

Seed banks and seedling establishment on an experimental productivity gradient

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A major cause of the lower species richness of nitrogen enriched grasslands with high productivity is the lower colonization rate of such areas by new species. To determine what role the soil seed bank might play, we performed greenhouse germination tests of soil sampled in experimental plots that had received various rates of annual nitrogen addition for 11 yr. Species composition of the soil seed bank depended on the rate of N-addition, even though the species richness and the overall density of viable seeds in soil were independent of N-addition. The proportion of the species shared between the seed bank and the above-ground vegetation decreased with N-addition. The density of forb seedlings emerging in field plots was negatively correlated with the nitrogen input, productivity, and litter depth, but the probability of seedling survival from May through August was not different among treatments. Thus, suppression of seed germination, rather than limitation of the availability of seeds in soil or seedling survivorship, was the primary mechanism of lower colonization rates in the high-N plots. Burning in spring decreased litter depth and promoted germination of forbs, especially in no-N control plots. However, germination was still very low in the high-N plots even after fire decreased litter depth to a level similar to that of controls.

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In several natural and experimental systems, it has been documented that high soil nutrient availability and high productivity result in lowered species diversity (Tilman 1982, 1988, Bakker 1989, Huston 1993, Tilman and Pacala 1993). Nitrogen is the most limiting resource in many plant communities including old field communities on the sandy soils of Cedar Creek Natural History Area (CCNHA). Nitrogen addition from 1982 to 1993 in replicated plots in three fields at CCNHA resulted in higher productivity, strong dominance by the grass *Agropyron repens* (up to 80% of above-ground biomass in high-N plots, Fig. 1) and a decline in plant species richness (Tilman 1987, 1990, 1993). These changes were accompanied by micro-environmental changes, including decreased light at

the ground level and greater litter accumulation. Tilman (1993) found that this decline in plant species diversity was the result of both a decrease in local colonization rates and an increase in local extinction rates. Further, the decline in colonization with nitrogen addition was explained statistically better by litter mass rather than by above-ground plant biomass or light penetration. Thus, litter accumulation may be an important mechanism of competitive suppression by *Agropyron*. Inhibition of seedling emergence by litter has been shown in other studies (Werner 1975, Goldberg and Werner 1983). Experimental litter removal has consistently increased stem density (Monk and Gabrielson 1983, Carson and Peterson 1990, Facelli 1991). Casual observations at Cedar Creek suggest that dicot

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seedlings rarely emerge through the thick litter in plots receiving high nitrogen addition. However, litter may not be the only mechanism for this apparent colonization limitation of diversity. Seedling colonization may be limited by alternative mechanisms that affect local seed production, seed rain, germination, and seedling survival.

Most studies of the effect of nitrogen addition on plant community composition and diversity have overlooked the viable seed populations in soil, even though they are significant components of plant communities (Harper 1977). The dynamics of soil seed populations are likely to depend on multiple factors, including density of local seed rain, seed predators in soil, and chemical and physical environments that affect dormancy and survival of seeds (Roberts 1981, Williams 1984, Baskin and Baskin 1989, Thompson 1992). Nitrogen addition may modify many of these. The increased importance of above-ground competition and changes in disturbance regimes with N-addition affect the density of adult plants (Tilman 1987) and their reproductive output. Dominance by *Agropyron* in high-N plots may limit the density of local seed rain by other species. Although seeds of many species can survive for long periods in soil, the density of viable seeds declines exponentially when local seed rain is prevented (Roberts and Feast 1973, Roberts 1981, Williams 1984). The main sources of mortality of seeds in soil are fatal germination (germination without seedling emergence above ground), predation, attack by microorganisms, and physiological damage (Roberts 1981, Louda 1989, Murdoch and Ellis 1992, Thompson 1992, Kremer 1993). Higher soil nitrate and ammonium concentration in N-added plots may break physiological dormancy and stimulate germination of some species. However, higher root respiration in N-added productive plots may result in higher CO₂ and lower oxygen concentrations that may inhibit germination of some species (Egley 1986, Karssen and Hilhorst 1992). Higher food availability for various root and litter feeders might change the seed mortality due to ingestion by these organisms as well as the vertical mixing regime of seeds in the soil (Fenner 1985). Although many of these changes likely to occur with N-addition may lead to a decline of the seed bank size in many species, the overall effects of N-addition on soil seed densities are unclear (Williams 1985, Milberg 1992). With the decline of above-ground species richness, species richness in the seed bank is likely to decline as well (Milberg 1992). However, species composition or abundance of individual species in the soil seed bank may not follow the changes in the above-ground vegetation, given the great discrepancies generally observed between above-ground vegetation and soil seed banks.

In this report, we examine how factors that affect colonization differ along an experimental productivity gradient created by nitrogen addition, and address the

following questions. 1. How does the soil seed bank community change when productivity and community structure change in response to nitrogen addition? 2. Which aspect of seedling colonization, seed germination or seedling survival, is suppressed more by deep litter accumulation in productive communities?

In order to examine the species richness and the abundance of seeds in soil, we quantified the density of viable seeds in 0–4 cm deep soil in plots that had received three contrasting levels of annual nitrogen addition for 11 yr (1982–1993) in a successional field abandoned 36 yr earlier (1957). We examined the abundance and survival of dicotyledonous seedlings by directly tagging and counting seedlings in sub-plots in the same plots. Because litter accumulation was hypothesized to be the most influential factor limiting seedling colonization, we expected that burning litter before the beginning of the growing season would enhance seedling emergence and survival. Annual burning treatment was initiated in 1992 to provide a test of this prediction. We quantified litter depth, and examined the correlations between seedling density and litter depth in burned and unburned plots that had been receiving contrasting N-addition rates.

Methods

Site and experimental plots

The study site was an old field (36 yr after abandonment in 1993, referred to as Field B in Tilman (1987, 1993) on a nitrogen-poor sandy soil in central Minnesota, USA, inside the Cedar Creek Natural History Area of the University of Minnesota (a Long Term Ecological Research site). As part of the experiment reported in Tilman (1987), six replicates of 4 m × 4 m plots inside mammalian exclosures received eight different levels of nitrogen addition annually since 1982. These plots were disturbed by disking once before the nitrogen addition in the first year, but not thereafter. Soil disturbance by pocket gophers (*Gyomys hirsarius*) that occasionally invaded the fences was kept low by removal with traps. As reported in Tilman (1987), nitrogen is the only limiting mineral nutrient in soil at the study site. Above-ground dry biomass of each plant species in each plot has been estimated by clipping vegetation in a narrow strip (10 cm × 300 cm) in August every year. For the study of soil seed bank and seedling emergence reported here, we chose replicate plots of three addition rates of ammonium nitrate: control without nitrogen addition (No-N), Medium (5.4 g m⁻² yr⁻¹ of N), and High (17.0 g m⁻² yr⁻¹ of N). These correspond to treatment I, E, G, respectively in Tilman (1987, 1990). In April 1992 before emergence of any green foliage or seedlings, three of the six replicates of each treatment were randomly burned to decrease litter

depth. Burning was repeated in April 1993. Thus, we had three replicates each of six different treatment combinations.

Estimation of viable seed density in soil

Species richness and density of viable seeds in soil were estimated by germinating seeds from a combined sample of six, 4-cm deep, 5.8-cm diameter cores collected from each unburned plot on 7 October 1993. Three cores were taken at 20 cm from each of the two edges not used for clip-sampling of vegetation. Each core was sliced to separate the upper 0–1 cm layer (including compacted litter) and the 1–4 cm mineral soil layer. Plots burned in spring of 1992 and 1993 were not sampled for seed banks. The total area sampled per plot was 158.4 cm². This sample area should contain 40–60% of the plateau number of species in the species – area relationship for the soil seed bank at the study site (Kitajima and Tilman, unpubl.). Since there was a steep decline of seed density of most species with soil depth (Kitajima and Tilman, unpubl.), the 0–4 cm depth layer is representative of the total seed bank community.

Samples were kept in plastic bags at 3–5°C for five weeks of cold stratification, and then spread out to be 0.5 cm thick on the top of a 3-cm deep mix of sterilized sand and vermiculite in a glass house. Large rhizomes sampled with soil were removed after rinsing with a mist-sprayer above the spread-out sample soil to remove any seeds on the surface of the rhizomes. Temperature of the room was at 20 ± 5°C. Natural day length was supplemented with sodium halogen lamps to the light period of 14 h per day. Samples were watered daily, and emerging seedlings were recorded and removed when they were large enough for identification to species. Seedlings in apparent bad health were removed and identified at least to forb (dicotyledonous species) or grass (monocotyledonous species). The surface of each soil sample was stirred once in the 3rd week to encourage further germination. Observation was terminated after 5 weeks when all seedlings were large enough for species identification. Nomenclature of the species followed Gleason and Cronquist (1991).

Quantifying natural emergence and survival of seedlings in the field

In May 1992, approximately 3 weeks after the first burning treatment, we tagged seedlings of forbs with colored plastic picks, identified the species whenever possible, and counted them, in 8 subplots (0.2 m × 0.2 m each) in each of the three replicate plots of each treatment. Seedlings were sought and identified under litter as well. However, we did not count grass seedlings

in the field because it was difficult to tell seedlings from sprouts from rhizomes. Litter was sampled from two circular areas of 12 cm diameter in each plot. Mean litter depth in the field was calculated from bulk volume measured in 5-cm diameter glass cylinders. Litter dry mass was measured after drying at 60°C for three d. Mean litter depth and litter dry mass were strongly correlated with each other ($r^2 = 0.88$, $n = 36$). In August 1992, we counted the number of surviving seedlings, as well as the number of unmarked newly emerged seedlings.

Statistical analysis

JMP statistical package and SAS v6.7 (both from SAS Institute) were used for statistical analysis. Estimated soil seed densities of individual species were transformed to $\log_{10}(\text{number} + 1)$ for improved homogeneity of variance before ANOVA. Vertical distribution of seeds in soil were examined in two ways. First, for each species or life-form group (pooled for N-treatments), the ratio of seed number per unit volume of soil between two layers (density in 0–1 cm divided by the density in 1–4 cm) was calculated. Second, for each life-form group, the effect of N-addition on vertical seed distribution (what proportion of seeds in the 0–4 cm depth belongs to the 0–1 cm layers) was examined by a log-likelihood chi-square test for a contingency table (SAS CATMOD Procedure, 2 soil depth layers × 3 N-addition levels, with the total count of emerged seedlings from soil samples for each treatment combination entered in each cell). For correlation analysis between seed density or field seedling density and litter depth, mean litter depth for each plot was used. Because of the strong correlation between litter depth and litter dry mass, only the results of correlation analysis using litter depth were reported. For analyses of seedling density or survival in the field, seedling numbers were totaled for each plot, giving three replicates per treatment combination. Seedling numbers were transformed to $\log_{10}(\text{number} + 1)$ before ANOVA and correlation analyses because it corrected for the normality of the distribution and improved the homogeneity of variance. Seedling survival probabilities were arcsine-square-root-transformed before ANOVA (Sokal and Rohlf 1981). We treated the N-addition level as an ordered categorical variable and burning treatment as a nominal categorical variable in ANOVA.

Results

Effect of N-addition on above-ground vegetation

The trends in the above-ground vegetation reported earlier (Tilman 1987, 1990) were holding in 1992 and

1993. Total above-ground plant biomass increased with N-addition rate (mean \pm s.e. = 202 ± 19 , 250 ± 23 , and 471 ± 49 g/m² for No-, Medium-, and High-N treatments, respectively, in August 1993). Relative abundance of *Schizachyrium scoparium* and *Agropyron repens* were negatively and positively correlated with the rate of N-addition (Fig. 1). These differences were accompanied by decrease in species richness with N-addition. Number of species recorded in above-ground biomass samples were 17, 9, and 6 in 1992 and 19, 14, and 10 in 1993 in No-, Medium-, and High-N treatments, respectively. Burning in Aprils 1992 and 1993 did not have significant effects on above-ground vegetation in these plots in Augusts 1992 and 1993 in terms of productivity, relative abundance of *Schizachyrium* and *Agropyron*, or species richness (2-way ANOVA).

Effect of N-addition on species composition and density of viable seeds in soil

The density of viable seeds in 0–4 cm soil (number per m²) did not differ among nitrogen addition treatments for forbs, grasses, or all species combined (Table 1). This result did not change when 0–1 cm and 1–4 cm depth layers were analyzed separately. There was a weak tendency for seed density of annual forbs to decrease with N-addition (ANOVA for log-transformed data, $P < 0.06$). The total number of species or the number of species in each life-form group also did not differ among treatments. Thus, neither overall density nor species richness of viable seeds in soil was lower in plots receiving high rate of N-addition. However, seed density of four individual species differed among treatments (Table 1). Among annual forbs, seed density of *Erigeron canadensis* and *Mollugo verticillata* decreased with N-addition, while seed density of *Polygonum con-*

volulus increased with N-addition. Other annual or perennial forbs did not show significant differences. Among grasses, only *Schizachyrium* showed a significant decrease in soil seed density with N-addition. No seeds of *Schizachyrium* were found in high-N plots where its adults were rare (Fig. 1). Likewise, no seeds of *Agropyron* were found in samples from control plots where its adults were rare (Fig. 1). However, its soil seed density did not increase as dramatically as did its vegetation cover with N-addition.

The number of viable seeds per unit volume of soil was much higher in the 0–1 cm layer than in the 1–4 cm layer (Fig. 2A). Seed density of grasses had a steeper decrease with depth than forbs (Fig. 2B, C). In 2-way ANOVA, neither effects of N-addition nor the interaction between N-addition and soil depth were significant for all species, just grasses or just forbs. Seed density of most individual species decreased with soil depth (density ratios between the upper and lower layers > 1 in Table 1), except for *Mollugo verticillata* and *Rumex acetosella*, which had much lower seed density in 0–1 cm than in 1–4 cm (density ratio < 1 in Table 1). Patterns of seed distribution with soil depth differed among life-form groups. The ratio of seeds in the 0–1 cm layer to seeds in the 1–4 cm increased with N-addition for forbs (log-likelihood chi-square = 21.5, $P < 0.001$), but it decreased for grasses (log-likelihood chi-square = 17.8, $P < 0.0001$). The trend for some individual species were different from the trend of their group. For example, 74% of *Polygonum* seeds were sampled from 0–1 cm layer in the control plots, which decreased to 61% and 46% in medium and high-N plots, respectively.

Although neither species richness nor total seed density differed among treatments, the species composition and the relative abundance of species in the soil seed bank diverged with N-treatment (Table 2). The proportion of shared species was lower between No-N and High-N than between No-N and Medium-N or between Medium-N and High-N treatments. Pearson's correlation coefficient, counting abundance of missing species as zero, was much higher between No-N and Medium-N treatments, than between other contrasts (Table 2). This divergence was statistically independent of changes in above-ground vegetation with N-treatments, since there was no significant correlation between species abundance between the above-ground vegetation and the soil seed bank.

In all treatments, species composition differed greatly between the soil seed bank and the above-ground vegetation. In any replicate plot or treatment, species abundance in the soil seed bank (seed number per unit volume of soil) was not correlated with the species abundance above ground (biomass per unit area in August 1993). None of the ten common species that were found at least in eight plots in above-ground vegetation or soil seed samples exhibited any significant

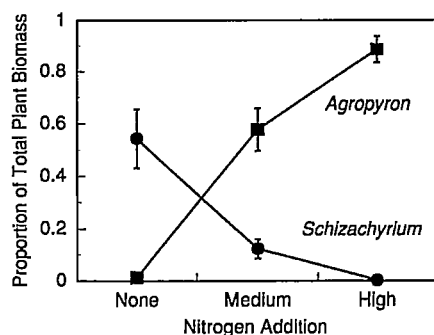


Fig. 1. Relative abundance (proportion of total above-ground plant biomass) of two dominant grass species, *Agropyron repens* (circles) and *Schizachyrium scoparium* (squares) in August 1993 at three contrasting rates of annual nitrogen addition. Means \pm s.e. for $n = 6$ plots per N-addition level, including both burned and unburned plots, since the effect of burning was not significant in 1993.

Table 1. Estimated density of viable seeds per square meter in 0–4 cm deep soil under three treatments (mean \pm s.e., $n = 3$ plots per treatment). A species is listed if actual total count of seeds was higher than 9 ($\geq 63 \text{ m}^{-2}$) or its seeds were present in all plots in one treatment. Three nitrogen addition treatments received 0, 5.4, 17.0 g N m^{-2} per year for 11 yr. Significant difference among treatments by ANOVA is indicated by * ($P < 0.05$). Number in () following the species name or group is its ratios of seed density per litter in 0–1 cm to that in 1–4 cm (all N-addition treatments combined). Total number of species in each group is indicated in []

Species (seed density ratio)	Nitrogen addition			
	No	Medium	High	
All species combined (3.9) [No. spp.]	8922 \pm 927 [22]	6987 \pm 1665 [26]	7575 \pm 2365 [25]	
Forbs (2.3)	5176 \pm 641 [14]	4545 \pm 704 [16]	4608 \pm 2084 [17]	
Annual forbs (1.3)	3324 \pm 401 [7]	2882 \pm 779 [7]	1557 \pm 180 [6]	
<i>Mollugo verticillata</i> (0.3)	2399 \pm 239	1704 \pm 753	400 \pm 105	*
<i>Polygonum convolvulus</i> (4.0)	126 \pm 36	652 \pm 105	547 \pm 128	*
<i>Chenopodium album</i> (4.8)	21 \pm 21	358 \pm 201	337 \pm 248	
<i>Erigeron canadensis</i> (21.3)	337 \pm 21	42 \pm 21	126 \pm 96	*
<i>Hedeoma hispida</i> (2.1)	189 \pm 96	84 \pm 56	84 \pm 56	
<i>Lepidium densiflorum</i> (8.0)	210 \pm 111	21 \pm 21	0 \pm 0	
Perennial forbs (4.3)	1747 \pm 770 [7]	1368 \pm 168 [9]	2630 \pm 2095 [11]	
<i>Rumex acetosella</i> (0.3)	1010 \pm 359	337 \pm 172	316 \pm 222	
<i>Achillea millefolium</i> (15.4)	252 \pm 193	484 \pm 295	168 \pm 84	
<i>Verbascum thapsus</i> (22.5)	358 \pm 358	231 \pm 76	126 \pm 96	
<i>Gnaphalium obtusifolium</i> (4.5)	42 \pm 21	168 \pm 138	105 \pm 56	
Grasses (8.1)	3745 \pm 926 [8]	2441 \pm 560 [11]	2967 \pm 718 [8]	
Annual grass	[1]	[1]	[1]	
<i>Setaria glauca</i> (19.5)	252 \pm 222	42 \pm 21	21 \pm 21	
Perennial grass (7.9)	2420 \pm 547 [7]	1789 \pm 358 [9]	2673 \pm 601 [7]	
<i>Poa pratensis</i> (18.9)	1515 \pm 359	778 \pm 297	1389 \pm 789	
<i>Panicum oligosanthes</i> (4.0)	44 \pm 21	358 \pm 201	820 \pm 324	
<i>Agrostis scabra</i> (3.4)	484 \pm 201	358 \pm 180	189 \pm 36	
<i>Agropyron repens</i> (∞)	0 \pm 0	126 \pm 73	63 \pm 63	
<i>Schizachyrium scoparium</i> (∞)	126 \pm 36	21 \pm 21	0 \pm 0	*

association of distribution (Fisher's exact test for presence and absence in plots) nor significant correlation of abundance (Pearson's correlation coefficients). Fraction of the species shared between the seed bank and the above-ground vegetation decreased with N-addition (Fig. 3, $P < 0.03$, with and without arcsine square-root transformation). Conversely, the fraction of the species found only from the seed bank increased with N-addition ($P < 0.03$). Thus, the similarity of the species composition between the soil seed bank and the above-ground vegetation decreased with N-addition.

Effect of N-addition and burning on litter and forb seedling density

Nitrogen addition and burning treatments had significant effects on litter depth and litter dry mass (Fig. 4). Without burning, litter was much deeper in N-added plots. There was a significant interaction between N-addition and burning. Burning greatly decreased the litter depth in N-addition plots but had little effect in the

control plots. Litter depth and litter mass per unit area were highly correlated with each other ($r^2 = 0.88$, $P < 0.00001$, $N = 36$ samples), and their response to burning differ only slightly.

N-addition and burning had significant effects on density of forb seedlings in the field (Fig. 5). There was no significant interaction between N-addition and burning at any time. N-addition had negative effects on seedling density in both unburned and burned plots. Although burning enhanced seedling emergence, seedling density was still very low in plots receiving high rates of N-addition. Density of newly emerged seedlings in May depended strongly on N-addition, but only weakly on burning (Fig. 5A, $P < 0.001$ and 0.06, respectively). The same trend held in August, both in terms of density of seedlings surviving from May through August (Fig. 5B) and total number of seedlings in August (Fig. 5D). The effect of burning on seedling emergence continued until August (Fig. 5C), but the negative effect of N-addition on newly found seedlings in August was weak ($P < 0.06$). There was no effect of

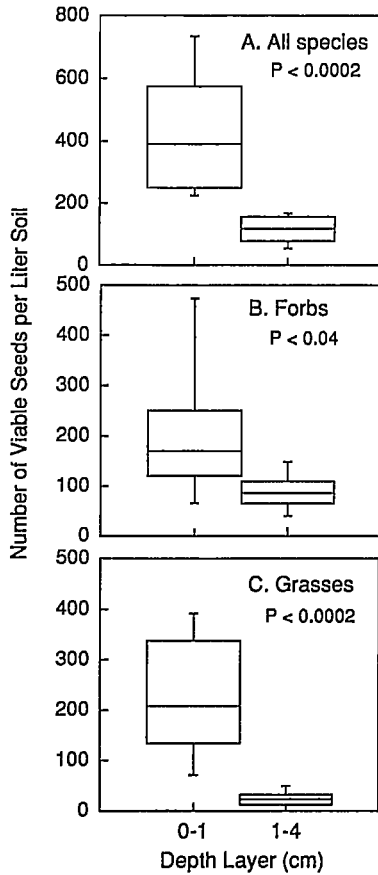


Fig. 2. Density of viable seeds (number per liter) at different depth layers of soil (0-1 cm including litter and 1-4 cm) for all species (A), forbs (B), and grasses (C). Each box plot indicate median ± 25 percentile (box) and ± 40 percentile (line) of $n = 9$ plots. Since there was no N-addition effect, all treatments were combined for t -test of the difference of seed density between depth layers (significance level indicated by P).

N-addition, burning, or their interaction on the probability of seedling survival from May to August (2-way ANOVA on arcsine square-root transformed data, model $F = 0.27$). These trends were created by seedlings

Table 2. Similarity of the soil seed bank communities among three N-addition treatments. Percentage similarity (= number of species common in two plots divided by total number of species present in either plot, upper part of the diagonal) and Pearson's correlation coefficient of species abundance between each pair of treatment (lower part of the diagonal). Total number of species in the analysis is 34 spp., of which 19 (56%) were encountered in all three treatments. *** $P < 0.0005$, ** $P < 0.005$, * $P < 0.05$.

	No-N	Medium-N	High-N
No-N (22 spp.)	-	66%	62%
Medium-N (26 spp.)	0.87***	-	70%
High-N (25 spp.)	0.36*	0.41*	-

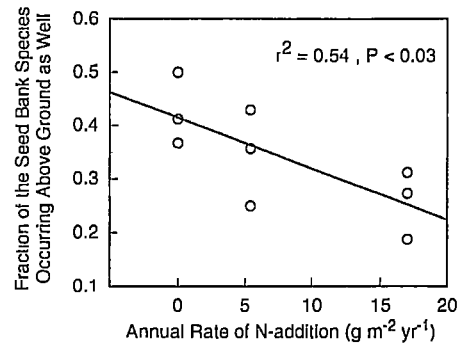


Fig. 3. Fraction of the species found in the soil seed bank that also occurred in the above-ground vegetation for field plots receiving different rates of annual N-addition (3 replicate plots per treatment). Regression is also significant when the fractions are arcsine square-root transformed.

of a very small number of species (Table 3), especially, *Polygonum convolvulus* and *Rumex acetosella*.

Correlation between litter depth and seed or seedling density

Total seedling density was negatively correlated with litter depth throughout the growing season (Table 4). In contrast, litter depth was not correlated with total

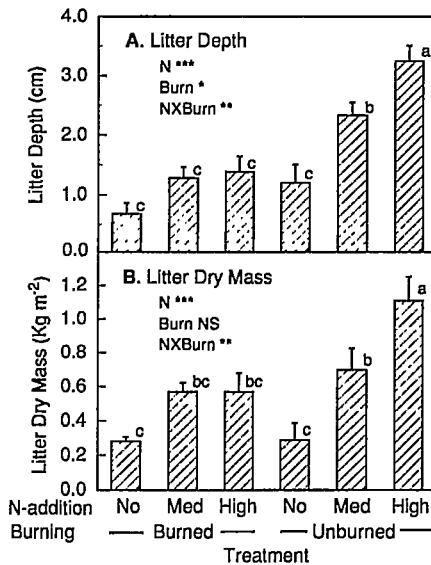
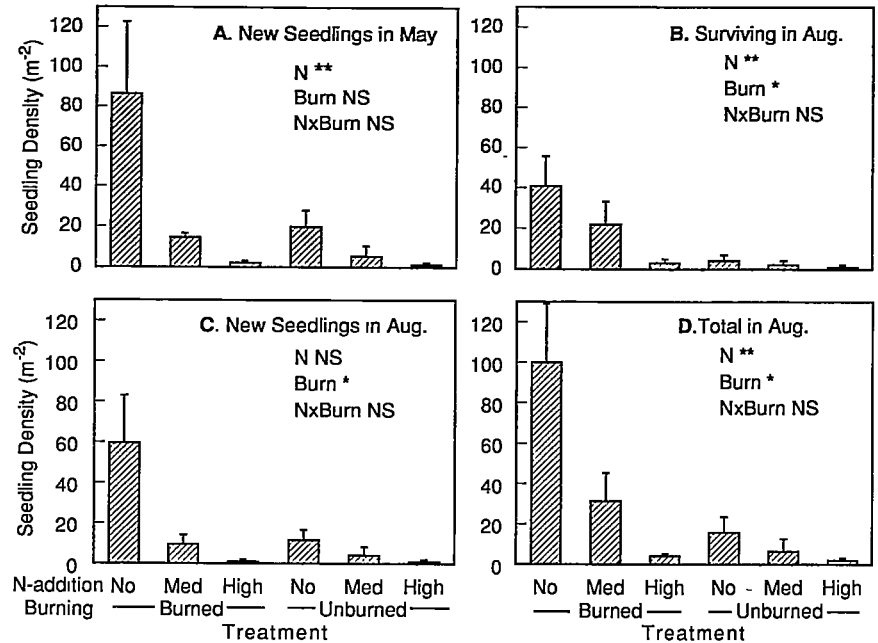


Fig. 4. Effects of N-addition and burning treatments on litter depth (A) and litter mass per unit area (B). Mean (bar) and s.e. (line) for six samples per treatment combination. Results of 2-way ANOVA are indicated as *** $P < 0.0005$, ** $P < 0.005$, * $P < 0.05$. Two treatments with a same letter are not significantly different from each other at $P = 0.05$ by Tukey-Kramer HSD for multiple comparisons.

Fig. 5. Effects of N-addition and burning on the density of new seedlings tagged in May (A), seedlings surviving in August (B), new seedlings in August (C), and total of surviving and new seedlings in August (D). Mean (bar) and s.e. (line) of three plots per treatment combination. Effects of N-addition, burning, and their interaction were tested by 2-way ANOVA (** $P < 0.005$, * $P < 0.05$).



density of viable seeds in soil for all species combined (Fig. 6A). Soil seed density of *Polygonum convolvulus* and *Rumex acetosella*, two species most abundant as seedlings in the field, showed opposite correlation with litter depth (Fig. 6B, C). Soil seed density of *Polygonum* increased with litter depth, while that of *Rumex* decreased with litter depth. The results were similar if litter mass was used instead of litter depth for these analyses.

Discussion

Because species richness of above-ground vegetation declined as productivity increased in response to N-addition (Tilman 1987, 1990, 1993), we anticipated that the seed bank species richness and seed abundance would also have declined. As species composition above ground diverged in response to N-addition treatments (Inouye and Tilman 1988), species composition in the soil seed bank diverged as well (Table 2). However, the changes in soil seed banks did not directly reflect the changes above ground. Similarity of species composition between the above-ground vegetation and the soil seed bank were low and further decreased with N-addition (Fig. 3). Overall, there was no difference in the total density or species richness of soil seed banks among N-addition treatments. Thus, low seedling emergence in N-addition plots in the field was caused by the low levels of seed germination, rather than by depletion

of viable seeds in the soil. Accumulation of litter in N-addition plots was negatively correlated with seedling density, and was a likely mechanism for inhibition of seed germination. However, litter was not the only mechanism, since seedling emergence was still very low in N-addition plots after litter depth was decreased by burning. The expectation of high seedling mortality in N-addition plots was not supported. Although low light availability at ground level in the dense stand of *Agropyron* dominated plots was expected to increase seedling mortality, survivorship of forb seedlings tagged in May was not different among treatments. Below, we discuss how responses of coexisting species with contrasting regeneration traits might have contributed to these overall results.

Response of soil seed banks to N-addition

Lack of response of total soil seed density to N-addition was the result of contrasting responses by different species; viable seed density in 0–4 cm depth decreased after 10 yr of N-addition for some species (*Mollugo*, *Erigeron*, *Rumex*, *Schizachyrium*) but it increased for some others (*Polygonum*, *Panicum*). The available pool of species in the field differed not only in adult abundance and seed rain density, but also in seed size, dispersal mechanisms, control mechanisms of physiological dormancy, and degree of protection against seed predators (Fenner 1985, Baskin and Baskin 1988,

Table 3. Total count of seedlings of forb species in the field for each treatment (24 subplots = 0.96 m² per treatment).

Burning	Burned			Unburned		
	No	Med.	High	No	Med.	High
Nitrogen addition						
<i>Polygonum convolvulus</i>	78	34	1	11	2	2
<i>Rumex acetosella</i>	24	1	0	4	0	0
<i>Chenopodium album</i>	9	0	4	0	4	0
<i>Ambrosia artemisiifolia</i>	1	0	0	0	0	0
unidentified	10	0	0	8	1	0

Washitani and Masuda 1990). Thus, it is likely that the soil seed-bank populations of different species responded differently to changes in soil fertility and productivity. Although total seed density and species richness did not change, the relative abundance of species changed with the N-addition treatment over the 10 yr (Table 1).

A decrease in the soil seed density of most species following N-addition was expected, because the monodominance by *Agropyron* in high-N plots was likely to have lowered the local seed rain of other species. This negative effect is likely to be stronger on species whose soil seed populations consist mostly of the current year's seed rain. One way to estimate which have only such "transient" seed banks (Thompson and Grime 1979) is to examine the ratio of seed density in the shallow layer to that in the deeper layer (Table 1). For example, all seeds of *Agropyron* and *Schizachyrium*, two perennial grasses dominant at high and low ends of the soil fertility gradient, respectively, were found only within 0-1 cm deep soil in the plots where adults are dominant, with none from the deeper layer (Fig. 1, Table 1). Thus, the changes in seed density of these species corresponded to the changes in adult density with N-addition. In a few other species with highly skewed seed distribution to the surface layer (*Erigeron*, *Verbascum*, *Setaria*), seed density tended to decrease with N-addition as well, but with less clear relationship to adult abundance which decreased from sporadic (in control plots) to non-existent (in High-N plots) with N-addition.

Two species, *Mollugo verticillata* (annual forb) and *Rumex acetosella* (perennial forb) had increasing seed density with soil depth. This indicates that a large fraction of their soil seed populations existed as deeply

buried dormant seeds. In these species as well, the total seed density per unit area significantly decreased with N-addition. Thus, the density of local seed rain had not been sufficient to replenish the loss of seeds from the seed bank due to germination without emerging above ground, predation, or other causes of mortality. Because physiological dormancy of seeds tends to remain intact at the deeper layer (Thompson 1992, Mohler 1993), mean depth of seed distribution is expected to increase without new seed input (Milberg 1995). In control plots, 92% and 87% of all *Mollugo* and *Rumex* seeds in a given area existed in the 1-4 cm layer, which increased to 100% in High-N plots (none in the 0-1 cm layer). These seeds in the deep layer might be largely the survivors of the seeds input from the transient increase of reproductive plants of these species in response to disturbance and N-addition during the first few years of the experiment (Tilman 1983: Fig. 2, Tilman 1987: Fig. 8).

Polygonum, a twining vine, was the only species that showed significant increases of soil seed density with N-addition. Because the fraction of seeds in the 0-1 cm layer decreased with N-addition (74, 61, and 44% in No-, Medium-, and High-N levels, respectively), the increase of total seed density with N-addition reflects sporadic, rather than constant seed rain during the 10 yr of N-addition. The successful reproduction of this annual species in N-added plots perhaps owes to its growth form. Creation of gopher mounds was not completely prevented by periodical trapping, and mound occurrence was higher in high-N plots (Tilman 1983, Inouye et al. 1987). However, a given bare opening suitable for colonization lasts for a very short period in N-added plots because of vegetative invasion by rhizomes of the dominant, fast growing *Agropyron*. *Polygonum* could climb up *Agropyron* as it rapidly closed up each gopher mound. N-addition changes disturbance regimes by pocket gophers, increases soil heterogeneity (Tilman 1983, Inouye et al. 1987), and leads to chaotic dynamics of above plant biomass (Tilman and Wedin 1991). Different disturbance regimes should favor different species in soil seed bank communities, as demonstrated in many studies in agricultural fields and grasslands where different tillage or

Table 4. Pearson's correlation coefficients (*r*) between litter depth and log(seedling density + 1) at different time. *N* = 18 (3 plots × 3 N-addition levels × 2 Burning treatments).

	<i>r</i>	<i>P</i>
New seedlings in May	-0.66	0.004
Surviving seedlings in August	-0.56	0.019
New seedlings in August	-0.47	0.056
Total seedlings alive in August	-0.61	0.010

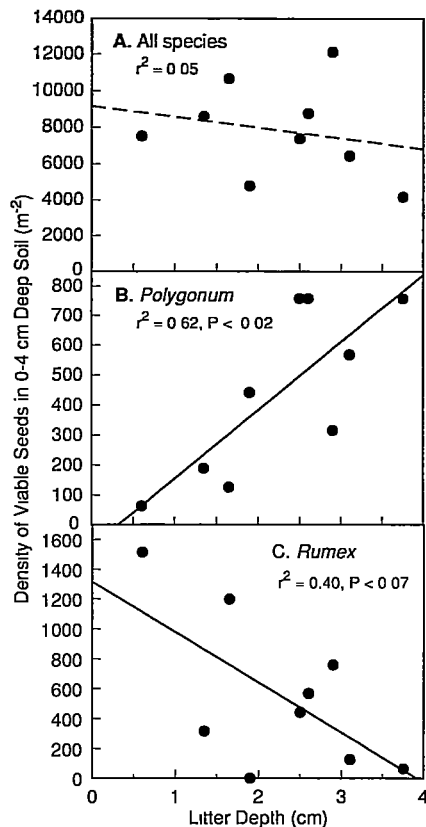


Fig. 6. Relationship between litter depth and density of viable seeds in soil for all species combined (A), *Polygonum convolvulus* (B), or *Rumex acetosella* (C). $N = 9$ plots. The weak regression for all species (A) is shown as a broken line.

grazing patterns favor different species (Roberts 1981, Milberg 1992, 1995, Mohler 1993).

Factors controlling seedling density in the fields

When all species were combined, density of seedlings were less than 1% of the density of viable seeds per unit area. This was much lower than the 3–6% typically observed in agricultural fields (Roberts 1981, Forcella et al. 1993). Seedling density was lower in higher N plots, even though density of viable seeds did not differ significantly among N-addition treatments. This low seedling density relative to seed density indicates the predominance of seed dormancy, especially in N-added plots. *Polygonum* and *Rumex* accounted for the majority of forb seedlings. This decrease in seedling density of *Rumex* with N-addition and litter accumulation could be partially caused by its decrease in abundance in the soil seed bank (Table 1, Fig. 6C). However, for *Polygonum*, seed density increased with N-addition and litter accumulation (Table 1, Fig. 6B), and its low seedling density in N-added plots must be due to germination inhibition rather than lack of seed availability.

It has been demonstrated repeatedly that litter inhibits germination or increases mortality of seedlings (Sydes and Grime 1981, Gross and Werner 1982, Monk and Gabrielson 1985, Carson and Peterson 1990). In this study, overall seedling density was correlated with litter depth that was modified by N-addition and burning treatments. Burning in the spring increased the seedling emergence in all N-addition levels; however, seedling density was still extremely low in high-N plots even though litter depth was decreased to a level similar to control plots (Figs 4, 5). Why was seedling emergence still low in N-added plots after burning had decreased litter depth? There are three plausible explanations. First, heat from burning large amounts of litter in N-added plots perhaps killed viable seeds in the litter layer and in the shallow layer of soil (Muegler 1956 cited in Major and Pyott 1966, Cheplick and Quinn 1987). Unfortunately, we did not examine the soil seed bank right before and after the burning and can not test this possibility. Second, factors associated with N-added plots other than litter depth per se might be responsible for inhibition of germination. For example, chemical inhibitors of germination in aqueous extracts of *Agropyron* litter (Hamilton and Buchholz 1955, Werner 1975, Stowe 1979) may have accumulated in the soil and suppressed seed germination even after burning decreased litter depth. Third, early seedling mortality may have been so high in N-added plots with or without burning that we missed some seedlings completely. For example, we observed a great number of crane fly larvae (suborder Nematocera) hatching in soil samples from N-added plots when they were incubated in the green house. High density of such detritus feeders in productive N-addition plots may result in high mortality for germinating seeds.

It was surprising to find no difference in seedling survivorship between N-addition treatments or burning treatments. It might be thought that lowered light level under dense *Agropyron* canopy in N-added plots would lower the seedling survival as suggested by results of Goldberg and Werner (1983). Also it might seem that seedlings emerging through thick litter may exhaust all seed reserves for hypocotyl and epicotyl extension, and thus have higher mortality (Fenner 1985, Molofsky and Augspurger 1992). These negative effects of litter accumulation were probably small for *Polygonum*, a vine with relatively large seeds (3–4 mg excluding seed coats), which was the most common species as seedlings in the study plots.

Significance of soil seed banks in plant community dynamics

N-addition changed the relative abundance of plant species in the soil seed bank, without a detectable change in the total number of species or the total seed

density of the seed bank. Our results contrasts to those in Milberg (1992) which found that seed bank species richness decreased in response to N-P-K fertilization for 35 yr. The relative abundance of species in the soil seed bank differed greatly from the relative abundance of the vegetative plants producing seeds in control plots, as has been found in many other communities (Roberts 1981, Rice 1989, Thompson 1992). The proportion of species shared between the soil seed bank and the above-ground vegetation further declined with N-addition. The generality of this discrepancy may be a strong indication of a trade-off between competitive ability and colonization ability in the plant community (Tilman 1994). Two grass species that are competitively dominant at contrasting nitrogen regimes, *Schizachyrium* (dominant on poor soil) and *Agropyron* (dominant on rich soil) have very few seeds in soil. In contrast, many other species of various life forms have large number of seeds available for colonization of gaps created by disturbance either by frequent dispersal of numerous seeds or by long viability of seeds in soil (Grime 1989). This trade-off between the ability to disperse vs compete, whether the dispersal be in space or in time, can allow the coexistence of numerous species (Skellam 1951, Horn and MacArthur 1972, Tilman 1994). This idea is in general agreement with the concepts of regeneration niches and strategies (Grubb 1977, Grime 1989), and the ecological function of fugitive species (Platt 1975). Examination of soil seed banks and seedling colonization adds depth to our understanding of the response of plant communities to resource availability. In total, our results show that the lower species richness of more productive plots resulted more from the inhibition of germination than from lower seed bank densities or lower plant species richness in the seed bank.

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