

Recovery of plant diversity following N cessation: effects of recruitment, litter, and elevated N cycling

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Abstract. Plant species richness has declined and composition shifted in response to elevated atmospheric deposition of biologically active nitrogen over much of the industrialized world. Litter thickness, litter nitrogen (N) content, and soil N mineralization rates often remain elevated long after inputs cease, clouding the prospects that plant community diversity and composition would recover should N inputs be reduced. Here we determined how N cycling, litter accumulation, and recruitment limitation influenced community recovery following cessation of long-term N inputs to prairie-like grasslands. We alleviated each of these potential inhibitors through a two-year full-factorial experiment involving organic carbon addition, litter removal, and seed addition. Seed addition had the largest effect on increasing seedling and species numbers and may be necessary to overcome long-term burial of seeds of target perennial grassland species. Litter removal increased light availability and bare sites for colonization, though it had little effect on reducing the biomass of competing neighbors or altering extractable soil N. Nonetheless, these positive influences were enough to lead to small increases in species richness within one year. We found that, although C addition quickly altered many factors assumed favorable for the target community (decreased N availability and biomass of nearby competitors, increased light and site availability), these changes were insufficient to positively impact species richness or seedling numbers over the experimental duration. However, only carbon addition had species-specific effects on the existing plant community, suggesting that its apparent limited utility may be more a result of slow recovery under ambient recruitment rather than from a lack of a restorative effect. There were dramatic interactions among treatments, with the positive effects of litter removal largely negated by carbon addition, and the positive effects of seed addition generally amplified by litter removal. It remains unclear whether each mechanism explored here will induce community recovery, but over different temporal scales. Long-term monitoring will help resolve these remaining questions. Regardless, our results suggest that reversal of species loss and compositional shifts from N deposition in prairies may be more inhibited by habitat fragmentation, recruitment limitation, and long-term suppression of fire than from continued effects of elevated N.

Key words: carbon addition; diversity; grassland; invasion; litter inhibition; nitrogen deposition; prairie restoration; recovery; recruitment limitation.

INTRODUCTION

Research has demonstrated that chronic N deposition at rates over much of the industrialized world can reduce plant species diversity and alter community composition (Bobbink et al. 1998, Stevens et al. 2004, Clark and Tilman 2008). What is unclear, however, is the potential reversibility of these shifts in ecosystem structure and function should N inputs be reduced, and which are the

dominant processes that inhibit ecosystem recovery (Bakker and Berendse 1999, Clark et al. 2009).

Theory predicts that the reversibility of terrestrial eutrophication depends on the dominant mechanisms regulating community dynamics and the presence or absence of hysteretic behavior. In communities without hysteretic behavior that are structured by a competition–colonization trade-off (Levins and Culver 1971), loss of poor colonizers in favor of opportunistic invasives suggests that recruitment limits recovery. In communities structured by resource-ratio competitive interactions (Tilman 1982), shifts in resource availabilities to a prior state will enable recovery of the original community when hysteretic tendencies are absent, but may not be sufficient when hysteric tendencies are present (Suding et al. 2004). Multiple community process operating simultaneously over different spatial and temporal scales can generate both hysteretic and

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non-hysteretic behavior from a range of study systems and approaches (Schroder et al. 2005). Thus, understanding the interactions among community processes and their relative contributions toward regulating community dynamics is critical in advancing our understanding of ecosystems.

Meta-analyses across herbaceous ecosystems find that N enrichment generally increases N cycling and plant production (Gough et al. 2000, Elser et al. 2007), favoring species proficient in rapid aboveground biomass accumulation such as *Agropyron repens* over *Schizachyrium scoparium* in Minnesota old fields (Tilman 1987), *Brachypodium pinnatum* over *Carex* spp. in Dutch calcareous grasslands (Bobbink 1991), and *Betula nana* over *Ledum palustre* in Alaskan arctic tundra (Chapin et al. 1995). Greater aboveground biomass reduces light levels below the canopy and drives a reduction in species richness (Hautier et al. 2009), with native, leguminous, perennial, and rare species especially susceptible to loss (Suding et al. 2005). The generality and reversibility of this process however is yet unclear; and, it is unlikely that increasing light to prior levels is sufficient to stimulate community recovery after long periods of seed burial for many target grassland species. This likelihood implies the presence of hysteretic behaviors, where multiple negative feedbacks between individual processes co-regulate community dynamics (Suding et al. 2004).

Compared to research on N enrichment, studies focused on recovery following cessation of N inputs to terrestrial ecosystems are much fewer, and suggest that manipulative strategies may be required to reverse some of the effects of prior fertilization. Observational studies of prior fertilization experiments find that, although plant tissue nutrient content, soil pH, and soil-extractable inorganic N pools may recover (Boxman et al. 1995, Power et al. 2006, Clark et al. 2009), plant composition and phenology, as well as microbial populations and activity may not (Strengbom et al. 2001, Nordin et al. 2005, Power et al. 2006, Standish et al. 2007a). Indeed, net N mineralization was elevated 20 years after N treatments ceased to a shortgrass steppe community (Vinton and Burke 1995) and 12 years after treatments ceased to an acid-prairie grassland (Clark et al. 2009). This lack of recovery of nutrient cycling likely maintained the persistence of the new community. Restoration of previous soil conditions, however, is no guarantee that the composition of the original community will recover. Seeds of the original species may not be viable in the seed bank, available through dispersal, or able to successfully germinate and establish in the eutrophied community (Bakker and Berendse 1999, Willems 2001). Recent research on the recovery of community composition is equivocal, with research from the United Kingdom (Eschen et al. 2007), Australia (Standish et al. 2007a, b), and the Netherlands (Kardol et al. 2008) suggesting that restoration of soil conditions is not sufficient to induce community

recovery, while research from Switzerland (Eschen et al. 2007), Minnesota (Blumenthal et al. 2003), and greenhouse studies (Eschen et al. 2006) suggest otherwise. Thus, direct comparisons of multiple mechanisms under field conditions is needed to determine the dominant mechanisms preventing community recovery, as all of them are logically defensible.

In total, this body of work suggests that several interacting factors must be in place for the re-establishment of a plant community following reduction of N inputs, including (1) the presence of propagules of the target community, (2) suitable germination conditions, and (3) favorable competitive conditions for establishment and growth (Bakker and Berendse 1999). In this study we examine the individual and interacting influences of these three mechanisms in regulating community dynamics. These are articulated in the following hypotheses.

Hypothesis 1: High rates of N cycling maintain conditions unfavorable to species of the original community.—Several studies have found evidence of elevated N cycling long after inputs cease (Power et al. 2006, Clark et al. 2009). Methods have been developed to reduce N availability including sod removal, increased frequency of fire, or addition of organic carbon. Of these, carbon addition shows the greatest promise for increasing target native species relative to nontarget invasive species (Morgan 1994, Blumenthal et al. 2003). Carbon addition induces microbial immobilization of soil N, reduces availability of soil N, and reduces plant biomass, often favoring natives over invasives (Zink and Allen 1998, Torok et al. 2000, Blumenthal et al. 2003, Eschen et al. 2007). However, most studies to date have combined carbon addition with disturbance and/or seed sowing, which confounds release from recruitment limitation with changes in soil conditions and prevents comparisons of their relative effects.

Hypothesis 2: The presence of a thick litter layer prevents the re-establishment of species of the original community.—Litter plays a key role in structuring plant communities, through its physical inhibition of growth, reduction in light levels to the soil, storage of nutrients, and facilitation of elevated pathogen levels (Facelli and Pickett 1991). Removal of the litter layer, a process normally carried out by fires in herbaceous systems, may be required to open up germination sites, increase light levels at the soil surface, and reduce ecosystem N stores to create favorable competitive conditions (Goldberg and Werner 1983, Tilman 1993, Foster and Gross 1998).

Hypothesis 3: Seed limitation prevents the re-establishment of species of the original community.—With or without favorable resource and physical conditions in place, a lack of viable propagules either in the seed bank, the existing community, or in the regional species pool could limit the re-establishment of the original community (Zobel 1991). Many herbaceous communities are recruitment limited (Grubb 1977), even species-rich savannah at our site (Foster and Tilman 2003),

suggesting that recruitment limitation may be especially severe in species-poor communities that result following long-term N enrichment.

Most studies to date have looked at one of these factors (high N availability, litter inhibition, recruitment limitation) and their associated mitigating treatments (C-addition, litter removal, propagule addition) in isolation, even though their influences on community dynamics likely interact (Suding et al. 2004). Here we report the results of a two-year field experiment, where we add carbon, remove litter, and add seed, in factorial combination, to explore these interactive dynamics in the recovery of a grassland following long term N addition.

MATERIALS AND METHODS

Site description and experimental history

The experiment is located in an old field (Field C) of the Cedar Creek Ecosystem Science Reserve (CCESR, formerly Cedar Creek Natural History Area), approximately 45 km north of Minneapolis, Minnesota, USA, that was last cultivated to corn 48 years earlier (Tilman 1987). Plant production at CCESR is primarily limited by N availability (Tilman 1984). Upon initiation of the experiment in 1982, the field was dominated by a diverse mixture of perennial prairie grasses and forbs, including *Schizachyrium scoparium*, *Artemisia ludoviciana*, *Stipa spartea*, *Sorghastrum nutans*, *Solidago nemoralis*, *Aster azureus*, *Ambrosia coronopifolia*, and *Carex* spp. (Tilman 1984, 1987). Six large plots were established in 1982 (20 × 50 m, 2-m walkway in between, termed "macroplots") to explore the effects of long-term N addition under ambient rates of herbivory (experiment E004; details *available online*).² There were two replicates of three treatments each, randomly assigned to the plots, including no nutrient addition (control), and addition of non-N nutrients (P, K, Ca, Mg, S, and trace metals) plus N at either 54 or 170 kg N·ha⁻¹·yr⁻¹. Non-N nutrients were added to ensure primary limitation by N according to Tilman (1987). Previous studies have documented no effect of adding non-N nutrients on the plant community in these N-poor fields (Tilman 1987, Clark 2007). Additional site and experimental details are available in prior publications (Grigal et al. 1974, Tilman 1987).

The macroplots of this experiment are adjacent to another experiment in this field that received similar treatments beginning in 1982 in smaller plots (4 × 4 m; Tilman 1987). N addition to both experiments caused declines in species richness and shifts in plant community composition as reported elsewhere (Tilman 1987, Wedin and Tilman 1996). In short, species-rich assemblages (12–16 species/m²) dominated by native species of C₄ grasses and forbs were replaced by species poor

assemblages (3–5 species/m²) that were dominated by invasive C₃ grasses. In the macroplots, nutrient addition treatments were ceased in 1994 to observe the dynamics of recovery. Upon initiation of the factorial experiment highlighted here, previously fertilized macroplots were dominated by five C₃ species (>80% total cover), including two invasive exotic grasses (*Agropyron repens* and *Poa pratensis*), two native perennial forbs that are common in disturbed sites (*Ambrosia coronopifolia* and *Artemisia ludoviciana*) and one native sedge (*Carex* spp.). Following earlier protocols, sedges are not identified to species (Tilman 1987). By 2004–2005, nearby control macroplots (<50 m) that had never received fertilizer closely resembled the floristic composition prior to N addition, and were still a diverse (~12 species/m²) assemblage of C₄ grasses and forbs which were primarily dominated by *Schizachyrium scoparium* (43% cover; Dybzinski 2007).

Experimental design and treatments

In 2004, we set up a manipulative experiment in one of the two macroplots that had previously received 54 kg N·ha⁻¹·yr⁻¹ to explore the mechanisms inhibiting community recovery. We subdivided this macroplot into 56 plots (2 × 3 m each), using 30 cm tall aluminum flashing driven 20 cm into the ground. Eight treatments with seven replicates each were randomly assigned to plots. Treatments included no manipulation (control, CTL), organic carbon addition (O), litter removal (L), seed addition (S), and all factorial combinations. Carbon was hand broadcasted as commercial sucrose (42% carbon) every two weeks from early April to early September in 2004 and 2005, for an annual total of 10 applications of 200 g C/m² each (2000 g C/m² total). Carbon added at these annual rates to a nearby agricultural field has been shown to decrease soil nitrate concentrations, increase light penetration to the soil surface, and favor the production of native prairie species over weedy exotics (Blumenthal et al. 2003). Litter was removed annually prior to the first C-addition treatment by gentle manual raking, then dried at 60°C and weighed. Raking caused minimal soil disturbance. Seeds from local genotypes (Prairie Restorations, Princeton, Minnesota) of 10 mid- and late-successional native species were added at 0.5 g·m⁻²·species⁻¹ using methods similar to Tilman (1997). The species mixture included one legume (*Lespedeza capitata*), three C₄ graminoids (*Andropogon gerardii*, *Schizachyrium scoparium*, *Sorghastrum nutans*), and six forbs (*Achillea millefolium* (*lanulosum*), *Artemisia ludoviciana*, *Asclepias tuberosa*, *Liatrus aspera*, *Solidago nemoralis*, *Solidago rigida*). These species are relatively common in late successional grasslands at Cedar Creek, are generally lost with N addition, and were all present in nearby control macroplots in the field (Dybzinski 2007). Only *Artemisia ludoviciana* was present with >1% relative abundance in the previously fertilized macroplot at the initiation of the experiment. Of the remaining nine

² (<http://www.cedarcreek.umn.edu/research/exper/e004/e004.html>)

species, three were rare (<1% relative abundance in 2004: *Achillea millefolium (lanulosum)*, *Andropogon gerardii*, *Lespedeza capitata*), and the remaining six were absent. Seeds were added once in spring (April 2004) and again in the fall (October 2004) to allow for winter stratification.

Measurements

A suite of above- and belowground measurements was made in 2004 and 2005 to assess the impact of treatments on community recovery. In short, we measured changes in resource conditions and site availability (soil inorganic N availability, light, germination sites) and their effects on plant community structure (plant cover, plant biomass, numbers of germinating seedlings).

We measured light penetration early and mid-season in 2004 (6 May and 21 July, respectively, not measured in 2005) in triplicate per plot using a 1 m long Decagon integrating ceptometer (Decagon Devices, Pullman, Washington, USA). Measurements were taken on a cloudless day within two hours of solar noon, above and below the litter layer, and above the canopy for full sun. Averages for the three replicate measurements are reported here as the percentage penetration of full sun at the surface of the litter layer and at the soil surface (below the litter layer).

We estimated monthly N availability in the soil surface in 2004 and 2005 with ion exchange resins (IER) using protocols commonly employed at our site (Hobbie 2005). Resin bags were buried in triplicate in each plot in non-harvest areas at 5–10 cm depth for five-week intervals from mid June to late September in 2004, and in the middle of the growing season in 2005 (mid June to mid July). Replicate samples were averaged in each plot. Total N availability was calculated as the sum across the three months. Soil samples (2.54 cm diameter, 10 cm depth, three per plot), were collected on 18 June (2005), dried (102°C for 48 hours), and weighed for soil water content.

We estimated plant community responses near the time of peak standing biomass (mid July), using percent cover (2004 and 2005), destructive harvests (2005), and seedling counts (2004 and 2005). Cover estimates were made by species in the central 1 × 1 m section of each plot using area cards as references. All cover estimates were done by the same person to control for bias. In 2005, we clipped vegetation at the soil surface in a 10 cm × 1.5 m strip adjacent to the central cover quadrat using electronic clippers. Plant material was returned to the lab, separated into litter and green plant material, and the green tissue was further separated to species. All biomass (litter and green shoots) was dried to constant weight (60°C), and weighed. Species richness was calculated as the number of species in the 1.5 m × 10 cm strip. In 2004 (10 July) and 2005 (20 July), we counted the number of seedlings in the total area of each plot just prior to taking cover and/or biomass measure-

ments. Seedlings were identified by the presence of cotyledon(s) and identified to species when possible.

Statistical analyses

For aggregate percent cover data (i.e., green, litter, bare ground) and percent light penetration, treatment effects differed through time and were analyzed separately by time period (cover, 2004 or 2005; light, May or July). Cover responses of individual species did not differ with treatment between years; thus, percent cover data for 2004 and 2005 were combined into a single analysis that included a factor for year (higher order interactions were examined and none were found). Variance stabilization transformations were explored (logit, arcsine square root) for percentages and did not qualitatively change the results or reduce the heteroscedasticity, so analyses on percentages are reported here. Two plots were removed from all analyses in 2005 due to ant hill formation (one O plot and one OS plot). All responses were initially examined using a full factorial model (type III sum of squares) with terms for organic carbon addition (O), litter removal (L), seed addition (S), and all factor combinations. Models were then reduced to exclude nonsignificant terms ($P > 0.05$), the results of which are reported here.

RESULTS

Light

In May, litter removal increased light penetration to the soil surface by 54%, and decreased light penetrating through the canopy by 16% (Table 1, Fig. 1). Between May and July, average light levels declined markedly across treatments, by 30% at the litter surface (from 81% to 51%) and by 17% at the ground surface (from 46% to 29%). In July, light penetrating the canopy to the litter surface increased with carbon addition by 33% and was unaffected by other treatments (Table 1). Light penetration to the soil surface increased with carbon addition (+15%) and litter removal (+12%), and interactively with their combination (+43%; Table 1). Light incident on the soil surface in the OL and OLS treatments was nearly double that of any other treatment combination. There was a weak O × S interaction (Table 1, Fig. 1), as light incident on the soil surface increased with carbon addition (+17%), was unaffected by seed addition, and increased more with their combination (+23%).

Available inorganic N

Total resin-N in 2004 was strongly reduced with carbon addition and was generally unaffected by other treatments (Table 1, Fig. 2). There was a nonsignificant trend for reduced soil N with seed addition ($P = 0.064$). Analyses of N availability for each period gave similar results, with only carbon addition having a significant effect ($P < 0.01$ for each month, analyses not shown). In the single collection for 2005, resin-N was again reduced by organic carbon addition and was unaffected by other treatments (Table 1), though it was higher in absolute

TABLE 1. Analytical results for light, dissolved inorganic nitrogen (DIN), and plant community responses measured in 2004 and 2005 in a model including organic carbon addition (O), litter removal (L), seed addition (S), and significant interactions.

Category	Response	O	L	S	OL	OS	LS	OLS	Model	R ²
2004										
Light	May %PAR (ground surface)	ns	+***	ns	ns	ns	ns	ns	***	0.89
	May %PAR (litter surface)	ns	-***	ns	ns	ns	ns	ns	***	0.48
	July %PAR (ground surface)	+***	+***	ns	**	*	ns	ns	***	0.77
	July %PAR (litter surface)	+***	ns	ns	ns	ns	ns	ns	***	0.76
DIN	Total (June–September)	-***	ns	ns	ns	ns	ns	ns	***	0.50
Plant	Percent cover (green)	-**	ns	ns	ns	ns	ns	ns	*	0.14
	Percent cover (litter)	+**	ns	ns	ns	ns	ns	ns	*	0.14
2005										
DIN	June–July	-***	ns	ns	ns	ns	ns	ns	***	0.34
Plant	Percent cover (green)	-***	ns	ns	ns	ns	ns	ns	**	0.29
	Percent cover (litter)	ns	ns	ns	ns	ns	ns	ns	ns	0
	Percent cover (bare ground)	+***	ns	ns	ns	ns	ns	ns	***	0.33
	Biomass (green)	-***	ns	ns	*	ns	ns	ns	**	0.25
	Biomass (litter)	ns	-***	-*	ns	ns	ns	ns	***	0.42
	Species richness	-**	ns	+*	*	ns	ns	ns	***	0.27
	Number of seedlings	ns	+*	+***	*	ns	ns	*	***	0.63

Notes: Light responses are separated for each month (May or July) and for incidence either to the litter or the soil surface. DIN is total N availability summed over the three collection periods in 2004, and for the single collection period in 2005. PAR stands for photosynthetically active radiation; %PAR = 100 in full sun (i.e., not in the shade, not under the plant canopy). So, %PAR (ground surface) means the percentage of incident sunlight (relative to full sun) that hits the ground surface; e.g., %PAR(ground surface) = 30%, means that 30% of full sunlight was incident on the ground surface. Plant responses include the percent cover in 2004 and 2005 of green shoots, litter, or bare ground, the dry biomass in 2005 of green shoots and litter, the species richness as estimated by clipped vegetation in 2005, and the number of seedlings counted in 2005. There was little bare ground recorded in 2004 (no significant relationships). The direction of relationship (+, positive; -, negative) is indicated for significant main effects only. Also indicated are the level of significance, the amount of variance explained in the model (R²), and the significance of the overall model F value after removing nonsignificant terms.

* P ≤ 0.05; ** P < 0.01; *** P < 0.001; ns, P > 0.05.

magnitude in 2005 than in 2004 (averaging 164 and 610 μg N/g dry resin in plots that did and did not receive carbon respectively).

Plant cover and biomass

In 2004, organic carbon addition reduced plant cover by 10% (from 47% to 37%) and increased litter cover by

10% (from 53% to 63%; Table 1). There were only trace amounts of bare ground recorded in 2004 (no significant effects), and no other treatments significantly affected percent cover responses (Table 1).

In 2005, cover of plant material decreased with organic carbon addition by 12% (from 48% to 36%), cover of litter was unaffected by treatment (average of

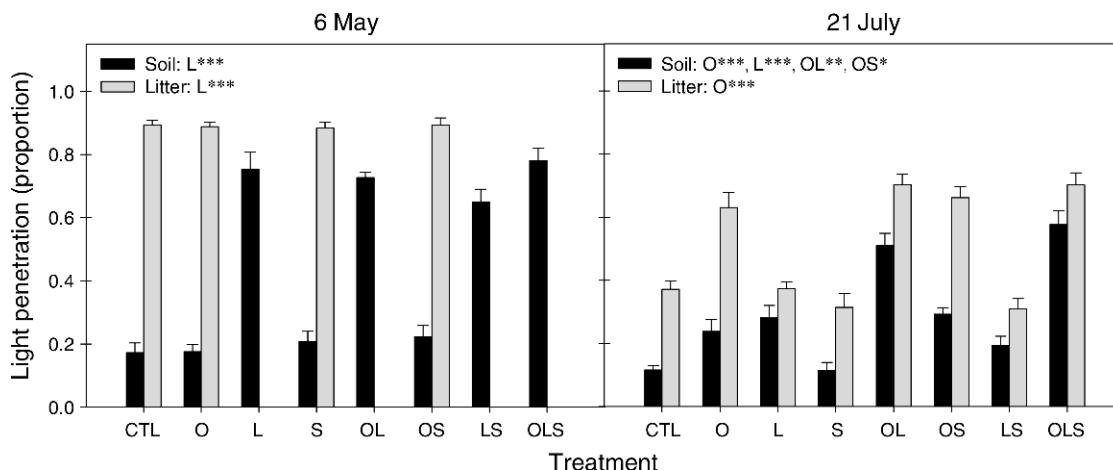


FIG. 1. The proportion of incident light that penetrated the canopy to the litter surface (gray bars) and the soil surface (black bars) early and mid-season averaged for each treatment (+SE). Treatments are control (CTL), carbon addition (O), litter removal (L), seed addition (S), and all combinations. Significant terms from analytical results (Table 1) are indicated next to the key in each panel. Incidence of full sun was 1477 ± 30 and 1522 ± 11 μmol photons/m² for May and July, respectively.

* P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001.

48%), and cover of bare ground increased with carbon addition by 14% (from 2% to 16%). Though no other treatments significantly affected gross cover estimates (Table 1), litter removal tended to increase bare ground cover ($P = 0.075$, from 6% to 12%). Aside from bare ground and litter, which increased and decreased respectively between 2004 and 2005, there were no differences between the two years in the effect of treatment on percent cover.

Aboveground plant biomass was slightly reduced by carbon addition and greatly reduced with litter removal and carbon addition combined (Fig. 3, Table 1). No other treatments significantly altered aboveground biomass. The biomass of litter removed averaged 156 and 35 g/m² in 2004 and 2005, respectively in L plots. There was a nonsignificant O × S interaction ($P = 0.072$) indicating that although carbon addition tended to increase litter biomass and seed addition had little effect, their combination tended to reduce litter biomass (Fig. 3).

The same five species previously mentioned dominated the field in 2004 and 2005 and were the only species to make up more than 1% in cover and 3 g/m² in biomass averaged across the experiment (*Agropyron repens*, *Ambrosia coronopifolia*, *Artemisia ludoviciana*, *Carex* spp., and *Poa pratensis*). *Agropyron* biomass decreased with carbon addition and litter removal combined and was unaffected by seed addition (Fig. 3, Table 2). *Carex* biomass responded with complex O × S and O × L interactions, increasing with organic carbon or seed addition alone (though relatively unaffected by their combination), and decreasing with organic carbon addition if the litter layer had also been removed. *Artemisia* also had a significant O × S interaction, though in the opposite direction. *Artemisia* biomass was reduced by addition of organic carbon or seed, though their combination had no additional effect than either individually (litter removal had no effect). *Poa* biomass was reduced by carbon addition; and, though carbon addition and litter removal together appeared to amplify biomass reduction (Fig. 3), this was nonsignificant ($P = 0.1$). *Ambrosia* biomass increased with litter removal, decreased with carbon addition, and was unaffected with their combination (Table 2, Fig. 3). Species cover responses generally gave qualitatively similar results to biomass responses, with the exception that *Agropyron* cover was reduced with addition of organic carbon alone (C. M. Clark, unpublished data).

Species richness

Species richness as estimated by clipped biomass in 2005 increased with litter removal or seed addition, and was unaffected by carbon addition alone (Fig. 4). However, carbon addition in combination with litter removal decreased species richness, leading to a significant O × L interaction (Table 1, Fig. 4). Analyses of richnesses by functional group generally were not robust to model assumptions, but the increase in richness with

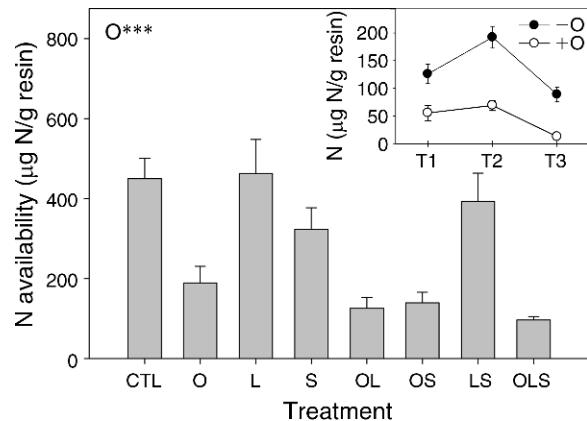


FIG. 2. Total resin N in 2004 summed across the three months of collection averaged for each treatment (+SE). The inset shows temporal dynamics averaged over all treatments that did receive carbon (+O) and those that did not (-O) for the three periods of collection (T1, 10 June–14 July; T2, 14 July–19 August; T3, 19 August–21 September). Treatment abbreviations are from Fig. 1. Significant terms from analytical results (Table 1) are indicated.

seed addition tended to be from legumes and forbs, while the increase with litter removal and decrease with carbon addition tended to be from forbs (C. M. Clark, unpublished data).

Seedling numbers

Only seven seedlings were recorded in 2004, with no relationships among treatments. In 2005, the number of emergent seedlings responded strongly to treatments (Table 1, Fig. 5). Seedling number increased dramatically with seed addition, especially when the litter layer was also removed, while litter removal and carbon addition alone or in combination had only minor effects on increasing seedling numbers. Carbon addition did not affect the number of seedlings in plots that only received seed; however, if the litter layer had also been removed, carbon addition did reduce seedling numbers, resulting in a significant three-way interaction (Table 1).

DISCUSSION

Restoration of species diversity following long-term N enrichment to herbaceous systems involves a process of community re-assembly promoting increases in target seedling numbers and species richness. Our results suggest that, over the short time duration of this study, recovery to target diversity levels (~12–16 species/m² of late successional forbs and C₄ grasses; Tilman 1987, Dybzinski 2007) is hindered primarily by seed limitation, and that sustained high levels of N and litter contribute less to inhibition of community recovery. Similar responses have been found in other studies. Indeed, one-time seed addition to an abandoned hay field in the tall-grass prairie region was sufficient to increase sown C₄ grasses and legumes and decrease nonsown resident species even after 6 years (Foster et al.

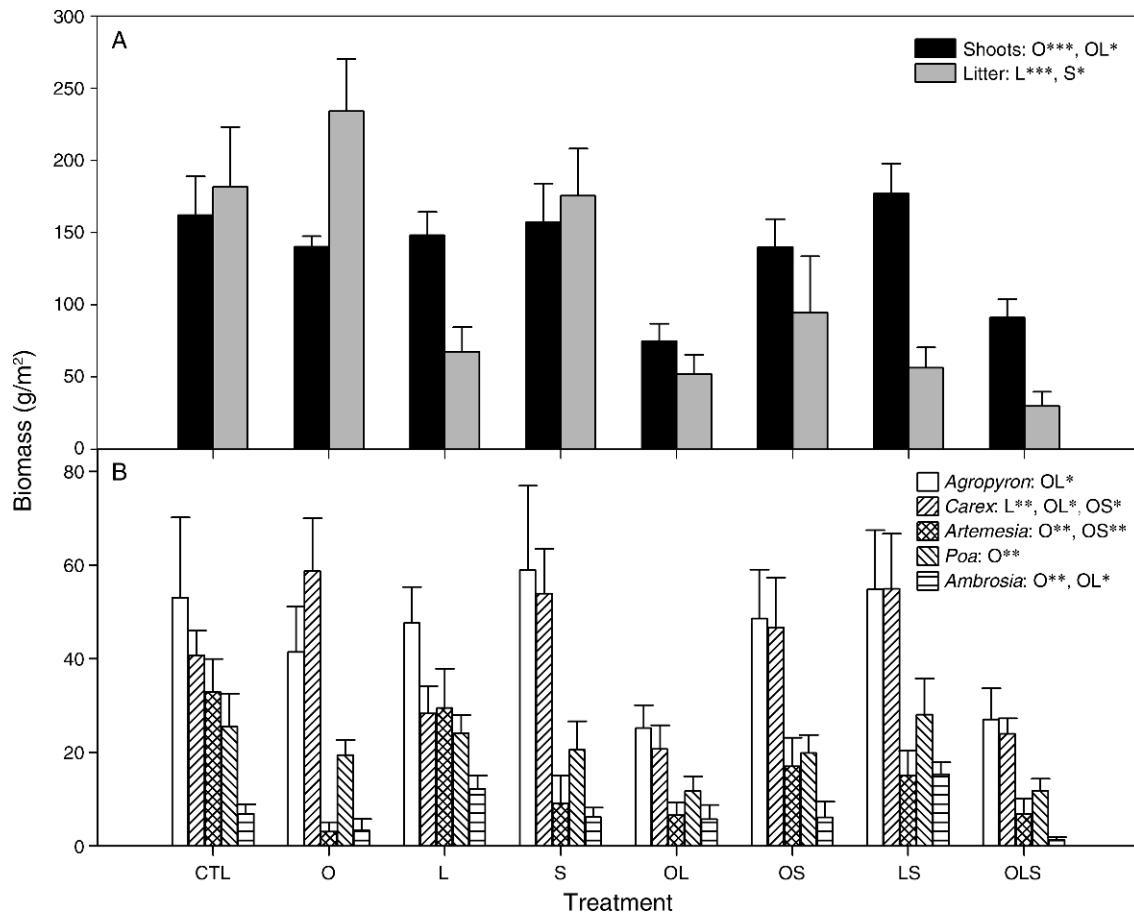


FIG. 3. Average biomass in 2005 (+SE): (A) total aboveground living biomass (shoots) and litter; (B) shoots of the five dominant species in the field, by treatment. Treatment abbreviations are from Fig. 1. Significant terms from analytical results (Table 1) are indicated in the keys.

2007). Furthermore, research from the United Kingdom (Eschen et al. 2007), Australia (Standish et al. 2007a, b), and the Netherlands (Kardol et al. 2008) similarly report that restoration of soil conditions may not be sufficient to induce community recovery. Thus, the relatively short distances that must be traversed from the nearby control macroplot (<50 m) may be enough to prevent recovery 12 years after treatments cease under ambient seed rain, highlighting the importance of local interactions and spatial structure for these sessile organisms (Tilman 1994). Several lines of evidence suggest that seed limitation might be strong. First, many target species lost following 10 years of N addition (range of

treatments: 10–170 kg N·ha⁻¹·yr⁻¹) do not recover in abundance 10 years after N treatments cease (Clark and Tilman 2008). Second, these target species do not generally bank seeds in the long term seed bank (Kitajima and Tilman 1996). Third, even unfertilized species-rich savannah at this site has been found to be seed limited, and one-time seed addition to these fields appears to have sustained impacts on community structure for nearly a decade or longer (Tilman 1997, Foster and Tilman 2003). Whether or not seed addition leads to sustained increases in species and seedling numbers in previously fertilized fields in this system remains to be seen. Regardless, this work combined with

TABLE 2. Analytical results for biomass responses for the five dominant species in 2005.

Species	O	L	S	OL	OS	LS	OLS	Model	R ²
<i>Agropyron repens</i>	ns	ns	ns	*	ns	ns	ns	*	0.11
<i>Ambrosia coronopifolia</i>	–**	ns	ns	*	ns	ns	ns	***	0.26
<i>Artemisia ludoviciana</i>	–**	ns	ns	ns	**	ns	ns	***	0.28
<i>Carex</i> spp.	ns	–**	ns	*	*	ns	ns	**	0.23
<i>Poa pratensis</i>	–*	ns	ns	ns	ns	ns	ns	*	0.10

Note: See Table 1 for explanation.

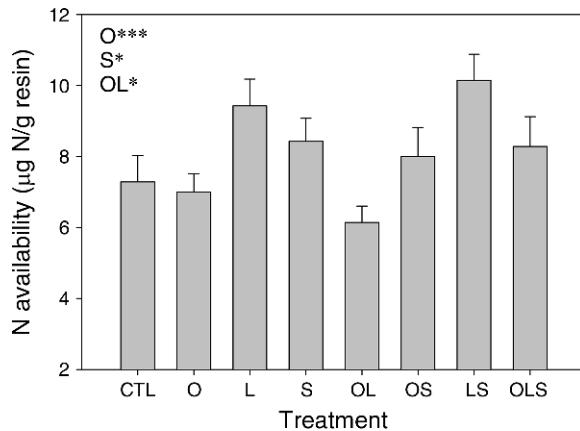


FIG. 4. Average species richness (+SE) in 2005, by treatment, from 10 cm × 1.5 m harvest areas. Treatment abbreviations are from Fig. 1. Significant terms from analytical results (Table 1) are indicated.

our observations of increased species and seedling numbers when seed was added to an intact thick litter layer (Fig. 5), and of a 72% decline in *Artemisia* with seed addition (even though it was included in the seed rain, Fig. 3), suggest that the addition of seed of several dominant grasses may be sufficient to initiate competitive displacement in these fields.

Many studies have found that a thick litter layer can regulate species numbers from physical impediment, shading, and increased mortality (Facelli and Pickett 1991, Tilman 1993, Foster and Gross 1998, Weltzin et al. 2005), which together tend to reduce species gains and increase losses especially for forbs (Tilman 1993, Xiong and Nilsson 1999). We found that litter removal increased species richness, but that seedling numbers were only amplified under elevated, not ambient, seed rain. These apparently contradictory findings likely resulted from an increase of annuals that had been missed in the seedling assays that occurred later in the season (July), rather than from an increase of slow-growing target species from the seed bank (Kitajima and Tilman 1996). It is not surprising that litter removal had no effect on soil N availability within two years, although one might expect that over longer time periods, N availability may eventually decline following removal of N-rich litter of these opportunistic species (Berendse et al. 1992, Clark et al. 2009). Though we cannot disentangle the exact mechanisms by which litter influenced species richness and seedling emergence, our study suggests a modest increase in species numbers likely from increases in annuals from the long-term seed bank rather than from colonization by target-perennial species.

Our observed lack of a richness or seedling increase with carbon addition may be due to the short duration of study, as species in the nearby (<50 m) control macroplot had insufficient time to recolonize these plots. Lack of a response was not from a lack of changes in

resource levels, as carbon addition was the only treatment that led to large and rapid changes in both aboveground and belowground resource availabilities. Natural recolonization of Minnesota old fields by these target species following agricultural abandonment can take several decades (Inouye et al. 1987, Tilman 1990), a process that may be further slowed when nutrients are added and biomass is not removed either from harvest, fire, or grazing. Indeed, unassisted community recovery of three Australian old-fields only occurred in the field in which soil conditions had recovered and only decades after abandonment (Standish et al. 2006, 2007a). Nonetheless, species-specific responses of the extant community to changes in resource levels also appeared to be triggered with C addition, indicating a trend toward recovery. Of the five initial dominants, both nonnative species (*Agropyron* and *Poa*) and both native species common in disturbed areas (*Artemisia* and *Ambrosia*) tended to decrease with addition of organic carbon. In contrast, the native sedge (*Carex*), which normally coexists with the target native forbs and C₄ grasses, increased with carbon addition (Fig. 4). These antagonistic responses explain why there was only a small and nonsignificant decrease in total biomass, and suggest a potential for recovery following carbon addition. Even though promising, this potential remains undetermined, as most other studies that report beneficial impacts from carbon addition include some type of initial or recent disturbance (tillage, mowing, or burning) combined with direct sowing of target species (McLendon and Redente 1992, Zink and Allen 1998, Blumenthal et al. 2003, Eschen et al. 2007), and the few that do not have found conflicting effects (Paschke et al. 2000, Eschen et al. 2007).

The negative impacts from carbon addition to plots without a litter layer were unequivocal, in terms of reducing richness and negating the increase in seedlings, though explanations for the effects are unclear. We speculate that the observed high light levels from

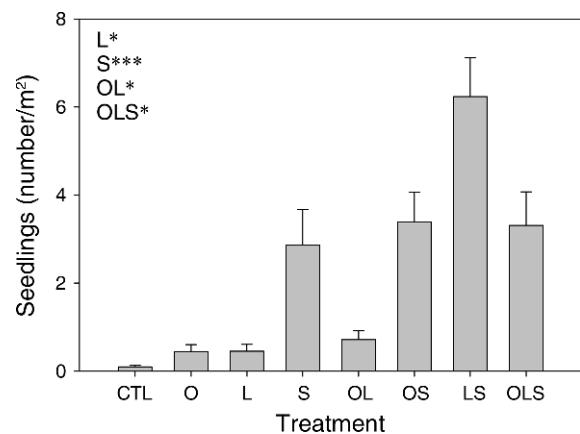


FIG. 5. Average number of seedlings (+SE) counted in 2005 by treatment. Treatment abbreviations are from Fig. 1. Significant terms from analytical results (Table 1) are indicated.

combined litter removal and carbon addition may have been too unfavorable for successful germination and growth. Alternatively, the small additional decline in soil nitrate (in OL as opposed to O plots; Fig. 2) may have crossed some threshold indicative of strong plant–microbial competition for soil N (Wedin and Tilman 1996), leading to the large observed decline for all five dominants and negating the restorative impacts from litter removal. Examination of gross N transformation rates and germination trials would help elucidate the role of these processes.

Specific considerations regarding soil acidification and the amount and form of carbon addition warrant attention, but probably do not explain the conclusions presented here. Because base cations had been added along with N since the experiment was initiated (Tilman 1987), soil pH had changed very little with N addition or cessation (range: 5.1–6.1; Clark et al. 2009). As such, we did not assess the role that changes soil pH may have played in this study. However, we acknowledge that in other systems soil pH may play a substantial role in the process of N-induced diversity decline and the potential for recovery (Roem et al. 2002, Horswill et al. 2008). Second, although the amount and form of carbon added likely influenced the findings from this study, both were expected to maximize the impact of added C. Blumenthal et al. (2003) tilled in varying amounts of C that was primarily sawdust (0–3346 g C/m², 94% sawdust), finding that the effects of C addition generally increased with more C, decreasing soil nitrate, increasing light, decreasing weed biomass, and increasing prairie species biomass. We used C addition at 2000 g C/m², intentionally exceeding Blumenthal et al.'s (2003) reported thresholds for increases in C₄ prairie grasses (1000 g C/m²) and C₃ prairie grasses and forbs (529–1675 g C/m²), relative to weeds in order to maximize the potential for effect. We used 10 applications (200 g C/m² each) of 100% sucrose because we did not want to disturb the site by tillage, and because topical addition of sucrose plus sawdust to an unplanted UK field (1000 g C/m²) had no effect on the relative abundances of legumes, forbs, or grasses (Eschen et al. 2007).

Implications for theory and for restoration

Resource levels and propagule availability have been hypothesized to jointly influence species coexistence (Chesson 2000) and site invasibility (Davis et al. 2000, Fridley et al. 2007). Davis et al. (2000) hypothesized that sites are most easily invaded when propagule availability and high resource levels coincide. Our results support this hypothesis, as species richness and seedling numbers were greatest under the combined influences of litter removal and seed addition in plots that did not receive carbon. Further, reduced soil nitrate levels are likely to affect seedlings of target species more strongly than established adults (of either nontarget or target species), as no root system or storage tissues have yet developed. Hautier et al. (2009) recently showed direct experimental

evidence that light competition was the primary driver of diversity loss following N addition. If light limitation were also the dominant mechanism preventing recovery, then litter removal or carbon addition alone should have yielded most of (and similar) restorative influences through each of their effects on increasing light. However, our observed lack of a restorative effect of carbon, lack of a seedling response from litter removal alone, and the prevalence of recruitment limitation in both natural and fertilized fields (this study; Kitajima and Tilman 1996, Foster et al. 2007, Standish et al. 2007a), suggest otherwise. Rather, hysteretic effects from multiple interacting factors that operate in a spatially structured habitat suggest that restoring prior nutrient conditions is insufficient to promote community recovery, and that the reduction of soil nutrient levels may be the result of rather than a precursor for community recovery.

From a conservation perspective, all three treatments had rapid effects on the recovery of some community properties (e.g., carbon addition on resources, seed addition on community structure, litter removal on some of both), and none influenced all responses. This suggests that a combination of treatments might be necessary to stimulate recovery, to manage multiple self-reinforcing processes (Davis et al. 2000, Suding et al. 2004, Seastedt et al. 2008). Alternatively, it may be that each individual mechanism explored here will lead to community recovery, though over different temporal scales. However, whether the mechanisms and their combinations explored in this study will lead to the same final state via different pathways, or different states altogether, remains to be seen. Regardless, our results suggest that over the short-term seed limitation is the dominant process preventing community recovery. In total, these studies warn that systems sensitive to species loss from N-induced light limitation likely have hysteretic tendencies that we hypothesize will increase in magnitude with the temporal duration of N deposition (from seed mortality in the seed bank) and its spatial extent (from slow recolonization), necessitating additional management to reverse.

Conclusions

Our study found that the recovery of herbaceous ecosystems following reduction in regional N deposition is hindered primarily by seed limitation over the short term, and that diminishing contributions to inhibition are made by litter inhibition and sustained high levels of N. Indeed, we found that although C addition quickly altered many factors assumed favorable for the target community (decreased N availability, increased light, increased bare sites, and decreased biomass of nearby competitors), these changes were insufficient to positively impact species richness or seedling numbers. However, only carbon addition had strong and species-specific effects on the existing plant community, suggesting that its apparent limited utility may be more

a result of slow recovery under ambient recruitment rather than from a lack of a restorative effect. Long-term monitoring will help resolve these remaining questions. Reversal of species loss and compositional changes from regional N deposition appear to be more impacted by habitat fragmentation and long term suppression of fire than from continued effects of elevated N cycling.

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