

Establishment limitation reduces species recruitment and species richness as soil resources rise

M. HENRY, H. STEVENS, DANIEL E. BUNKER*, STEFAN A. SCHNITZER† and WALTER P. CARSON*

338 Pearson Hall, Department of Botany, Miami University, Oxford, OH 45056, USA, *A234 Langley Hall, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA, and †Department of Biological Sciences, University of Wisconsin–Milwaukee, Milwaukee, WI 53201, USA

Summary

1 At local spatial scales, species richness tends to fall as productivity rises. Most explanations have focused on increased extinction, but, instead, we test experimentally whether increased soil fertility reduces recruitment. Specifically, we test whether variation in recruitment is due to source limitation, germination limitation or establishment limitation, and how litter accumulation and seed predation contribute to these processes.

2 We established four crossed experimental treatments in a perennial-dominated early successional plant community over 3 years. We added seed of 30 species, manipulated access by selected seed predators, removed litter and added slow release fertilizer at four levels (0, 8, 16 and 32 g N m⁻²).

3 Species recruitment and richness both decreased with increasing fertility, but, counter to our expectations, we found that neither seed additions nor litter removal could counteract the negative effects of fertilizer.

4 Seed additions increased seedling density at all fertilizer levels, and seed predation appeared to have no influence on seedling densities. In spite of high seedling densities at all fertilizer levels, final stem density declined by 70% as fertilizer increased. A strong stem density–species richness relationship suggests that declines in final stem density caused more than half of the decline in species richness along this fertility gradient.

5 These results suggest that establishment limitation, i.e. the reduction of growth and survival from seedling to adult, controls species recruitment in highly fertile sites.

6 The high degree of recruitment limitation commonly observed in productive habitats suggests that high productivity causes establishment limitation, thereby isolating these communities from the regional species pool. We suggest that such isolation provides a mechanism to explain why the species composition of productive communities exhibits higher variability than the composition of less productive communities within the same regional source pool.

Key-words: canonical lognormal species abundance distribution, colonization, competition, diversity, extinction, immigration, litter, productivity, richness, seed predation

Journal of Ecology (2004) **92**, 339–347

Introduction

The number of species observed in small samples frequently increases and then declines as productivity (g C m⁻² y⁻¹) rises from zero along natural productivity gradients (Stevens & Carson 1999b; Waide *et al.* 1999). Theories aimed at explaining the decline of richness at high productivity have generally assumed that productivity varies due to variation in resource availability

(Mittelbach *et al.* 2001). Indeed, declines in species richness and increases in productivity are ubiquitous along experimental nutrient gradients (DiTomaso & Aarssen 1989; Gough *et al.* 2000). What controls the loss of species along both natural and experimental productivity gradients remains unknown (Mittelbach *et al.* 2001). Here we use an experimental nutrient gradient to test selected mechanisms recently proposed to control richness along such gradients.

Rates of extinction and recruitment determine the number of species in a local community (MacArthur & Wilson 1963; Grace 2001; Hubbell 2001). Both theory

(Tilman 1982; Holt *et al.* 1994; Kassen *et al.* 2000) and experiments (Bohannan & Lenski 2000; Rajaniemi 2002; Stevens & Carson 2002) have focused on how competition and predation influence the productivity–diversity relationship via effects on the rates of local extinction (Rajaniemi 2003). In contrast, few studies have assessed the role of recruitment by measuring variation in recruitment rates along fertility gradients, or testing how competition or predation may cause such variation. Tilman (1993) and Wilson & Tilman (2002) showed that recruitment rate falls as soil resources and standing crop biomass rise. Foster (2001) used seed additions along naturally occurring gradients of standing crop biomass to demonstrate experimentally that recruitment falls as standing crop biomass increases. Thus recruitment limitation appears to contribute to patterns of plant species richness along productivity gradients, but the mechanisms underlying this phenomenon have remained untested.

A variety of mechanisms, operating at several life-history stages, may contribute to variation in recruitment rate (Nathan & Muller-Landau 2000). Recruitment limitation may result from any or all of *source limitation* (recruits fail to arrive at a site due to reduced fecundity of adults or reduced dispersal of propagules), *germination limitation* (site conditions prevent or reduce seed germination or increase seed mortality), and *establishment limitation* (seedlings fail to mature).

Biotic and abiotic conditions of fertile, nutrient-rich habitats are inextricably linked to conditions associated with high productivity, and these conditions appear likely to reduce all stages of recruitment. Fertile and productive habitats have been characterized by low light levels, heavy litter layers, high levels of above-ground competition or increased competition intensity and seed predation (e.g. Carson & Peterson 1990; Reader & Beisner 1991; Wilson & Tilman 1991). Such studies, however, do not distinguish the mechanism whereby these factors reduce overall recruitment.

We tested how reduced recruitment limits species richness at high fertility in a perennial-dominated early successional plant community by adding seed of 30 species in small plots with and without cages, with and without litter at various points along an experimental fertility gradient. We define recruitment as the absence of a species in the first census and its presence in the last census. These experiments tested hypotheses arising from each of the possible mechanisms that may contribute to reduced recruitment.

Source limitation. Increasing fertility reduces fecundity of subordinate species and seed additions would therefore eliminate negative effects of fertilizer on all recruitment parameters (i.e. seedling density, species recruitment and species richness).

Germination limitation. Increasing fertility reduces germination through effects on germination cues and seed survival and seed additions should therefore not eliminate or ameliorate the negative effects of fertilizer. If heavy litter layers or high levels of seed predation in

fertile sites contribute to reduced germination, then litter removal and predator exclosures would increase recruitment parameters to a greater degree in fertile sites than unfertile sites. If established vegetation in fertile sites reduces germination, then we should observe a negative effect of fertility on seedling density where litter has been removed.

Establishment limitation. Established individuals in fertile sites prevent seedling growth and survival, so the probability of a seedling dying will increase with fertility. Seed additions should thus eliminate the negative effect of fertilizer on seedling density, but the probability of a seedling dying should increase with fertility, causing final stem density to decline with increasing fertilizer. Seed additions should not eliminate the negative effects of fertilizer on species recruitment or species richness.

Methods

We conducted this study at the Pymatuning Laboratory of Ecology in north-western Pennsylvania, USA (UTM Zone 17, N 4610737, E 552046). Soils at the study site are poorly drained French Silt Loams (USSCS 1979). Average annual precipitation of 106 cm is distributed fairly evenly throughout the year. In October 1995, we sprayed with herbicide and ploughed a 2-ha portion of an oldfield that had previously been abandoned for more than 10 years. By the fourth year of succession, *Agropyron repens*, *Solidago rugosa*, *Euthamia graminifolia*, *Rubus allegheniensis* and *Rubus flagillaris* (Gleason & Cronquist 1991) dominated the area used in this experiment (Stevens 1999). In April 1996, we established 48, 6 × 4 m main plots, divided into two blocks of 24 main plots each. Each main plot contained two 1 × 1 m split plots, which were each divided into two 1 × 0.5 m split-split plots (see Fig. 1 for details). In late spring of each year (1996–99), we fertilized main plots at four different rates (Osmocote® slow release fertilizer, Scott-Sierra Agricultural Products, Marysville, OH, USA, 18-6-12 NPK, at rates of 0, 8, 16 and 32 g N m⁻² year⁻¹). Fertilizer resulted in linearly proportional increases in mean above-ground biomass from c. 350 g dry biomass m⁻² in control plots to nearly 800 g dry biomass m⁻² in high fertility plots (Stevens 1999). This resulted in approximately an order of magnitude decline in subcanopy light levels (Stevens 1999; Stevens & Carson 2002). In November of 1997 and 1998, we removed by hand all standing and fallen litter in one of each pair of split-split plots (Fig. 1). Litter from 1997 was dried for 3 days at 60 °C to a constant mass and weighed.

In late April 1998 and again in late February 1999 we added 2.0 g of seeds of each of 30 species (see Table 1) to one of each pair of split plots. We obtained seeds from a seed supplier (Ernst Conservation Seeds, Meadville, PA, USA) located c. 10 km from the field site. This supplier collects local wild seed, using multiple parents from multiple sites to ensure a diverse genetic stock, and also sows crops of selected native species using this diverse stock. We chose species that were likely to establish

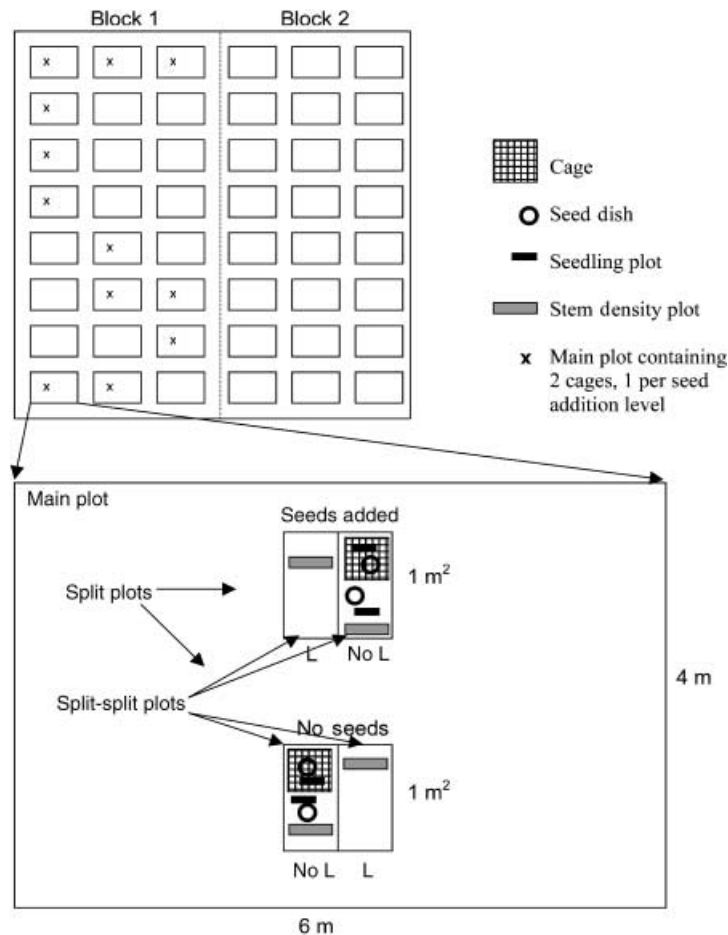


Fig. 1 Plot layout. Each block (eight rows \times three columns) contained six main plots (4 \times 6 m) at each of four fertilizer levels. Each main plot contained two 1 \times 1 m split plots for seed addition treatments. Each split plot was divided in half for litter treatments, and cages and seed dishes were established within split-split plots. All treatments were assigned randomly to plots.

in this habitat, and, in many cases, that we observed previously at our study site (Stevens 1999; Stevens & Carson 1999a). After seed addition in spring 1999, we randomly selected three main plots at each fertilizer level in block 1 and excluded rodents and birds by placing a small cage (15 W \times 30 L \times 10 H cm, with 0.61 cm mesh) in each of 24 litter removal split-split plots in block 1 (Fig. 1). We placed the edges of each cage 2 cm into the soil to prevent access of voles and mice. We used cages only in litter removal plots for two reasons. First, because we could then measure the direct effect of cage rather than cage + litter (Reader & Beisner 1991; Reader 1993). Secondly, because we did not count seedlings in any litter control plots (removing and replacing the litter in order to count seedlings had the potential to alter the effects of the litter itself; Long *et al.* 2003), the effect of cages on seedlings could only be assessed in no-litter plots. When the cages were established, we also placed small dishes containing 15 seeds each of *Daucus carota*, *Hypericum perforatum*, *Panicum clandestinum* and *Cornus racemosa* inside and immediately outside the cage ($n = 48$ dishes). Dishes were collected after 3 days to assess seed predation rates (Mittelbach & Gross 1984; Hulme 1994). In May 1999, while most seedlings were < 1 cm in height, we removed the cages for the remainder of the

experiment. At that time we counted seedling density in a randomly selected 2 \times 10 cm strip within each cage and in an adjacent randomly selected area within the same split-split plot ($n = 48$ strips). Thus our cage treatment evaluated seed predation and early seedling predation by mammals and birds, rather than post-establishment seedling predation on seedlings > 1 cm.

We estimated colonization and extinction of species within plots from changes in species presence and absence between 1997 and 1999. In early September of each year, we visually estimated percentage cover of each species and of litter in each split-split plot. Only ramets originating in split-split plots were included, rather than any species with parts overhanging the plot. This grid-based approach may tend to give relatively steep species–area slopes (Williamson 2003), but we took this approach to reduce the variability associated with presence–absence estimates in a canopy that was regularly moved in light wind and by field workers. This choice should not hinder comparisons of richness–density slopes because all samples are based on similar sized plots. We estimated cover of each species independently, and total percentage cover frequently exceeded 100%. In 1999, we also counted stem density in a randomly placed 2 \times 30 cm strip within each split-split plot.

Table 1 Numbers of split-split plots (out of 96 per treatment) containing species used for seed addition treatment. Two grams of seed of each species were added to plots in 1998 and 1999. A, P, B refer to annual, perennial, biennial; N refers to nitrogen fixing

Traits	Seed	Addition	Control
Tall forbs (> 1 m)			
<i>Daucus carota</i>	P	6	4
<i>Lespedeza capitata</i>	P, N	0	0
<i>Melilotus alba</i>	A, N	0	0
<i>Melilotus officinalis</i>	A, N	0	0
<i>Solidago altissima</i>	P	25	28
<i>Solidago gigantea</i>	P	42	40
<i>Solidago rugosa</i>	P	89	89
Short forbs (< 1 m)			
<i>Achillea millefolium</i>	P	0	0
<i>Bidens cernua</i>	A	0	0
<i>Bidens frondosa</i>	A	0	0
<i>Chamaecrista fasciculata</i>	A, N	0	0
<i>Chrysanthemum leucanthemum</i>	P	0	0
<i>Hypericum perforatum</i>	P,	2	2
<i>Rudbeckia hirta</i>	B		
<i>Rudbeckia lacinata</i>	P	0	0
<i>Trifolium hybridum</i>	P, N	0	0
<i>Trifolium pratense</i>	P, N	0	0
Grasses			
<i>Andropogon gerardii</i>	P, C4	0	0
<i>Elymus canadensis</i>	P, C4	2	1
<i>Elymus virginicus</i>	P, C4	0	0
<i>Panicum clandestinum</i>	P	1	1
<i>Panicum virgatum</i>	P	0	0
<i>Schizachyrium scoparium</i>	P, C4	2	0
Woody			
<i>Acer rubrum</i>	P	2	3
<i>Cornus amomum</i>	P	0	0
<i>Cornus florida</i>	P	0	0
<i>Cornus racemosa</i>	P	9	3
<i>Rhus glabra</i>	P	0	1
Vine			
<i>Clematis virginiana</i>	P	0	0

General linearized models (R_Core_Development_Team 2003) estimated treatment effects on species recruitment (1997–99), May 1999 seedling density, seed removal rates, September 1999 stem densities and September 1999 species richness. The glmmPQL function (glmmPQL function in the MASS library for R, Venables & Ripley 2002) assumes multivariate normal random effects and uses penalized quasi-likelihood estimation (Lin & Breslow 1996). We modelled most responses with a Poisson distribution with a log link function. Only seed removal (fraction of seeds removed) was modelled with binomial error and a logit function. We used hierarchical mixed models because of the physical nesting of litter treatments (or cages) within seed addition treatments within fertilizer treatments within blocks (Fig. 1). To model treatment effects on species richness, we included stem density as a covariate. We standardized stem density as $\ln(x) - \text{mean}[\ln(x)]$, where $x = \text{stems per } 60 \text{ cm}^2 + 1$. Transforming stem density simplified interpretation and adding 1.0 allowed us

to include five plots with meaningful zero counts. In addition, this transformation maximized model fit, relative to others or no transformation.

We used an interactive modelling approach to hypothesis testing and estimation of treatment effects. Fitting statistical models to data using appropriate techniques (e.g. conditional F -tests, Akaike's information criterion) provides more accurate and more predictive parameter estimates of effect sizes than uncritical acceptance of models suggested by a particular experimental design (Venables & Ripley 2002; Pinheiro & Bates 2000; Crawley 2002). Following standard procedures (Venables & Ripley 1999; Pinheiro & Bates 2000; Crawley 2002), minimally adequate (i.e. maximally parsimonious) models were determined by sequential backwards removal of non-significant terms based on conditional F -tests of ANOVA terms. Random effects were eliminated or retained on the basis of AIC values using reduced maximum likelihood methods. Where we present treatment effects in the text, we use predicted values of the response variables estimated from the models.

Each of our hypotheses may be supported, in part, by the absence of particular treatment effects. For instance, the failure of seed additions to eliminate the negative effect of fertilizer on species richness would be consistent with both germination limitation and establishment limitation. Given a standard statistical null hypothesis (e.g. $\alpha_1 = 0$), noisy data or low replication favours accepting no effect of added seeds, and low signal-to-noise ratios (small effect sizes/large variance) reduce power and increase the probability of not observing significant effects (Hoenig & Heisey 2001). To provide a conservative and robust test for the absence of selected treatment effects, we used estimates of relevant slope parameters to test whether seed and litter treatments could eliminate negative fertilizer effects. For instance, we tested the null hypothesis that $\beta_1 + \alpha_1 = 0$, where β_1 is the (negative) slope of the fertilizer effect, and α_1 is the added effect on this slope due to added seeds. Noisy data or low replication would cause this test to favour accepting the null hypothesis that the seed treatment can eliminate the negative effect of fertilizer on richness. We provide below many such tests, providing clear bounds on the information available from our data. To perform these tests, we typically had to add back into our models terms that were not significant and were excluded from our most parsimonious models. In all cases, we provide the means and standard errors of all relevant re-fit parameters (e.g. Table 2).

Results

Litter biomass per 0.5 m² plot increased with increasing fertilizer ($F_{1,46} = 29.23$, $P < 0.0001$), from 113.5 g 0.5 m⁻² in controls (101.0–127.6, 95% CLs) to 196.3 g 0.5 m⁻² in the high fertility plots (160.1–240.8, 95% CLs). This result was consistent with earlier data on above-ground biomass (see Methods). Species recruitment declined with increasing fertilizer ($F_{1,45} = 13.53$, $P =$

Table 2 Key hypothesis tests. Final models for each response variable (bold) are minimally adequate models, where all terms or their higher order interactions are significant ($P < 0.05$) and excluded terms were not significant on the basis of standard ANOVA conditional F -tests ($P > 0.05$). Parameters $\alpha_0, \beta_0, \gamma_0, \tau_0, \mu$ are intercepts, and $\alpha_1, \beta_1, \gamma_1, \tau_1, \delta_1$ are effects on slopes. Selected tests (e.g. $\beta_1 + \alpha_1 = 0$) evaluate whether a seed, litter or cage treatment eliminates the negative effect of fertilizer. Note that if parameters had been excluded from the final (minimal) model, they were added back in order to test the relevant hypothesis. Values of particular parameters may vary depending upon the presence or absence of other terms in the model. All t -tests were two-tailed

Treatment	Model (fixed effects)	Random effects	Test H_0	Parameter 1 mean \pm SE, d.f.	Parameter 2 mean \pm SE, d.f.	$ t $	P
Recruitment							
Fertility (β)	$Y = \exp(\beta_0 + \beta_1 + \alpha_0)$	Block/mainplot	$\beta_1 = 0$	$-0.0177 \pm 0.00483, 45$	0	3.68	0.0006
Seed (α)	$Y = \exp(\beta_0 + \beta_1 + \alpha_0)$	Block/mainplot	$\alpha_0 = 0$	$0.115 \pm 0.0572, 143$	0	2.01	0.0464
Seed (α)	$Y = \exp(\beta_0 + \beta_1 + \alpha_0 + \alpha_1)$	Block/mainplot	$\beta_1 + \alpha_1 = 0$	$-0.0185 \pm 0.00554, 45$	$0.00141 \pm 0.00508, 142$	2.27	0.0121
Litter (γ)	$Y = \exp(\beta_0 + \beta_1 + \alpha_0 + \gamma_0 + \gamma_1)$	Block/mainplot	$\beta_1 + \gamma_1 = 0$	$-0.0135 \pm 0.00544, 45$	$-0.00836 \pm 0.00504, 141$	2.95	0.0018
Seedling density							
Fertility (β)	$Y = \exp(\beta_0 + \beta_1 + \alpha_0)$	Mainplot	$\beta_1 = 0$	$0.00360 \pm 0.0167, 10$	0	0.302	0.834
Seed (α)	$Y = \exp(\alpha_0)$	Mainplot*	$\alpha_0 = 0$	$0.922 \pm 0.370, 34$	0	2.49	0.0177
Cage (τ)	$Y = \exp(\alpha_0 + \tau_0)$	Mainplot	$\tau_0 = 0$	$-0.206 \pm 0.395, 33$	0	0.520	0.606
Seed predation on <i>Daucus carota</i>							
Fertility (β)	$Y = \frac{\exp(\beta_0 + \beta_1 + \tau_0)}{1 + \exp(\beta_0 + \beta_1 + \tau_0)}$	Mainplot	$\beta_1 = 0$	$0.0182 \pm 0.0119, 10$	0	1.53	0.1566
Seed (α)	$Y = \frac{\exp(\alpha_0 + \tau_0)}{1 + \exp(\alpha_0 + \tau_0)}$	Mainplot	$\alpha_0 = 0$	$-0.213 \pm 0.262, 34$	0	0.812	0.4226
Cage (τ)	$Y = \frac{\exp(\tau_0)}{1 + \exp(\tau_0)}$	Mainplot	$\tau_0 = 0$	$0.993 \pm 0.278, 35$	0	3.57	0.0011
Final stem density							
Fertility (β)	$Y = \exp(\beta_0 + \beta_1 + \gamma_0)$	Block	$\beta_1 = 0$	$-0.0392 \pm 0.00622, 185$	0	6.30	< 0.0001
Seed (α)	$Y = \exp(\beta_0 + \beta_1 + \alpha_0 + \alpha_1)$	Block	$\beta_1 + \alpha_1 = 0$	$-0.0416 \pm 0.00914, 183$	$0.00446 \pm 0.0125, 183$	2.39	0.0086
Litter (γ)	$Y = \exp(\beta_0 + \beta_1 + \gamma_0)$	Block	$\gamma_0 = 0$	$0.280 \pm 0.127, 185$	0	2.201	0.0289
Litter (γ)	$Y = \exp(\beta_0 + \beta_1 + \alpha_0 + \alpha_1 + \gamma_1)$	Block	$\beta_1 + \gamma_1 = 0$	$-0.0307 \pm 0.00922, 184$	$-0.0153 \pm 0.0126, 184$	2.95	0.0017
Species richness							
Stem density (δ)	$Y = \exp(\mu + \delta_1 + \beta_1)$	Block	$\delta_1 = 0$	$0.262 \pm 0.0222, 185$	0	11.812	< 0.0001
Fertility (β)	$Y = \exp(\mu + \delta_1 + \beta_1)$	Block	$\beta_1 = 0$	$-0.0144 \pm 0.00179, 185$	0	8.055	< 0.0001
Seed (α)	$Y = \exp(\mu + \delta_1 + \beta_1 + \alpha_0 + \alpha_1)$	Block	$\beta_1 + \alpha_1 = 0$	$-0.0142 \pm 0.00243, 183$	$-0.000403 \pm 0.00319, 183$	3.643	0.0001
Litter (γ)	$Y = \exp(\mu + \delta_1 + \beta_1 + \gamma_0 + \gamma_1)$	Block	$\beta_1 + \gamma_1 = 0$	$-0.0141 \pm 0.00236, 183$	$-0.000434 \pm 0.00435, 184$	3.683	0.0001

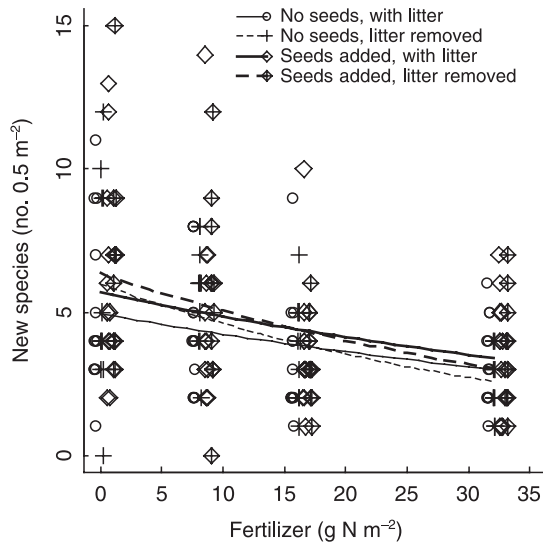


Fig. 2 Fertilizer significantly decreased recruitment between 1997 and 1999, and seed additions enhanced recruitment at all fertilizer levels (see text and Table 2). In all plots, fitted lines are not based on the minimally adequate model (Table 2) but rather a model with all possible interactions. Therefore, not all lines represent significant treatments. Points have been jittered in the X direction only, to facilitate inspection of data.

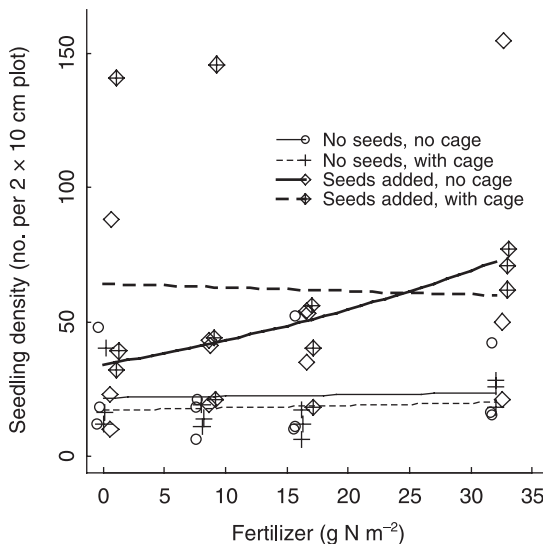


Fig. 3 Seed additions more than doubled seedling density, and neither fertilizer, cage, nor their interactions had any effect on seedling density.

0.0006). New species recruited into high fertility plots (32 g N) at roughly half the rate as in control plots (2.8 vs. 5.0 species; Fig. 2, Table 2). Seed additions enhanced species recruitment by 12% at all fertilizer levels ($F_{1,143} = 4.037$, $P = 0.0464$; Fig. 2, Table 2). However, seed additions did not significantly influence the slope of the fertilizer effect (seed \times fertilizer interaction, $F_{1,138} = 0.0786$, $P = 0.780$). Further, the negative slope of the fertilizer–recruitment relation was more than an order of magnitude steeper than the added positive effect on that slope due to seed additions ($\beta_1 = -0.0185$ vs. $\alpha_1 =$

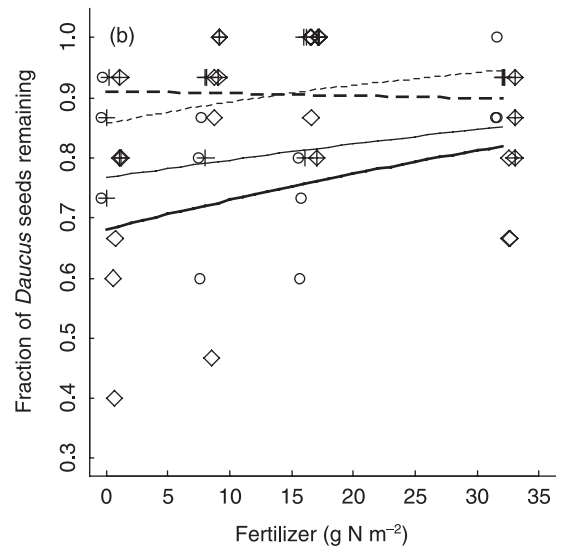
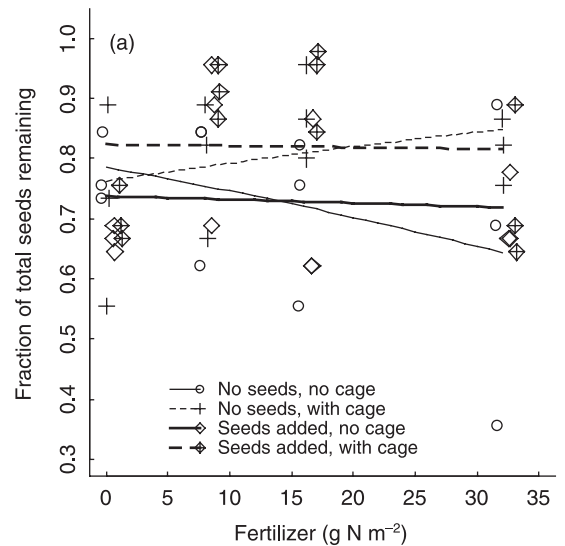


Fig. 4 Fraction of seeds remaining in dishes for each treatment combination. (a) Total of four species of seeds (15 seeds per species \times four species = 60 seeds per dish). Cages appeared to reduce seed loss. (b) Only *Daucus* showed a significant response to cages (see Results), accounting for the effect of cages on total seed removal.

0.00141; $t = 2.27$, $P = 0.0121$, Table 2). Litter removal had no significant effect on mean recruitment levels, and, like seed additions, had no effect on the slope of the fertilizer–recruitment relation (Table 2).

Seed additions increased seedling density by 174% ($F_{1,35} = 19.59$, $P = 0.0001$), and neither cages nor fertilizer had any significant effect (Fig. 3, Table 2). The effect of adding seeds was an order of magnitude greater than the estimated effect of fertilizer and more than three times greater than estimated effects of cages (Table 2). Cages increased the total number of seeds in dishes by 11% ($F_{1,35} = 5.35$, $P = 0.027$; Fig. 4), but this was due entirely to the 16% increase of *Daucus carota* seeds ($F_{1,35} = 12.74$, $P = 0.0011$; Fig. 4, Table 2). We observed no evidence of mammals getting into the cages, which were buried at least 2 cm into the soil.

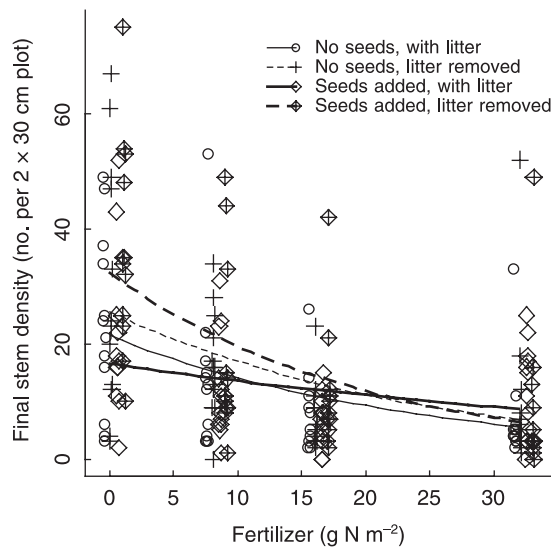


Fig. 5 Final (late season) stem density declined by 70% as fertilizer increased, and litter removal increased stem density by approximately 32% above control densities. Seed additions had no effect.

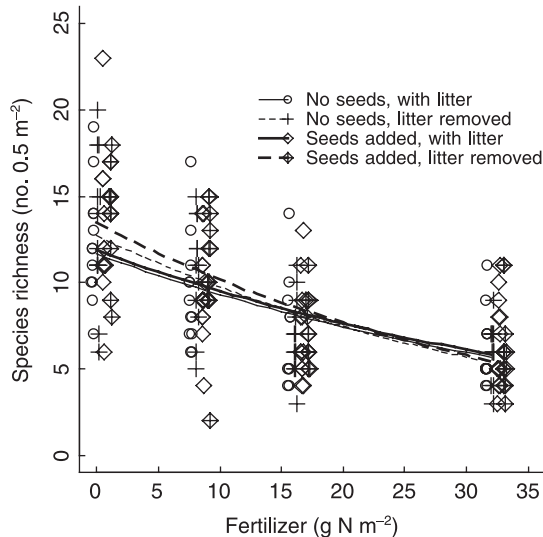


Fig. 6 Species richness declined by 54% with increasing fertilizer, and neither seed additions nor litter removal appeared to ameliorate these effects.

Final (late season) stem density declined by 70% as fertilizer increased to 32 g N ($F_{1,185} = 39.75$, $P < 0.0001$; Table 2, Fig. 5). Litter removal increased stem density by 32%, regardless of fertilizer level ($F_{1,185} = 4.845$, $P < 0.029$; Table 2, Fig. 5). Litter removal did not influence the relation between fertilizer and final stem density (interaction term eliminated from the model, Table 2), and the relevant t -test showed that the maximum possible effect of litter removal on the fertilizer–density relation was significantly smaller than the negative effect of fertilizer (Table 2). Seed additions did not enhance final stem density or influence fertilizer–density relations, and maximum possible effects were smaller than the negative effect of fertilizer.

Overall, species richness declined by 54% as fertilizer increased ($F_{1,185} = 64.88$, $P < 0.0001$; Table 2, Fig. 6), and this negative effect of fertilizer was not eliminated by seed additions or litter removals (Table 2). Neither the main effects of seed additions nor litter removal, nor the interactions between these and fertilizer, were significant and were eliminated from the model (Table 2). In particular, the effects of seed addition and litter removal on fertilizer–richness relations were significantly smaller than the negative effects of fertilizer (Table 2, $P = 0.0030$, $P = 0.0029$, respectively), with estimated effect sizes very close to zero (respective means \pm SE = -0.0005 ± 0.004 , -0.0004 ± 0.004 ; Table 2).

Discussion

The observed decline in recruitment rates with increasing fertility appeared to result from declining establishment with increasing fertility. Although seed additions more than doubled seedling density, neither seed additions nor litter removal eliminated or appeared to significantly moderate the negative effects of fertilizer on species recruitment and species richness. The absence of a significant effect of fertilizer on seedling density combined with the strong negative effect of fertilizer on final stem density is consistent with hypotheses proposing that relatively diffuse competitive interactions (Goldberg & Miller 1990; Miller 1994; Stevens & Carson 1999b) among growing individuals predominate the dynamics of this system. Taken together, our results support the hypothesis that it is establishment limitation that limits diversity. They are also consistent with theories focusing on plant resource competition that emphasize species' differential effects on resources and their responses to those resources by relatively mature individuals (Tilman 1982; Goldberg 1987; Goldberg & Miller 1990; Cahill 1999; Stevens & Carson 1999a,b; Rajaniemi 2002; Stevens & Carson 2002).

Mechanisms controlling local richness may vary along gradients where the range of productivity is sufficiently broad (Stevens & Carson 1999b; Weiher 1999; Virtanen *et al.* 2001). In particular, source limitation may generally be important in unproductive sites (Foster & Tilman 2003), and establishment limitation may become relatively more important in productive sites (Xiong *et al.* 2003). Foster (2001) showed that seed additions enhanced species richness in low or moderate above-ground biomass ($< 250 \text{ g m}^{-2}$) but not in high biomass plots ($> 250 \text{ g m}^{-2}$). The mechanism preventing seed additions from augmenting the community is unclear and has been attributed to both above-ground biomass and litter accumulation. Foster and Gross (1998) manipulated litter and found that only when litter was added in amounts equivalent to high fertility plots did litter cause large declines in species richness. Carson & Peterson (1990) found that high amounts of litter, equivalent to that in high fertility sites, suppressed annuals and biennials to a far greater extent than perennials. Tilman (1993) found that recruitment rate declined and

litter increased along a fertility gradient, and suggested that litter was the mechanism causing the observed declines in recruitment and species richness. In our system, above-ground biomass and litter were high ($> 200 \text{ g m}^{-2}$) (Stevens & Carson 1999a, 2002), consistent with the hypothesis that either alone could have prevented seed additions from augmenting control plots. In addition, the vast majority of species in this community were perennials that spread clonally, making their local dispersal less susceptible to the impacts of litter (Carson & Peterson 1990). Source limitation via seed may thus be relatively unimportant in productive sites because establishment limitation is so severe. Most theories predict that the rate at which richness declines should slow at sufficiently high productivity, flattening the negative slope of the richness–productivity curve (Grime 1979; Tilman & Pacala 1993; Stevens & Carson 1999b; Weiher 1999; Virtanen *et al.* 2001). If both source and germination limitation become unimportant at moderate productivity, the lack of variation in richness due to these mechanisms might account for a flattening of the richness–productivity curve (Grime 1979).

Establishment limitation provides one level of explanation for the observed decline in recruitment along this fertility gradient. Whether the decline in recruitment comes from a decline in the establishment of vegetative propagules or of seedlings in high fertility plots remains untested. Indeed, source and establishment limitation hypotheses should also apply to vegetative propagules, where asymmetric competition in adjacent plots (source limitation) or within a plot (establishment limitation) would limit species recruitment via vegetative expansion. Nearly all species in this study are perennials that spread via clonal expansion, and conditions associated with increased fertilizer levels may have suppressed rates of expansion for many species. This could be tested easily with appropriate barriers around replicate plots.

The relation between the number of species and the number of individuals in our study ($S = cN^z$, $z = 0.262$, Table 2) happens to be identical to that predicted by Preston's empirical fit of his canonical lognormal species abundance distribution ($S = I^z$, $z = 0.262$; equations 14–16, p. 190, Preston 1962). This power relation predicts that a 70% decline in density (as observed in our study) should cause a concomitant 31% decline in the number of species. This would leave 23% of the decline in richness to be explained by changes in relative abundance and composition. Although we do not have the requisite data to simulate random thinning (e.g. Stevens & Carson 1999a), we present these simple calculations here because they provide quantitative predictions regarding competing explanations for the loss of species along productivity gradients.

If recruitment is generally very low in productive communities (Huston 1999; Grace 2001), interesting consequences may follow. As immigration rates into productive communities decline, these communities are more effectively isolated from a larger regional species

pool. Neutral theory (Bell 2000; Hubbell 2001) predicts that rare stochastic events are more likely to affect isolated communities than connected communities. Stochastic events may include unusual initial conditions that occur by chance, or they may be the cumulative events associated with ecological drift (Hubbell 2001). Empirical studies indicate that productive communities vary more widely in their structure than less productive communities, given the same regional species pool (Chase & Leibold 2002; Condit *et al.* 2002). We suggest that intense recruitment limitation in productive habitats provides a mechanism to explain these empirical studies. We hypothesize that the structure of communities in productive habitats is more susceptible to stochastic processes (Hubbell 2001) because strong local interactions (Huston 1994; Cahill 1999; Stevens & Carson 1999a; Rajaniemi 2002) prevent immigration, and destroy linkage with the regional community.

Acknowledgements

We would like to thank Emily Greiner for extensive fieldwork, and the staff of the Pymatuning Laboratory of Ecology and the University Pittsburgh Department of Biological Sciences for logistical support. This work was supported by National Science Foundation (DEB 9903912), and a G. W. McKinley Research Grant. This is Pymatuning Laboratory publication no. 146.

References

- Bell, G. (2000) The distribution of abundance in neutral communities. *The American Naturalist*, **155**, 606–617.
- Bohannan, B.J.M. & Lenski, R.E. (2000) The relative importance of competition and predation varies with productivity in a model community. *The American Naturalist*, **156**, 329–340.
- Cahill, J.F. (1999) Fertilization effects on interactions between above- and belowground competition in an old field. *Ecology*, **80**, 466–480.
- Carson, W.P. & Peterson, C.J. (1990) The role of litter in an old-field community: impact of litter quantity in different seasons on plant species richness and abundance. *Oecologia*, **85**, 8–13.
- Chase, J.M. & Leibold, M.A. (2002) Spatial scale dictates the productivity-biodiversity relationship. *Nature*, **416**, 427–430.
- Condit, R., Pitman, N., Leigh, E.G., Chave, J., Terborgh, J., Foster, R.B. *et al.* (2002) Beta-diversity in tropical forest trees. *Science*, **295**, 666–669.
- Crawley, M.J. (2002) *Statistical Computing: an Introduction to Data Analysis Using S-Plus*. John Wiley and Sons, Chichester.
- DiTomasso, A. & Aarssen, L.W. (1989) Resource manipulations in natural vegetation: a review. *Vegetatio*, **84**, 9–29.
- Foster, B.L. (2001) Constraints on colonization and species richness along a grassland productivity gradient: the role of propagule availability. *Ecology Letters*, **4**, 530–535.
- Foster, B. & Gross, K.L. (1998) Species richness in a successional grassland: effects of nitrogen enrichment and litter. *Ecology*, **79**, 2593–2602.
- Foster, B.L. & Tilman, D. (2003) Seed limitation and the regulation of community structure in oak savanna grassland. *Journal of Ecology*, **91**, 999–1007.
- Gleason, H.A. & Cronquist, A. (1991) *Manual of Vascular Plants of Northeastern United States and Adjacent Canada*, 2nd edn. New York Botanical Garden, New York.

- Goldberg, D.E. (1987) Neighborhood competition in an old-field plant community. *Ecology*, **68**, 1211–1223.
- Goldberg, D.E. & Miller, T.E. (1990) Effects of different resource additions on species diversity in an annual plant community. *Ecology*, **71**, 213–225.
- Gough, L., Osenberg, C.W., Gross, K.L. & Collins, S.L. (2000) Fertilization effects on species density and primary productivity in herbaceous plant communities. *Oikos*, **89**, 428–439.
- Grace, J.B. (2001) The roles of community biomass and species pools in the regulation of plant diversity. *Oikos*, **92**, 193–207.
- Grime, J.P. (1979) *Plant Strategies and Vegetation*. John Wiley & Sons, Chichester.
- Hoening, J.M. & Heisey, D.M. (2001) The abuse of power: the pervasive fallacy of power calculations for data analysis. *Journal of American Statistical Association*, **55**, 19–24.
- Holt, R.D., Grover, J. & Tilman, D. (1994) Simple rules for interspecific dominance in systems with exploitative and apparent competition. *The American Naturalist*, **144**, 741–771.
- Hubbell, S.P. (2001) *A Unified Theory of Biodiversity and Biogeography*. Princeton University Press, Princeton, New Jersey.
- Hulme, P.E. (1994) Postdispersal seed predation in grassland – its magnitude and sources of variation. *Journal of Ecology*, **82**, 645–652.
- Huston, M.A. (1994) *Biological Diversity: the Coexistence of Species on Changing Landscapes*. Cambridge University Press, Cambridge.
- Huston, M.A. (1999) Local processes and regional patterns: appropriate scales for understanding variation in the diversity of plants and animals. *Oikos*, **86**, 393–401.
- Kassen, R., Buckling, A., Bell, G. & Rainey, P.B. (2000) Diversity peaks at intermediate productivity in a laboratory microcosm. *Nature*, **406**, 508–512.
- Lin, X. & Breslow, N.E. (1996) Bias correction in generalized linear mixed models with multiple components of dispersion. *Journal of the American Statistical Association*, **91**, 1007–1016.
- Long, Z.T., Mohler, C.L. & Carson, W.P. (2003) Extending the resource concentration hypothesis to plant communities: effects of litter and herbivores. *Ecology*, **84**, 652–665.
- MacArthur, R.H. & Wilson, E.O. (1963) An equilibrium theory of insular zoogeography. *Evolution*, **17**, 373–387.
- Miller, T.E. (1994) Direct and indirect interactions in an early old-field plant community. *The American Naturalist*, **143**, 1007–1025.
- Mittelbach, G.G. & Gross, K.L. (1984) Experimental studies of seed predation in old-fields. *Oecologia*, **65**, 7–13.
- Mittelbach, G.G., Steiner, C.F., Scheiner, S.M., Gross, K.L., Reynolds, H.L., Waide, R.B. *et al.* (2001) What is the observed relationship between species richness and productivity? *Ecology*, **82**, 2381–2396.
- Nathan, R. & Muller-Landau, H.C. (2000) Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution*, **15**, 278–285.
- Pinheiro, J. & Bates, D. (2000) *Analysis of Mixed Models*. Springer, New York.
- Preston, F.W. (1962) The canonical distribution of commonness and rarity: part I. *Ecology*, **43**, 185–215.
- Rajaniemi, T.K. (2002) Why does fertilization reduce plant species diversity? Testing three competition-based hypotheses. *Journal of Ecology*, **90**, 316–324.
- R_Core_Development_Team (2003) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- Rajaniemi, T.K. (2003) Explaining productivity-diversity relationships in plants. *Oikos*, **101**, 449–457.
- Reader, R.J. (1993) Control of seedling emergence by ground cover and seed predation in relation to seed size for some old-field species. *Journal of Ecology*, **81**, 169–175.
- Reader, R.J. & Beisner, B.E. (1991) Species-dependent effects of seed predation and ground cover on seedling emergence of old-field forbs. *American Midland Naturalist*, **126**, 279–286.
- Stevens, M.H.H. (1999) *Explaining why plant species richness declines as resource availability increases; tests of the important hypotheses*. PhD thesis, University of Pittsburgh, Pittsburgh, Pennsylvania.
- Stevens, M.H.H. & Carson, W.P. (1999a) Plant density determines species richness along an experimental fertility gradient. *Ecology*, **80**, 455–465.
- Stevens, M.H.H. & Carson, W.P. (1999b) The significance of assemblage level thinning for species richness. *Journal of Ecology*, **87**, 490–502.
- Stevens, M.H.H. & Carson, W.P. (2002) Resource quantity, not resource heterogeneity, maintains plant diversity. *Ecology Letters*, **5**, 420–426.
- Tilman, D. (1982) *Resource Competition and Community Structure*. Princeton University Press, Princeton.
- Tilman, D. (1993) Species richness of experimental productivity gradients: how important is colonization limitation? *Ecology*, **74**, 2179–2191.
- Tilman, D. & Pacala, S.W. (1993) The maintenance of species richness in plant communities. *Species Diversity in Ecological Communities: Historical and Geographical Perspectives* (eds R.E. Ricklefs & D. Schluter), pp. 13–25. University of Chicago Press, Chicago.
- USSCS (1979) *Soil Survey of Crawford County, Pennsylvania (by Michael Yaworski et al.)*; United States Department of Agriculture, Soil Conservation Service, in cooperation with the Pennsylvania State University College of Agriculture and the Pennsylvania Department of Environmental Resources, State Conservation Commission, National Cooperative Soil Survey, Washington, DC.
- Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S-PLUS*, 4th edn. Springer, New York.
- Virtanen, R., Muotka, T. & Saksala, M. (2001) Species richness–standing crop relationship in stream bryophyte communities: patterns across multiple scales. *Journal of Ecology*, **89**, 14–20.
- Waide, R.B., Willig, M.R., Steiner, C.F., Mittelbach, G., Gough, L., Dodson, S.I. *et al.* (1999) The relationship between productivity and species richness. *Annual Review of Ecology and Systematics*, **30**, 257–300.
- Weier, E. (1999) The combined effects of scale and productivity on species richness. *Journal of Ecology*, **87**, 1005–1011.
- Williamson, M. (2003) Species-area relationships at small scales in continuum vegetation. *Journal of Ecology*, **91**, 904–907.
- Wilson, S.D. & Tilman, D. (1991) Interactive effects of fertilization and disturbance on community structure and resource availability in an old-field plant community. *Oecologia*, **88**, 61–71.
- Wilson, S.D. & Tilman, D. (2002) Quadratic variation in old-field species richness along gradients of disturbance and nitrogen. *Ecology*, **83**, 492–504.
- Xiong, S.J., Johansson, M.E., Hughes, F.M.R., Hayes, A., Richards, K.S. & Nilsson, C. (2003) Interactive effects of soil moisture, vegetation canopy, plant litter and seed addition on plant diversity in a wetland community. *Journal of Ecology*, **91**, 976–986.

Received 9 June 2003

revision accepted 27 November 2003

Handling Editor: David Gibson