Long-lasting effects on nitrogen cycling 12 years after treatments cease despite minimal long-term nitrogen retention

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Abstract

Atmospheric deposition of biologically active nitrogen (N) has increased dramatically over the past 60 years, with far-reaching impacts on the structure and function of many ecosystems. Much research has examined the initial impacts of N enrichment; however, few studies have been multidecadal, and even fewer long-term studies have examined the longevity of N-induced impacts on N cycling after inputs cease. Here, we address this gap by reporting the state of key N pools and fluxes in a Minnesota grassland for plots that received N addition for 10 years and then none for 12 years, in comparison with plots that received annual N treatment for the entire 22 years. We found weak evidence for long-term N retention in plots that ceased receiving treatment; and in plots that continued to receive N over the 22-year period, retention that was high after 12 years (50–100% of inputs) was greatly reduced after 22 years (to 15%). In spite of this, net N mineralization rates remained elevated in plots that ceased receiving treatment 12 years prior, likely because N-rich litter maintained higher N-cycling rates. These results suggest (1) some systems do not retain much deposited N, with potentially large impacts on downstream habitats; (2) the previously reported high retention efficiencies for this and many other terrestrial ecosystems may be relatively short-lived as N sinks become saturated over time; and (3) the effects of even small amounts of retained N in N-limited environments may be particularly long-lasting. In total, these findings highlight the importance of long-term studies in evaluating the impacts of chronic N deposition to ecosystems, and urge additional research examining dynamics following N cessation to evaluate the reversibility of these impacts.

Keywords: eutrophication, nitrogen deposition, net N mineralization, N retention, recovery

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Introduction

Fossil fuel combustion and modern agriculture have increased inputs of biologically active nitrogen (N) over much of the industrialized world (Vitousek *et al.*, 1997; Galloway *et al.*, 2004). As N availability often limits the production, structure, and function of terrestrial ecosystems (Vitousek & Howarth, 1991; Vitousek *et al.*, 1997), these increased inputs may substantially impact terrestrial ecosystems globally (Sala *et al.*, 2000; Galloway *et al.*, 2004).

Many studies in a range of ecosystems have examined the effects of increased N inputs on biogeochemical cycling. Generally, N addition increases N cycling rates, often increasing aboveground plant biomass and tissue N content, soil N mineralization rates, and N losses through either gaseous or aqueous pathways (Pastor *et al.*, 1987a,b; Chapin *et al.*, 1995; Wedin & Tilman, 1996; Venterea *et al.*, 2003; Magill *et al.*, 2004; Hobbie, 2005). These changes in nutrient cycling are often accompanied by losses in plant diversity and shifts in community composition toward species that

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rapidly accumulate large stores of aboveground biomass (Tilman, 1987; Wedin & Tilman, 1996; Bobbink *et al.*, 1998). The long-term fate of added N, however, is found not in living tissue but in the organic or mineral soil (Nadelhoffer *et al.*, 1999, 2004; Barrett & Burke, 2002; Kaye *et al.*, 2002; Pilkington *et al.*, 2005b). Estimates of retention in plant tissue are often <20% of added N (Power *et al.*, 1998; Nadelhoffer *et al.*, 1999, 2004; Barrett & Burke, 2002; Pilkington *et al.*, 2005b), while retention in soil may exceed 70% for forest (Magill *et al.*, 2004; Nadelhoffer *et al.*, 2004) and herbaceous ecosystems (Wedin & Tilman, 1996; Kaye *et al.*, 2002; Phoenix *et al.*, 2003; Pilkington *et al.*, 2005b). However, lower estimates of soil retention (<30%) have also been reported (Wedin & Tilman, 1996; Tietema *et al.*, 1998; Phoenix *et al.*, 2003). Causes of this variation are unclear, as the dominant mechanism(s) of soil N retention are still unknown (Aber *et al.*, 1998; Magill *et al.*, 2004; Nadelhoffer *et al.*, 2004; Colman *et al.*, 2007).

Although many of the effects of N addition are well understood, the reversibility of N-induced shifts in ecosystem processes is largely unknown. How long does added N remain in ecosystems after inputs cease and where does it reside? Do the changes in biogeochemical cycling observed during the period of N addition persist after inputs stop? Limited information comes from a small number of artificial roof experiments in European forests (Boxman *et al.*, 1995, 1998) and observations of past fertilization experiments in forests (Koopmans *et al.*, 1995; Strengbom *et al.*, 2001; Nordin *et al.*, 2005; Chen & Hogberg, 2006), heathlands (Power *et al.*, 2006), and herbaceous ecosystems (Milchunas & Lauenroth, 1995; Vinton & Burke, 1995). Twenty years after N additions to a short grass steppe community ceased, net N mineralization and aboveground biomass were still elevated in formerly treated plots, although soil and microbial carbon (C) and N had recovered to control levels (Vinton & Burke, 1995). Fourteen years after N additions to a Swedish pine forest ceased, gross mineralization of N was three times higher than controls and indistinguishable from plots still receiving treatment (Chen & Hogberg, 2006). By contrast, only 2–4 years after roof installment to a Dutch pine forest, there were reductions in concentrations of nitrate in leachate, shifts in needle nutrient concentrations toward healthier levels, and reductions in net N mineralization (Boxman *et al.*, 1995, 1998; Koopmans *et al.*, 1995). Eight years after N addition ceased to a heathland ecosystem, Power *et al.* (2006) found continued effects of prior N addition on canopy height, canopy density, and microbial activity, while microbial biomass, extractable soil N, and total soil N had apparently recovered to control levels. Thus, the degree to which impacts of N addition are reversible (and for

which ecosystem properties) and the local biotic and abiotic properties that either impede or facilitate this recovery remain largely unknown. Knowledge on these topics will improve predictions of the long-term impacts of N deposition (Galloway *et al.*, 2004).

The long-term retention of added N and its impacts on nutrient cycling are interrelated, and together dictate the reversibility of N-induced changes. If ecosystems retain deposited N in readily available forms that are tightly cycled, effects on biogeochemical cycling may last long after inputs cease. If, on the other hand, systems retain N in unavailable forms, or in available forms that are readily lost, effects on biogeochemical cycling may be short-lived after N inputs cease. Thus, the reversibility of N-induced shifts in ecosystem function is influenced by both retention and cycling characteristics.

Here, we present the results of a long-term experiment in a prairie-like acid grassland of Minnesota where N was added at a range of rates for 10 years, after which N addition was stopped in half of the replicates. Much previous work has demonstrated that N addition to this site increases soil N availability, aboveground plant and litter mass and N content, and net N mineralization rates (Pastor *et al.*, 1987b; Tilman, 1987; Wedin & Tilman, 1996). These changes stimulate a shift in plant community composition and a sharp decline in diversity, from a species-rich assemblage of forbs and C_4 grasses to a species-poor community of invasive C_3 grasses such as *Agropyron repens* and *Poa pratensis* (Tilman, 1987; Wedin & Tilman, 1996). Following a decade of N addition, greater than 50% of the added N was retained in this N-poor system, suggesting that the impacts of N addition may be relatively large and long-lasting (Wedin & Tilman, 1996). The reversibility of these changes is unknown, and an observation of the recovery of the plant community has yielded mixed trends, with species numbers converging with controls without concomitant recovery in species abundances (Clark & Tilman, 2008).

This paper reports the state of key N pools and fluxes in 2003, for plots that received annual N treatment for 10 years (1982–1991) and then none for 12 years thereafter (1992–2003), and for plots that have received annual N treatment for the entire 22 years (1982–2003). The principle aims of this study are threefold: (1) determine whether the N retained after 10 years of N addition is still retained 12 years after treatments stopped, (2) determine to what extent this retention influences current N-cycling patterns, and (3) determine whether the high rates of retention observed after 10 years are sustained after 22 years of continued N addition. No study to date has examined the process of N enrichment and subsequent recovery for as long as

ours, providing an important benchmark for research on the recovery of nutrient cycling and storage following changes in N deposition.

Materials and methods

Study site and experimental treatments

The experiment is located in Cedar Creek Ecosystem Science Reserve (formerly Natural History Area), approximately 45 km north of Minneapolis, MN, USA (45°24′N, 93°12′W). Cedar Creek is located on a glacial outwash with nutrient-poor sandy soils (Grigal et al., 1974), resulting in plant production that is primarily limited by N availability (Tilman, 1984, 1987). Secondary succession occurs very slowly in these abandoned agricultural fields, and they are typically dominated by herbaceous vegetation for 50 years or more following abandonment (Inouye et al., 1987).

In 1982, an experimental grid was established in an old field (Field C, www.cedarcreek.umn.edu) that was last cultivated with corn in 1934 (Tilman, 1987). This field is part of a larger experiment at Cedar Creek that is described in more detail elsewhere (Tilman, 1987). The soil is a well-drained Zimmerman fine sand (93% sand) which is N-poor (N content, 701 $\mu g\,g^{-1}$; C:N, 14.6; percent organic matter, 2.38%), slightly acidic [pH(H_2O) = 5.8], with low cation exchange capacity (2.1 meq/100 g) and high base saturation (81%) (Grigal et al., 1974; Pastor et al., 1987b; Tilman, 1987). Before the experiment, the field was dominated by a diverse mixture of grasses and forbs, including *Schizachyrium scoparium*, *P. pratensis*, *Artemisia ludoviciana*, *Solidago nemoralis*, *Aster azureus*, *Ambrosia coronoptifolia*, *Sorghastrum nutans*, *Stipa spartea*, and *Carex* sp. (Tilman, 1987). The grid was thoroughly disked before establishment of 54 4 m × 4 m plots, with six replicates of nine treatments randomly assigned within the grid. Treatments consisted of no nutrient addition, addition of non-N nutrients (P, K, Ca, Mg, S, and trace metals), and addition of non-N nutrients plus one of the seven levels of N addition (10, 20, 34, 54, 95, 170, and 270 kg N $ha^{-1}\,yr^{-1}$). The highest N level (270 kg N $ha^{-1}\,yr^{-1}$) was not examined in the current study and is not discussed further. Non-N nutrients were added to ensure primary limitation by N as P_2O_5 (20 g $m^{-2}\,yr^{-1}$), K_2O_2 (20 g $m^{-2}\,yr^{-1}$), $CaCO_3$ (40 g $m^{-2}\,yr^{-1}$), $MgSO_4$ (30 g $m^{-2}\,yr^{-1}$), and trace metals (Cu, Zn, Co, Mn, and Mo) according to Tilman (1987). N was added as pelletized NH_4NO_3 (34-0-0) twice each year, with half added in mid-May, and half in late June. Background wet deposition for this site has been relatively constant over the period of available data (1997–2005, MN01, http://nadp.sws.uiuc.edu/), averaging 6 kg $ha^{-1}\,yr^{-1}$ (58% NH_4, 42% NO_3). The grid was enclosed by a fence to exclude large mammalian herbivores, primarily white-tailed deer (*Odocoileus virginianus*; Ritchie et al., 1998). By 1991, the grid had converged in plant species number and composition with an adjacent experiment that had received identical fertilizer treatment but was not initially disked (Inouye & Tilman, 1995). In 1992, nutrient addition was ceased in a randomly selected half of the replicates of each treatment in the previously disked grid to observe the dynamics of recovery.

In the summer of 2003, we constructed a detailed N budget on the grid that stopped receiving fertilizer in 1992. In short, we measured peak season total C and N pools in the soil, plant, and microbial compartments, monthly *in situ* net N mineralization rates, and soil-extractable N pools. Over the period from 1982 to 2003, mean annual precipitation (± 1 SD), growing season (April–September) mean precipitation, and growing season mean monthly maximum temperature were 817 ± 168 mm, 596 ± 141 mm, and 22.8 ± 1.2 °C, and in 2003 were 608 mm, 507 mm, and 22.7 °C, respectively.

Biomass harvests and total C and N pools

We harvested plant biomass using methods similar to Tilman (1987). For aboveground biomass, strips (0.1 m × 3 m) of clipped vegetation were collected from each plot near peak standing biomass (mid-July) and brought into the laboratory for sorting. Aboveground biomass was sorted to litter vs. living plant tissue, then plant tissue was sorted by species. For belowground biomass, four root cores (5 cm inner diameter, 30 cm deep) were taken per plot along the same strip that was clipped for aboveground biomass, composited, and washed over a 1 mm screen to separate roots from soil. Plant samples (roots, shoots, and litter) were dried at 40 °C for 48 h, and weighed. For soils, three cores (2 cm inner diameter, 30 cm deep) were taken per plot, sieved (2 mm), and composited, with a subsample taken to measure the water content (105 °C for 72 h). Soil, roots, shoots, and litter were then separately subsampled and ground before analysis of C and N via dry combustion using a Carlo Erba NA 1500 elemental analyzer (CE Elantech Incorporated, Lakewood, NJ, USA).

Net N mineralization and inorganic N pools

In 2003, we measured monthly *in situ* net N mineralization rates and extractable inorganic N over the growing season (late April to mid-September) following procedures similar to Wedin & Tilman (1996). PVC cores (5 cm inner diameter, 30 cm deep, one per plot per month) were driven vertically into the soil and loosely capped to allow aeration and prevent leaching loss.

Initial soil cores (2 cm inner diameter, 30 cm deep, three per plot per month) were collected, homogenized, and taken to the laboratory for subsampling and extraction. A root-free subsample (~ 20 g fresh soil) was weighed, mixed with 50 mL of 1 M KCl, agitated for 30 min, and allowed to settle overnight at 4 °C. The supernatant was collected and frozen until analysis of ammonium (NH_4^+) and total nitrite plus nitrate ($NO_2^- + NO_3^-$) using an automated flow-through analyzer (Alpkem, College City, TX, USA). Prior work at this site has compared samples processed with this methodology with immediately filtered samples and found no significant difference (D. Tilman, unpublished results). PVC cores were removed after approximately 4 weeks and processed similarly as above. This procedure was repeated for 5 months, with deployments on 29 April, 24 May, 21 June, 19 July, 21 August and collections on 26 May, 23 June, 21 July, 19 August, and 16 September.

Extractable soil inorganic N pools were measured using repeated shallow (0–30 cm) and deep (60–90 cm) soil cores every month during collections of initial N for net mineralization. Shallow cores were extracted with 1 M KCl using similar procedures as above to estimate total inorganic N in soil solution, and with deionized water to approximate N that is mobile following precipitation. Deep samples were extracted only with deionized water.

pH

Soil pH was measured each month on the supernatant from shallow 1 M KCl extracted soil samples using a pH meter (Orion 420A, Orion Research Inc., Beverly, MA, USA). Soil pH using 1 M KCl extractions is typically 1 unit lower than pH determined via water extraction (1 : 1, soil : solution) in the range of acidities examined in this study (Okusami *et al.*, 1987). However, direct comparison of our pH data with data from another study in the same field which used 1 : 1 soil : water slurries indicated that our pH values are lower by approximately 0.4 pH units (from Bradley *et al.*, 2006, data not shown).

Statistical analyses and calculations

For all analyses (JMP 5.1.1, SAS), we used ANCOVA models that included the rate of N addition (N_{add}), whether or not the plot ceased receiving treatment (N_{cess}), and their interaction ($N_{add} \times N_{cess}$) as main effects. Because of long-term exclusion of herbivores, the legume *Lathyrus venosus* had recently increased in abundance (Clark, 2007), likely impacting the N dynamics (Ritchie *et al.*, 1998). Thus, all analyses also included as a covariate the average abundance of *Lathyrus* over 2002–2003 in each

plot. Higher order interactions with *Lathyrus* were explored for all models and none were found. When a significant $N_{add} \times N_{cess}$ interaction was found (expected following recovery), additional analyses were run separately for plots that continue to receive N and those that do not. For extractable inorganic soil N pools (1 M KCl and water-extractable NO_3^- and NH_4^+ at depths of 0–30 cm and 60–90 cm), data were log-transformed to meet model assumptions and then averaged over the growing season to estimate mean inorganic soil N pools. All other analyses used untransformed data. We examined standard transformations for percentages (acrsine root, logit), but model assumptions did not appear violated and transformations did not improve the model fit or qualitatively change the results. Thus, we present analyses of untransformed percentages. For N mineralization, monthly values were summed to estimate growing season net N mineralization. Because of the relatively small sample size when averaged (or summed) across the growing season ($N = 48$) and low replication at each treatment rate ($n = 3$), detailed screening of influential data was performed. Plots that were highly influential (Cook's distance > 0.5), and/or outlying (studentized residual > 2.0) were screened for influence, and removed from the final analysis if they appeared to deviate from the general trends and qualitatively impacted the results (typically zero to two plots per analysis). Of the 29 ecosystem properties investigated in this paper, only one differed between plots receiving no nutrients and plots receiving all nutrients except N (pH, discussed below). Thus, these two treatments were combined for controls in all other analyses. Individual comparisons between treatments and controls use *post hoc* Dunnet contrasts. We calculated the percentage of added N that was retained (% retained) in individual pools of each plot (litter, root, shoot, and soil) as the difference in total N between each treated plot and the average of the controls, divided by the cumulative amount of N added to that plot, multiplied by 100. Throughout this paper, we refer to $+N$ and $-N$ plots as plots that continue to receive fertilizer treatment and those that do not, respectively.

Results

Biomass

No plant compartment examined (shoots, roots, litter) was elevated over controls in plots that ceased receiving N, although there were trends of increased shoot biomass with prior application rate. Shoot biomass increased with treatment for both $+N$ and $-N$ plots (Table 1); however, this increase was significantly weaker in $-N$ plots compared with $+N$ plots, and no $-N$

Table 1 Analytical results for key ecosystem compartments

Response	−N plots		+ N plots	
	N_{add}	*L. ven.*	N_{add}	*L. ven.*
Mass (g m^{-2})				
Shoots	* (+)	** (+)	*** (+)	ns
Litter	ns	* (+)	** (+)	ns
Roots	–	–	–	–
Total biomass	ns	ns	* (+)	ns
N concentration (%)				
Shoot %N	–	–	–	–
Litter %N	** (+)	*** (+)	*** (+)	*** (+)
Root %N	ns	*** (+)	*** (+)	*** (+)
Total biomass %N	ns	*** (+)	*** (+)	*** (+)
Soil %N	–	–	–	–
N content (g m^{-2})				
Shoot	ns	** (+)	** (+)	ns
Litter	ns	*** (+)	*** (+)	ns
Root	–	–	–	–
Total biomass	ns	*** (+)	*** (+)	*** (+)
Soil	–	–	–	–
Inorganic soil N (mg kg^{-1})				
Nitrate (0–30 cm)	** (+)	* (+)	*** (+)	* (+)
Nitrate (60–90 cm)	ns	*** (+)	*** (+)	ns
Ammonium (0–30 cm)	–	–	–	–
Ammonium (60–90 cm)	–	–	–	–

Results relate the rate of N addition (N_{add}) and the abundance of the legume *Lathyrus venosus* (*L. ven.*) separately for + N and −N plots for responses in which there was not a significant interaction between the rate of N addition, and whether or not treatment ceased (Fig. 1). Otherwise analyses not shown (–). Level of significance is indicated as ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; and direction of relationship is given in parentheses.

treatment rate was significantly elevated over controls (Fig. 1a). Increased abundance of the legume (*L. venosus*) in this and subsequent analyses was associated with increased biomass and N, a pattern that was especially evident at the 10 kg N ha^{-1} yr^{-1} treatment rate which had high legume abundances (Clark, 2007). Litter biomass increased with the rate of N addition only in + N plots and not in −N plots (Table 1, Fig. 1b). Root biomass showed no relationships with treatment (Fig. 1c). Total plant biomass (shoot + litter + root) increased with N addition primarily in + N plots and not in −N plots (Table 1, Fig. 2a).

N concentration

Although living plant compartments (roots and shoots) showed no association with treatment in −N plots, senesced litter and the soil indicated legacy effects from prior treatment. Shoot %N was an average of 0.4% higher in + N plots than in −N plots, independent of the treatment rate (Fig. 1d). Litter %N increased with N addition in both + N and −N plots, although significantly more so in + N plots (Table 1, Fig. 1e). Root %N was positively related to treatment only in plots that continued to receive treatment (Table 1, Fig. 1f). Across all treatments, total biomass %N was elevated only in + N plots and not in −N plots (Table 1, Fig. 2b). Although soil %N demonstrated no strong associations with treatments (no terms significant, analyses not shown), trends indicated that −N and + N plots were similar in soil %N, with both elevated over controls (Fig. 1j).

Total N pools

Inconsistent legacy effects on the biomass and N concentration of plant tissue in −N plots (e.g. trends of increased shoot biomass but not N content, opposite trends for litter) resulted in no sustained impact of the prior N treatment on plant N pools. Indeed, shoot total N increased across the N gradient in + N plots and not in −N plots (Table 1, Fig. 1g). Aside from the conspicuous increase in total litter N at the 10 kg N ha^{-1} yr^{-1} level, total litter N increased only in + N plots (Table 1, Fig. 1h). Pools of root N demonstrated no strong relationships with treatment (Fig. 1i). Total biomass N (root + shoot + litter) increased across the N gradient only in plots that continued to receive N (Table 1, Fig. 2c). Similar to N concentration, although total soil N demonstrated no significant relationships with treatment (analyses not shown, Fig. 1k), total soil N tended to be higher than controls in both −N and + N plots.

N retention

No plant compartments (litter, root, shoot, or plant) retained a significant amount of N in −N plots (Fig. 3). In addition, although significantly more N was retained in the litter, shoot, and plant compartments in + N plots than in −N plots, independent of the treatment rate, these retention percentages were all low (<20%). Conversely, there were trends of high N retention in the soil of −N plots and aggregated across compartments, contrasting very little retention in + N plots in any compartment, although these trends were not conventionally significant (Fig. 3).

Soil nitrate and ammonium pools

Water-extractable soil nitrate at 0–30 cm of depth increased significantly with N addition, up to 2.6-fold in
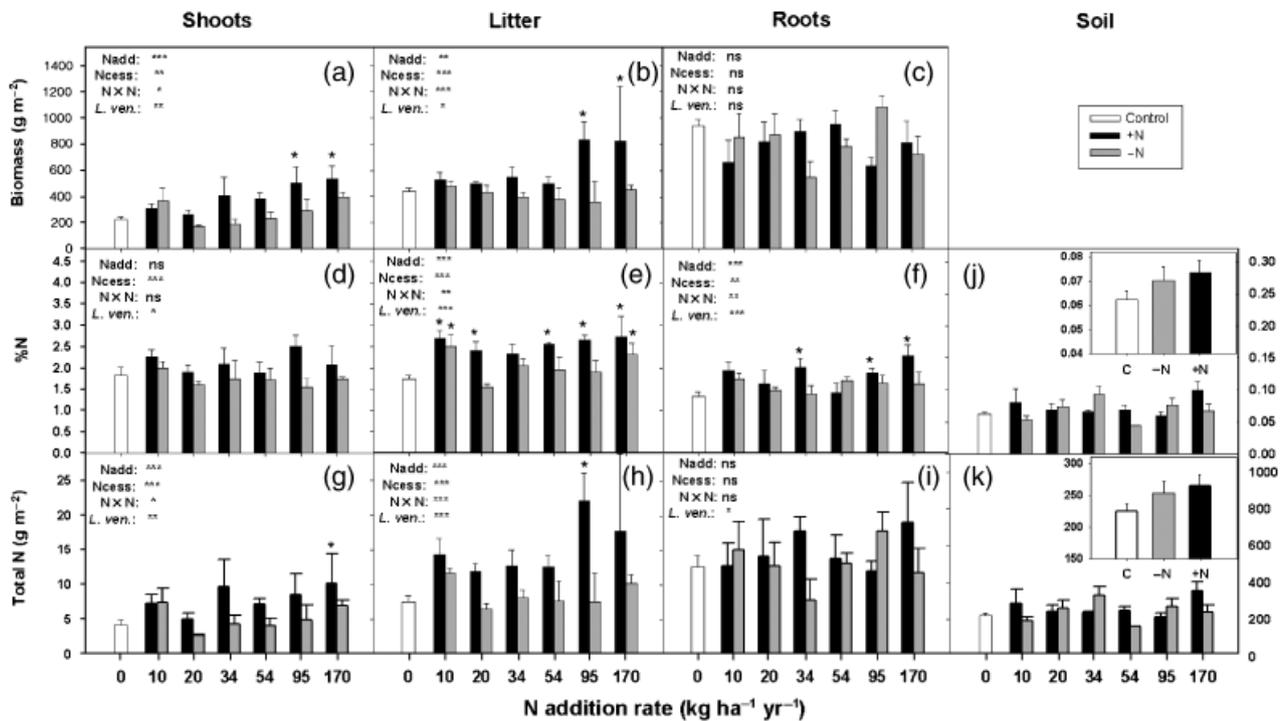
Fig. 1 Biomass, %N, and total N content by compartment. Shown are treatment averages (+1 SEM) in 2003 for shoot, litter, root, and soil compartments. Note changes in scale for soil properties. Analytical results are presented for the association between each particular response regressed on rate of N addition (N_{add}), whether or not treatment ceased (N_{cess}), their interaction (N × N), and the abundance of the legume *Lathyrus venosus* (*L. ven.*) included as a covariate. Level of significance is indicated as ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Significance of individual treatments relative to controls was also tested using Duncan's contrasts, with significance indicated above individual bars as *$P < 0.05$. Insert graphs for soil responses show mean values for control, −N, and +N plots.

−N plots and up to 29-fold in +N plots across the treatment gradient (Table 1, Fig. 4). At 60–90 cm of depth, nitrate increased with treatment only in +N plots (Table 1, Fig. 4). For ammonium, water-extractable pools were not associated with the rate of N addition, but were slightly higher in +N plots compared with −N plots at either depth (Fig. 4).

Patterns among treatments were qualitatively similar between 1 M KCl and water-extractable nitrate and ammonium (Fig. 4), even though mean ammonium concentrations were nearly five times higher in KCl-extracted soil samples. Thus, although the quantitative concentrations for ammonium were affected by extraction method, relationships with the variables of interest were not qualitatively affected for either N compound.

Net N mineralization

Annual net N mineralization rates showed sustained effects of prior N addition. Net N mineralization was elevated with N addition, unaffected by N cessation, and higher with higher abundances of the legume (Fig. 5). Results did not differ qualitatively when analyzing total N mineralized over the growing season

or monthly N mineralization (analyses not shown). Average nitrification : mineralization ratios were high (mean = 0.89), and not affected by treatment (model $F_{4,41} = 0.413$, $P = 0.798$; N_{add}, $F_{1,41} = 0.176$, $P = 0.677$; N_{cess}, $F_{1,41} = 0.032$, $P = 0.860$; $N_{add} \times N_{cess}$, $F_{1,41} = 0.144$, $P = 0.707$; *Lathyrus*, $F_{1,41} = 1.545$, $P = 0.221$).

Soil pH

Soil pH ranged very little across the field, reaching its lowest average value in plots not receiving any nutrients at all (5.1) and its highest average value in plots that continued receiving all non-N nutrients (6.1). At low treatment rates, the effect of additional cations on raising pH slightly outweighed the acidifying effect of added N, as +N plots had a higher pH than controls and than −N plots at the same treatment rate (by less than one pH unit). These trends were reversed at higher N addition rates, leading to a significant interaction term (model $F_{4,43} = 4.981$, $P = 0.002$; N_{add}, $F_{1,43} = 0.605$, $P = 0.441$; N_{cess}, $F_{1,43} = 6.602$; $N_{add} \times N_{cess}$, $F_{1,43} = 5.803$, $P = 0.020$; *Lathyrus*, $F_{1,43} = 3.921$, $P = 0.054$). Soil pH was the only variable examined that differed significantly between plots that received all nutrients except N and
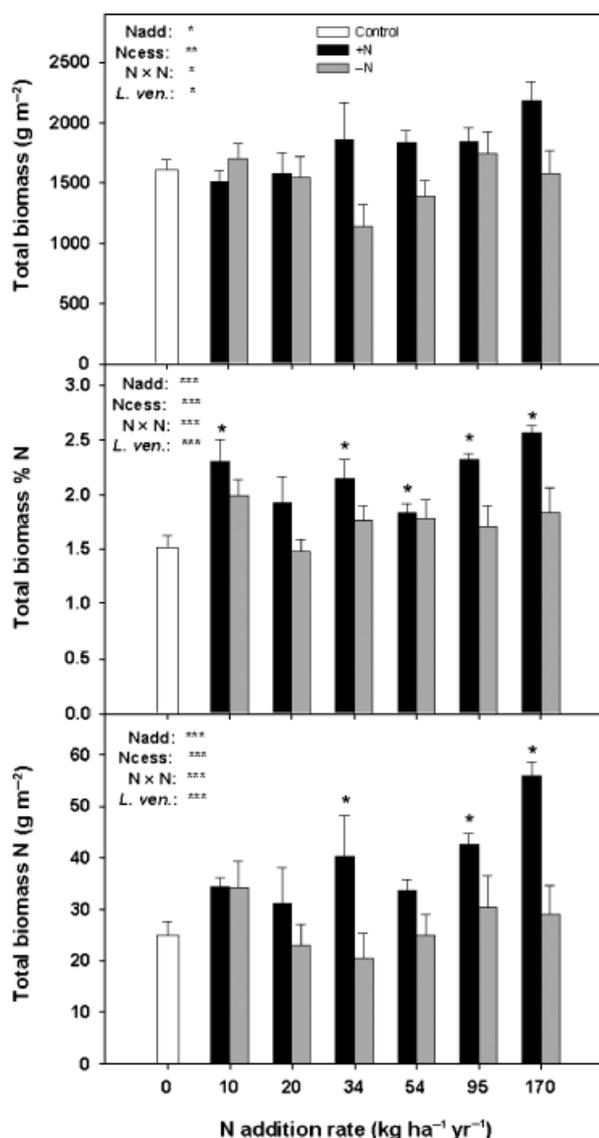
Fig. 3 Percentage of added N retained by 2003 in key ecosystem compartments. Shown is the average (+ 1 SEM) percentage N retained in + N plots and −N plots in the litter, root, shoot, plant (litter + root + shoot), and soil compartments, and aggregated (Total) on an area basis across compartments. Except where *P*-value is explicitly presented, analytical results and significance tests for each compartment are as in Fig. 1.

Fig. 2 Total plant biomass, %N, and N content. Shown below are treatment averages in 2003 (+ 1 SEM), where total represents the combined contribution of shoot, root, and litter compartments. Analytical results and significance tests are as in Fig. 1.

plots receiving no nutrients at all (one-way ANOVA, $P = 0.009$).

Discussion

N retention: patterns among −N and + N plots

We found no evidence of long-term retention of N in plant compartments for plots that ceased receiving treatment, and weak evidence for long-term retention in the soil. In plots that continued to receive N, soil retention that was high after 12 years (50–100% of
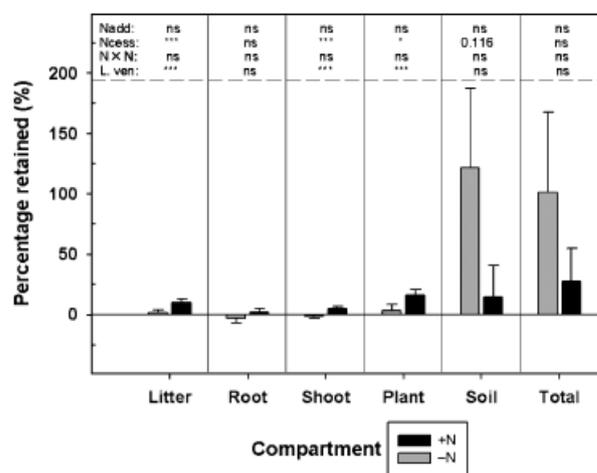
added N; Wedin & Tilman, 1996) declined greatly after 22 years (to 15%, this study). In addition, although several plant compartments retained a significant fraction of added N, these fractions were all low (all <17% of added N), and not related to the rate of N addition. These results highlight the critical importance of long-term experiments in evaluating the impacts of chronic N enrichment, and suggest that (1) retention efficiencies in various ecosystems may decline over time with continued N inputs as various sinks become saturated, and (2) the reduction in N pools following cessation of inputs may occur even though cycling rates remain elevated, suggesting delayed ecosystem recovery once inputs are reduced.

−N plots

The decline in plant N pools following cessation of N inputs resulted primarily from declines in aboveground biomass, aboveground N concentrations, and the mass of decomposing litter. During the period of N addition, all three ecosystem properties were elevated by two distinct mechanisms: (1) N addition increases all three properties for both native and exotic species at this site (Tilman & Wedin, 1991; Reich *et al.*, 2003), and (2) N addition shifts plant community composition toward species that produce higher amounts of more N-rich aboveground tissue (Tilman, 1987; Wedin & Tilman, 1996). The relatively modest changes in community composition following cessation (Clark & Tilman,
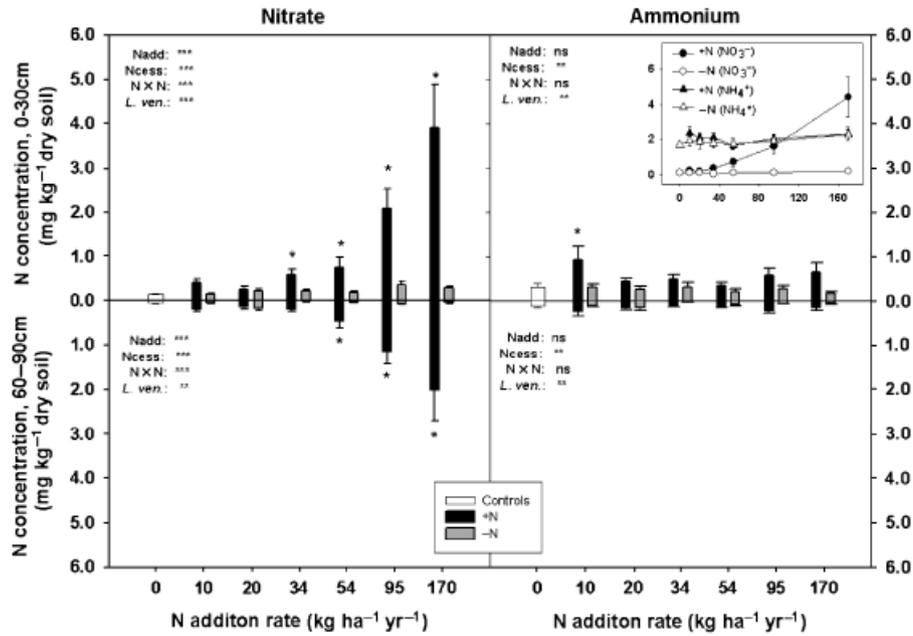
Fig. 4 Treatment and depth patterns of soil inorganic N. Treatment averages across the growing season are shown for water-extractable soil nitrate-N (left column) and ammonium-N (right column) at 0–30 cm (top row) and 60–90 cm (bottom row). Analytical results and significance tests are as in Fig. 1. For comparing extraction solution, the inset graph shows similar averages for 1 M KCl extractable nitrate (circles) and ammonium (triangles) at 0–30 cm depth for plots that continue to receive N (+ N, filled) and those that do not (−N, open).
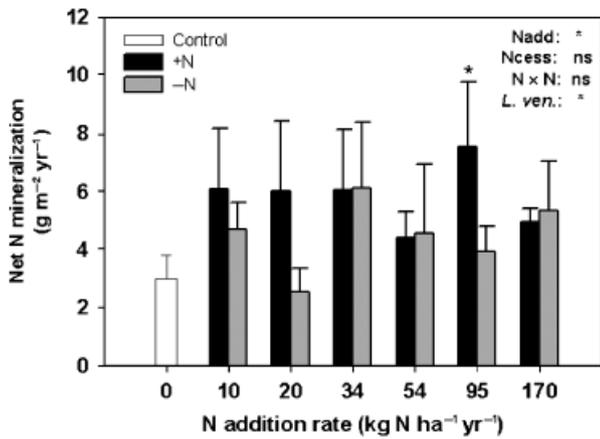

Fig. 5 Total annual *in situ* net N mineralization rates over April–September averaged by treatment (+ 1 SEM). Analytical results and significance tests are as in Fig. 1.

2008) suggest that reductions in biomass and foliar N concentration of the extant community, rather than a reversal of the compositional shifts previously observed, were likely driving the convergence in N pools between controls and −N plots. Our findings agree with several other studies, where both root and shoot C : N in a short grass steppe community were not different from controls in plots that had been previously fertilized; and, within 3 years after treatments ceased, foliar tissue

%N in a lowland heath (Power *et al.*, 2006) and a pine stand (Boxman *et al.*, 1998) had declined to control levels. These findings support the generalization that actively cycling plant pools are not long-term sinks for added N (Nadelhoffer *et al.*, 1999, 2004; Pilkington *et al.*, 2005b), as tissue contents from a variety of ecosystems often respond to both increases and decreases in N supply.

It is unclear what exact mechanism led to a reduction in plant N, as net N mineralization rates remained elevated in −N plots and indistinguishable from + N plots. However, it may be that much of the accumulated N was in excess of plant demand, stored as highly nitrogenous compounds (e.g. arginine), which were then rapidly metabolized to support continued growth once inputs ceased (Chapin *et al.*, 1986; Boxman *et al.*, 1998), and subsequently stabilized as recalcitrant soil N following tissue senescence.

The litter dynamics following N cessation suggest that although initial mass loss was likely due to increased decomposition of the N-rich substrates, maintenance of elevated litter N and lower litter mass was likely driven by microbial rather than plant processes. Litter mass loss at Cedar Creek (∼ 15% per year for grass litter; Hobbie, 2008) is stimulated by higher tissue N concentrations in decomposing substrates but shows relatively little response to inorganic N fertilization (Pastor *et al.*, 1987a; Hobbie, 2005, 2008). Tissue N

concentrations were elevated by shifts in composition as well as increases in tissue N in the extant community. Higher tissue N, along with reduced inputs of litter, likely drove an initial decline in litter mass. Sustained low litter mass 12 years later was likely driven by lower inputs and elevated litter N, even though new substrate inputs (i.e. recent shoots) were not elevated in N content. It is unclear what maintained elevated litter %N, but several processes likely contributed, including a possible lag between changes in litter %N (representing several years of growth) and shoot %N (representing 1 year of growth), more rapid mass loss of labile C in the litter of −N plots relative to controls due to plant compositional differences, or shifts in the microbial community. Regardless, our results clearly indicate that litter, although not a long-term sink in terms of total N, remains affected by treatment long after inputs cease with long-term impacts on N cycling.

There is weak evidence that soil was the location for much of the added N. The qualitative similarity in soil N content between −N and +N plots suggests that whatever sink strength was present in the soil may have been nearly saturated once N inputs ceased. Similar to our findings, research from a lowland heath (Power *et al.*, 2006) found that total soil N, although not statistically significant, was still 25% higher than controls 7 years after treatments stopped (13% higher in −N plots in our study). Research from shortgrass steppe found that soil N remained elevated 20 years after N and water treatments ceased, but not after N treatments ceased (Vinton & Burke, 1995). This mixed (or weak) evidence of retention following cessation apparently contradicts the consistently high N retentions found for this and other sites in studies over shorter durations (Wedin & Tilman, 1996; Magill *et al.*, 2004; Nadelhoffer *et al.*, 2004; Pilkington *et al.*, 2005b), but suggests that N considered retained after shorter term studies may be lost following N cessation and remineralization of previously retained N. Clearly, interpreting these and other retention efficiencies is problematic, because calculations by bulk analysis include the large and spatially heterogeneous mineral and organic soil layers (Wedin & Tilman, 1996; Magill *et al.*, 2004). Nonetheless, these mixed results highlight the critical need for more research on the dynamics of different ecosystem N pools following cessation of N treatment.

+ N plots

In light of prior research examining the impacts of N addition on nutrient stores and cycling, we will focus this discussion on the new insights gleaned from the long-term nature of this study.

Although N addition increased N stores in several plant tissue compartments, these increases were minor given the total N inputs over the experimental period. Low N retention aboveground (4.8%) was probably not only from a lack of perennial aboveground structures among species in the community, as similar low retentions have been reported in moorlands (4.3–22.6%; Pilkington *et al.*, 2005b) and forests (7–16%; Nadelhoffer *et al.*, 2004). A ^{15}N study in two temperate forests found that the moderate amount of N retained after 2 years in tree biomass (foliage, woody tissue, and fine roots) was substantially lower after 7 years (Nadelhoffer *et al.*, 2004), indicating that, as found here, plant biomass is a small and declining sink for chronic N inputs. The litter layer was also not found to be a large sink for added N (10.4%); and, although not often reported separately in other studies, similar low retentions were found in a moorland (Pilkington *et al.*, 2005b) and heath (Power *et al.*, 1998) site. Even so, the size of the plant N pools in our study was likely higher than might be expected following N enrichment to similar grasslands in this region as (1) fire which causes N losses from volatilization was suppressed, and (2) large herbivores which reduce aboveground N stores and belowground N cycling in this ecosystem (Ritchie *et al.*, 1998) were excluded. Thus, under typical burning and herbivory regimes for these grasslands, we might expect the plant N sink to be even lower than observed.

The most striking result from the +N plots is the large reduction in N retention in the soil after 22 years of continuous N addition compared with after 12 years. Three processes likely led to declining N retention (as a fraction of cumulative N inputs) in soils over time. First, long-term N addition reduced abundances of highly N-efficient C$_4$ species such as *Schizachyrium* [nitrogen use efficiency (NUE) = 203 g biomass g^{-1}N] and increased abundances of less efficient species such as *Agropyron* and *Poa* (NUE = 78 and 107, respectively; Wedin & Tilman, 1996), which were not completely offset by increased total production. Second, N addition may have saturated soil N sinks, leading to reductions in relative retention as N addition continued. The similarity between soil N pools in −N and +N plots suggests this may be occurring. Although speculative, we suspect that the sandy soils and low productivity at our site indicate a low potential for N retention via either biotic or abiotic mechanisms (Aber *et al.*, 1998; Kaye *et al.*, 2002; Davidson *et al.*, 2003). Third, methodological differences between this study and earlier work likely do not explain these differences. Previous N retentions were estimated on a portion of the same field that was untilled, while those from this study were on a portion of the field that was initially tilled (Tilman, 1987). However, this one time tillage event likely

increased soil N availability, explaining the higher production in the first few years (Tilman, 1987), and subsequently placed a higher demand on externally supplied N in the tilled compared with the untilled grid once this internal flush passed. Hence, we might expect higher retention efficiencies in the tilled rather than the untilled field. Thus, it appears likely that continued shifts in plant community composition (especially at lower N addition rates) combined with saturation of whatever sinks were present were primarily responsible for reduced N retention over time.

N retention: mechanisms of loss

The leaching of nitrate and organic N was likely the primary mechanism by which retention efficiencies declined in $+N$ plots over time and by which previously retained N was lost in $-N$ plots following cessation. The large increases in soil nitrate at both depths in $+N$ plots in our study, combined with reports of increased inorganic leaching with N addition at this (Dijkstra *et al.*, 2007) and other research sites (Phoenix *et al.*, 2003; Magill *et al.*, 2004), suggest this loss pathway may have been substantial. Others have observed that high leaching rates observed during N enrichment decline quickly (within 3 years) following reduced N inputs in forested sites (Boxman *et al.*, 1995, 1998; Bredemeier *et al.*, 1998), suggesting that this flush of N may have been fairly rapid at our site. However, this remains speculative and will require testing in other herbaceous systems for which temporal dynamics can be monitored. We did not measure dissolved organic N (DON) losses in this study, although research at this site (Dijkstra *et al.*, 2007) and in other nonforest communities (Pilkington *et al.*, 2005a) indicates that DON losses probably also contributed to N reductions.

Other loss pathways are not likely playing a large role either in maintaining low N retentions in $+N$ plots, or in reducing N stores in $-N$ plots after treatments stopped. We found that gaseous flux rates of N_2O and NO were negligible, as expected in these well-aerated sandy soils, and not significantly different between $+N$ plots and $-N$ plots (Clark, 2007). Although we did not measure ammonia volatilization, which can be substantial in agricultural fields (Matson *et al.*, 1997), the low soil pH in this field and low volatilization rates relative to inputs found in other grasslands ($<6\%$; Phoenix *et al.*, 2003) suggest that this loss pathway may be relatively minor. Finally, the suppression of fire, the long-term presence of the fence preventing N removal by large herbivores, and the relatively minor effects of insect herbivores on N losses in this system (Burt-Smith *et al.*, 2003), support our assertion that the primary loss pathway for this grassland is from leaching of organic and inorganic N.

N cycling: legacy effects of added N

Even though total pools of plant N had declined to control levels in $-N$ plots, trends of elevated N concentrations in the soil and litter, and their effects on increasing gross N mineralization and decreasing immobilization (Booth *et al.*, 2005), were maintaining high net N mineralization rates even 12 years after treatments ceased. This legacy was detectable in the surface soil nitrate pool of $-N$ plots which, although low in comparison with that of $+N$ plots, increased positively with prior treatment. Higher rates of net mineralization combined with negligible amounts of dissolved inorganic N (DIN) at 60–90 cm of depth suggest that this N was efficiently cycling in $-N$ plots. C_4 grasses did not demonstrate a strong increase in abundance after N addition ceased (Clark & Tilman, 2008), contributing to the maintenance of high N mineralization rates in $-N$ plots. Legacy effects on the microbial community as a result of N addition (Johnson, 1993; Bradley *et al.*, 2006) may also have contributed to lasting changes in N cycling, but remain to be studied at this site. Indeed, microbial activity remained elevated 6 years after treatments ceased in a lowland heath although microbial biomass showed no sustained effect (Power *et al.*, 2006). Explaining why net N mineralization rates in $+N$ plots were not higher than in $-N$ plots will require additional examination of how N cessation alters gross N cycling in addition to microbial composition. Earlier results from our site found elevated N mineralization only at higher N addition rates ($>54 \, \text{kg N ha}^{-1} \, \text{yr}^{-1}$; Wedin & Tilman, 1996). Although these differences may be partly attributable to spatial or temporal differences between sampling points, continued decreases in C_4 grasses and increases in C_3 grasses and legumes as N addition continued likely contributed to increases in N mineralization especially at lower treatment levels. Long-term effects of N enrichment have been reported in other systems as well, with elevated net N mineralization nearly 20 years after N treatments ceased in a shortgrass steppe community (Vinton & Burke, 1995), and elevated gross N mineralization rates 14 years after treatments ceased to a pine forest (Chen & Hogberg, 2006). These findings suggest that across a range of ecosystems, N cycling may be impacted decades after inputs cease. However, mixed results have also been reported following N cessation, with reduced net mineralization via increased immobilization in a spruce (Corre & Lamersdorf, 2004) and pine forest, (Koopmans *et al.*, 1995; Chen & Hogberg, 2006), and no effect of reduced N inputs to a Douglas fir forest (Koopmans *et al.*, 1995).

Thus, although effects of N addition on total pool sizes may be relatively transient, the effects on pool stoichiometry and thereby on nutrient cycling may last long after treatments cease, with site-specific variation based on coupled responses of plant and microbial communities to N cessation.

Conclusions

Understanding the long-term retention and recycling consequences of added N is critically important for evaluating the magnitude and duration of impacts from N deposition. Retention time and recycling from soil pools likely will influence many factors of global significance, including the potential for ecosystems to increase C storage in a future elevated CO_2 environment, the long-term consequences of N deposition on terrestrial nutrient cycles, as well as the release of nutrients to downstream freshwater and marine environments. The combination of our results suggests three broad conclusions. First, the magnitude and longevity of inorganic N retention may be relatively slight in some terrestrial habitats. This means that downstream freshwater and marine environments will be maximally impacted during periods of N deposition to these habitats, as well as during periods of N loss if deposition is reduced. Second, the retention characteristics of a habitat likely change during the period of N input, as many biotic and abiotic sinks become saturated or altered. Thus, the high retention efficiencies reported for shorter term studies for many terrestrial systems are likely to decline as systems become more N saturated with continued N loading (Aber *et al.*, 1998). Finally, and perhaps seemingly paradoxically, even small amounts of N retention may influence internal cycling long after inputs cease, with unknown periods of time required for recovery.

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