F_{1,74.3} = 3.37$; $P = 0.070$) and did not significantly affect non-sown richness in 2003 (year × clipping interaction; Table 1; Fig. 2c). Clipping did not significantly affect sown species richness (Table 1; Fig. 3c).

Fertilization decreased non-sown species richness from 10.3 to 7.6 on average (fertilizer main effect; Table 1). Fertilization decreased sown species richness in the wet year 2004 (slice: $F_{1,46.1} = 19.22$; $P < 0.001$), but sown richness was already low in the drought year 2003 and was not significantly decreased by fertilization (year × fertilizer interaction; Table 1; Fig. 3f).

Relationship between light penetration and species richness

The relationship between light penetration and species richness varied between drought and wet years (year × light interactions; Table 2). There was a significant, positive relationship between light penetration and non-sown richness in a wet year (2001 light: $t_{235} = 1.97$; $P = 0.0498$) and non-significant relationships in other years (Fig. 4a). There was also a significant, negative relationship between light penetration and sown richness in a drought year (2003 light: $t_{114} = -2.03$; $P = 0.045$) and a trend towards a positive relationship in a wet year (2004 light: $t_{115} = 1.72$; $P = 0.088$; Fig. 4b).

DISCUSSION

Two main conclusions can be drawn from this study. First, we did not find evidence to support the hypothesis that plant coexistence and species richness are controlled mainly by light levels in the presence of fertilization (Newman 1973; Tilman 1988), suggesting this hypothesis should be revisited. Instead, the results suggest that fertilization decreased plant species richness at least partly independent of light levels. Specifically, fertilization decreased plant richness whether there was a positive or negative relationship between light levels and richness. Also, the effects of the light manipulation treatments and fertilization on plant richness were additive (Table 1), whereas the light manipulation treatments were expected to interact with fertilization if light levels control species richness more in the presence than absence of fertilization (Newman 1973; Tilman 1988).

The role of light levels in controlling plant species richness at high soil fertility is important because it separates between two large bodies of theory (Grime 1973; Newman 1973; Tilman 1988; Grace 1990; Huston 1994). Several multi-species studies have been completed to attempt to separate between these two bodies of theory, but the results of field studies have differed from the results of a glasshouse study. Field results are suggestive of belowground competition increasing with fertilization (Rajaniemi 2002; Rajaniemi *et al.* 2003) or of fertilization affecting species richness partly independent of light levels (fig. 2B in Stevens & Carson 2002). Results of the elegantly designed glasshouse experiment of Hautier *et al.* (2009) suggest that light limitation controls the reduction in plant species richness due to fertilization.

Our study may help to explain some of the differences between previous studies since we found that environmental conditions could alter the importance of light levels in controlling plant richness. However, some differences between field studies and the glasshouse study remain unexplained. Specifically, if the glasshouse results are representative of the processes occurring in the field, then one would expect that declining light penetration would explain nearly all of the observed decline in plant species richness due to fertilization.

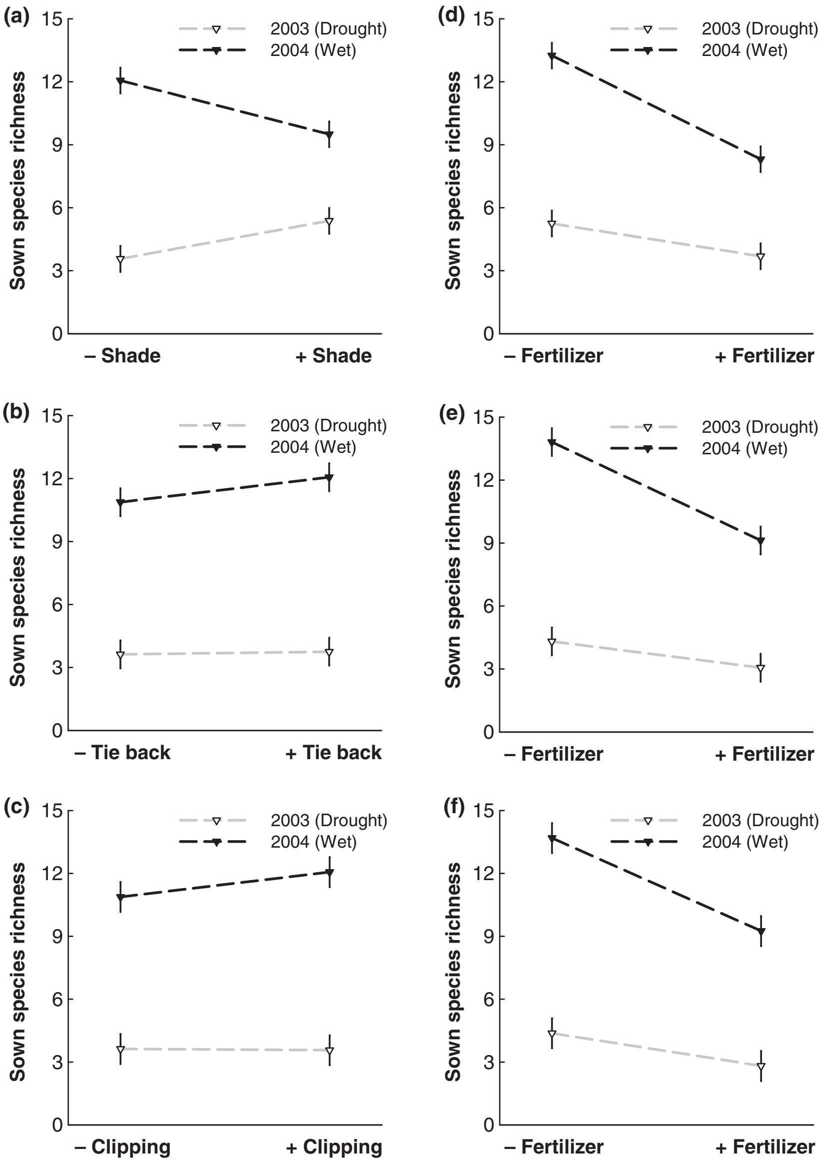

Figure 3 The response of sown plant species richness to shade cloth (a) and fertilization (d), vegetation tie-backs (b) and fertilization (e), and clipping (c) and fertilization (f) (lines are shown to visualize change from the absence to the presence of the treatment; ±1 standard error).

However, in the current study measured light levels explained much less of the decline in species richness than did the fertilization treatment (Table 2), and in previous studies measured light penetration in the field did not fully explain the decline in total plant richness due to fertilization (Rajaniemi 2002; Stevens & Carson 2002; Rajaniemi *et al.* 2003). The glasshouse study (Hautier *et al.* 2009) could have produced different results than field studies due to inherent differences between conditions in the glasshouse and the field. Water was never limiting in the glasshouse experiment, leading to a greater likelihood of finding light limitation. Also, the above-canopy light intensity (1.5 m above the ground) was 470 μmol m^{-2} s^{-1} in the glasshouse (Yann Hautier, personal communication), whereas we found an above-canopy light intensity of 1500+ μmol m^{-2} s^{-1}. A lower light intensity in the glasshouse than the field could accentuate any light limitation effects because light may already be limiting understory plant growth in the absence of fertilizer and may become extremely limiting in the presence of fertilizer. The glasshouse study (Hautier *et al.* 2009) was also completed in shallow soil (27 cm depth) which may have altered belowground competition from the deeper soil conditions typically found in the field. Whatever the reasons for the differences between the glasshouse and field results, it does not appear that light levels completely mediate the effects of fertilization on plant richness in the field.

If decreases in plant richness are at least partly independent of light under fertilized conditions, especially under variable environmental conditions, then some hypotheses of coexistence after fertilization must be revamped (Newman 1973; Tilman 1988). Specifically, predation, competition for resources other than light and other mechanisms may play a substantial role in the decline of plant richness after fertilization (Rosenzweig & Abramsky 1993; Rajaniemi *et al.* 2003; Stevens *et al.* 2004). More studies in the future should examine how mechanisms other than light penetration control plant richness after fertilization.

In this study, it appears that light levels were primarily driving the effects of the light manipulation treatments (shade cloth, vegetation tie-backs and clipping) on plant species richness. Light manipulation treatments have the potential to affect additional environmental variables, as well as light levels. However, there are several reasons

Table 2 ANCOVA analyses of species richness (significant effects are in bold)

	Non-sown richness		Sown richness	
Fertilizer (F)	$\mathbf{F_{1,57} = 38.69}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,55.8} = 35.41}$	$\mathbf{P < 0.001}$
Year (Y)	$\mathbf{F_{3,193} = 7.10}$	$\mathbf{P < 0.001}$	$\mathbf{F_{1,74.5} = 6.30}$	$\mathbf{P = 0.014}$
Y × F	$F_{3,185} = 1.34$	$P = 0.262$	$\mathbf{F_{1,62.6} = 7.19}$	$\mathbf{P = 0.009}$
Light (L)	$F_{1,117} = 0.26$	$P = 0.615$	$F_{1,69.4} = 0.08$	$P = 0.779$
Y × L	$F_{3,189} = 2.14$	$P = 0.096$	$\mathbf{F_{1,72.2} = 7.55}$	$\mathbf{P = 0.008}$

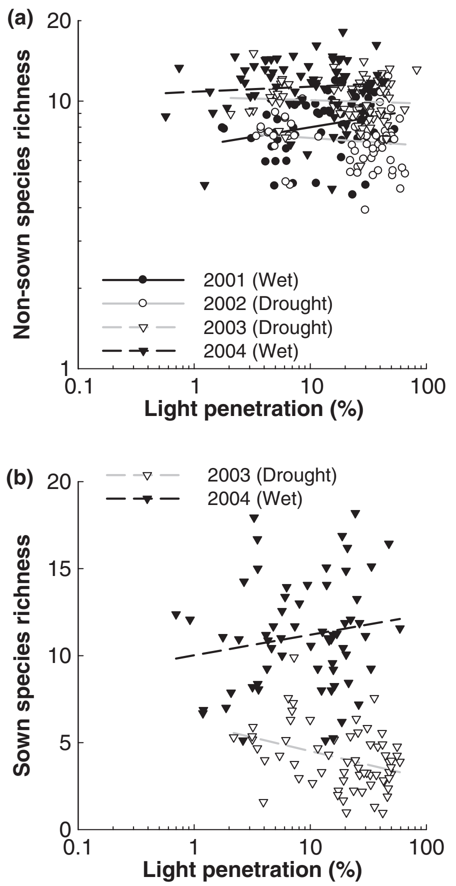

Figure 4 The relationship between non-sown plant species richness and light penetration (a) and sown plant species richness and light penetration (b). Data points show richness minus the effects of blocking and fertilization. Regression slopes are shown to aid in visualizing the year × light interactions.

why it is unlikely that the effects of light manipulation treatments on plant species richness are driven by these additional environmental variables (live aboveground biomass, available soil nitrogen, soil moisture and soil temperature) instead of by light levels.

First, the effects of light manipulation treatments on plant species richness varied from wet to drought years, similar to other studies where natural shade can either increase or decrease plant survival and seedling establishment depending upon environmental stress (Callaway & Walker 1997; Suding & Goldberg 1999). It seems unlikely that environmental variables such as available soil nitrogen would have different effects in different years. Second, all three of the light manipulation treatments affected plant species richness and light levels, as did fertilization, while none of the additional environmental variables, with the possible exception of soil temperature, were affected by all three of the light manipulation treatments (see Table S4). Soil moisture was not affected by shade cloth (see Table S4), which is similar to results from other studies (Williams *et al.* 1993; Stevens 1999; but see Davis *et al.* 1998). Third, none of the environmental variables except soil temperature showed a significant correlation with plant species richness (see Figure S7). Overall, this suggests that none of the additional environmental variables, except possibly soil temperature, were consistently confounding the effects of the light manipulation treatments on species richness. In the case of soil temperature, light penetration to the ground was almost certainly the main factor affecting this variable. Separating the effects of light limitation on richness from effects arising through changes in soil temperature would be very difficult outside a temperature controlled experiment, but such a study should be completed in the future.

The second main conclusion from this study is that low light levels only inhibit plant richness when water availability does not severely limit plant survival. This study is the first we are aware of that examines the relationship between fertilization and plant richness in wet and drought years. When droughts occur, it appears that low light levels facilitate higher plant richness. Although other studies have shown that plant growth can be either inhibited or facilitated by shade under different climatic conditions (Callaway & Walker 1997), this study is the first we are aware of that shows plant richness can be either inhibited or facilitated by low light levels under different climatic conditions.

More generally, previous models of the effects of light levels on biodiversity along a productivity gradient have made the implicit assumption that light will always limit plant species richness and survival at high soil fertility and productivity (Rajaniemi 2003 and references therein). However, long-term studies increasingly reveal that the effects of ecological phenomenon change over time depending upon environmental conditions (Vaughn & Young 2010 and current study). It may improve future models to include temporal variability and the possibility of light competition or facilitation depending upon environmental conditions.

Temporal variability in climatic conditions is predicted to increase in the future due to climate change (Knapp *et al.* 2008). There will be increased variability in precipitation and temperature, leading to higher incidence of drought and extreme rainfall events. Therefore, the droughts observed in this study, and the alternation of facilitation and competition due to low light levels, are likely to become even more common in the future.

Much of the world is now fertilized either intentionally through fertilizer application or unintentionally through atmospheric nutrient deposition (Clark & Tilman 2008). This fertilization will likely cause decreases in plant species richness at small scales and may lead to plant extinctions. The current study suggests that these decreases in plant biodiversity will be at least partly due to factors not related to light levels. Future studies should examine how these other factors can decrease plant biodiversity after fertilization.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 The effects of treatments on live aboveground biomass (error bars are + 1 standard error).

Figure S2 A flow chart representing the 4-factor incomplete factorial design (resulting in eight unique experimental treatments).

Figure S3 Photograph of a vegetation tie-back.

Figure S4 Average monthly temperature and precipitation as measured by a weather station located 200 m from the experiment.

Figure S5 The response of total plant species richness to shade cloth and fertilization (a), tiebacks and fertilization (b), and clipping and fertilization (c) in wet and drought years (+ 1 standard error).

Figure S6 Contrasts of the response of total plant species richness to drought vs. wet years in the presence of shade cloth and fertilization (a), tie-backs and fertilization (b), and clipping and fertilization (c) (+ 1 standard error).

Figure S7 The relationship between environmental variables and either sown species richness or plot-level total species richness.

Table S1 Characteristics of species sown in 2003 and 2004.
Table S2 Protocols used to measure different variables and to analyse and present data.
Table S3 Split-plot, repeated-measures ANOVA analyses of species richness (significant effects are in bold).
Table S4 The effects of treatments on different environmental variables (NA refers to data that were collected outside subplots with tie-backs; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

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