Inhibition of Nitrification Alters Carbon Turnover in the Patagonian Steppe

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ABSTRACT

Human activities are altering biodiversity and the nitrogen (N) cycle, affecting terrestrial carbon (C) cycling globally. Only a few specialized bacteria carry out nitrification—the transformation of ammonium (NH_4^+) to nitrate (NO_3^-), in terrestrial ecosystems, which determines the form and mobility of inorganic N in soils. However, the control of nitrification on C cycling in natural ecosystems is poorly understood. In an ecosystem experiment in the Patagonian steppe, we inhibited autotrophic nitrification and measured its effects on C and N cycling. Decreased net nitrification increased total mineral N and NH_4^+ and reduced NO_3^- in the soil. Plant cover ($P < 0.05$) and decomposition ($P < 0.0001$) decreased with inhibition of nitrification, in spite of increases in NH_4^+ availability. There were significant changes in the natural abundance of $\delta^{15}N$ in the dominant vegetation when nitrification was inhibited suggesting

that a switch occurred in the form of N (from NO_3^- to NH_4^+) taken up by plants. Results from a controlled-condition experiment supported the field results by showing that the dominant plant species of the Patagonian steppe have a marked preference for nitrate. Our results indicate that nitrifying bacteria exert a major control on ecosystem functioning, and that the inhibition of nitrification results in significant alteration of the C cycle. The interactions between the C and N cycles suggest that rates of C cycling are affected not just by the amount of available N, but also by the relative availability for plant uptake of NH_4^+ and NO_3^-.

Key words: carbon cycling; nitrification inhibition; nitrogen mineralization; semi-arid ecosystem; ammonium; nitrate; ^{15}N stable isotope; Patagonian steppe; Argentina; nitrapyrin.

INTRODUCTION

Over the last several decades, human activity has drastically altered the major global biogeochemical cycles, as well as affecting the diversity of organisms on Earth (Vitousek and others 1997b; Chapin and others 2000). Human impact on the nitrogen (N) cycle is of a magnitude similar to that of the disruption of the carbon (C) cycle, such that

anthropogenic inputs of fixed N currently equal the rates of natural fixation (Vitousek and others 1997a; Galloway and Cowling 2002). Recent studies have demonstrated the importance of the disruption of the N cycle for the functioning of ecosystems (Aber and others 1993; Wedin and Tilman 1996; Gundersen and others 1998). However, the importance of the interactions of N cycling with other biogeochemical cycles and with changes in biodiversity are only beginning to be understood (Nadelhoffer and others 1999; Sala and others 2000; Reich and others 2001; Schlesinger

and Lichter 2001). In particular, the control that N availability exerts on C turnover and storage could be a key factor in determining C sequestration as atmospheric carbon dioxide concentrations continue to increase (Hu and others 2001; Schlesinger and Lichter 2001; Gill and others 2002).

Nitrification plays a central role in the N cycle in terrestrial ecosystems, and a small group of bacteria and archaea are responsible for the transformation of ammonium (NH_4^+) to nitrate (NO_3^-) (Paul and Clark 1996, Leininger and others 2006). Studies of gross nitrification in forests have shown that the production of nitrate is much larger than previously thought (Stark and Hart 1997); this suggests that the movement of nitrate between soil and plants, and the control on nitrate supply, may be critical for determining C–N interactions at the ecosystem scale (Hart and others 1994). Although most plants in agricultural studies show a preference for the assimilation of NO_3^- over NH_4^+ in spite of the increased energy needed for the reduction of nitrate (Haynes and Goh 1978; Schlesinger 1997), it is not clear whether these patterns of N uptake have consequences for C cycling in natural ecosystems. The connection between the relative availability of different N species and its consequences for C cycling is not well understood and has only recently begun to be explored (Nordin and others 2001; McKane and others 2002; Falkengren-Grerup and others 2004).

The complexities of C–N interactions make it difficult to establish mechanistic connections, but one approach is through the direct manipulation of key processes to assess their impact on ecosystem functioning. To evaluate the significance of nitrification on C turnover, the ''removal'' of this functional group of nitrifying bacteria via chemical inhibition should highlight its importance for C cycling. We hypothesized that disruption of the N cycle, via the inhibition of autotrophic nitrification, would have direct consequences for all aspects of C cycling. Specifically, we suggested that a reduction in the availability of NO_3^- and changes in $NH_4:NO_3$ ratios would alter C fixation by vegetation and ultimately affect primary production, while increased NH_4^+ in the soil would increase the turnover and decomposition of organic matter.

We conducted a manipulative N-cycle experiment in the Patagonian steppe to test this hypothesis with respect to the effects of the inhibition of nitrification, and specifically the change in the available form of mineral N, on ecosystem processes. We applied a chemical inhibitor of autotrophic nitrification in undisturbed shrub–grass steppe and evaluated the effects of reduced nitrifi-

cation on N and C cycling. Coupled with the field data, we examined the consequences of changes in N availability on the uptake of N species of the major functional groups under controlled conditions. We evaluated changes in soil inorganic N, N transformations, stable N isotopes, plant preference for inorganic N species, and their effects on C cycling, including primary production and decomposition.

METHODS

Study Area

The Río Mayo experimental station is located in the Argentinean province of Chubut (45°41'S and 70°16'W) at 500-m elevation. Long-term mean annual precipitation of the site is near 155 mm and is strongly seasonal, with more than 70% of the precipitation falling in winter (Jobbágy and Sala 2000). Monthly mean temperature ranges from 15°C in January to 1°C in July. Soils are coarsely textured Aridisols, with high gravel content and low soil-water–holding capacity (Valle 1998). The vegetation is classified as semi-arid steppe, with near equal aboveground biomass of perennial bunch grasses and shrubs (Soriano and Sala 1983; Soriano and others 1994); these two functional groups account for more than 95% of the aboveground biomass of this community (Sala and others 1989; Golluscio and Sala 1993). Vegetative cover is discontinuous, with cover of 25% by grass species (dominant species, *Stipa speciosa*) and 12% by shrub species (dominant species, *Mulinum spinosum*) (Fernández and others 1991). Ten-year average aboveg net primary production of the site is 56 g m^{-2} y^{-1} (Jobbágy and Sala 2000).

Field and Laboratory Methods

Plots were chosen in a grazing exclosure that had been constructed in 1994, with a total of 16 circular plots measuring 25 m^2, each with a *Mulinum spinosum* shrub measuring approximately 1 m in diameter in the center. Plots were randomly assigned to control and inhibition treatments. We applied a chemical inhibitor of autotrophic nitrification, nitrapyrin (2-chloro-6-(trichloromethyl) pyridine) over a 2-year period from January 1998 to May 2000 at three time points during the year.

Nitrapyrin inhibits the metabolism of nitrifying bacteria, which are responsible for chemoautotrophic nitrification (Goring 1962; Kholdebarin and others 1998). Nitrapyrin has been shown to be an inhibitor of the ammonia monooxygenase (AMO) enzyme present in nitrifying bacteria by serving as

an alternative substrate (McCarty 1999). Laboratory and field experiments have shown the effect of nitrapyrin to be quite specific to nitrification inhibition; most other N transformations are relatively unaffected by the presence of the chemical, including net N mineralization and net ammonification (Sahrawat 1989; Crawford and Chalk 1992).

We applied 15 ml of emulsified nitrapyrin (N-serve 24E) dissolved in 1.5 L of water to the 25-m^2 area every 3–4 months during the 2-year period of study. Because nitrapyrin volatilizes on contact with air, we applied the water solution 3 cm below the soil surface, using a modified seeding machine. Additionally, to minimize disturbance effects on intact vegetation, we applied the solution under the shrub canopy and in the center of grass tussocks using a hollow metal stake fitted with plastic tubing to dispense the solution belowground. Control plots were manipulated in exactly the same way, except that water was added instead of the nitrapyrin solution. Nitrifying bacterial populations were notably reduced with additions of nitrapyrin solution, particularly in vegetated patches (López and others 2003).

Soil samples were taken from the 0–5-cm depth of the soil in three locations in each plot: bare soil, close to the grass tussocks, and below the central *Mulinum* shrub at each time point ($n = 48$)—in April (fall), October (spring), and December (summer) of both years of the study. At the December sampling date in the 1st year, we measured bulk density for each of the microsites, collecting soil in PVC tubes of 0–5 cm depth, returning the intact cores to the laboratory, and determining bulk density as the dry mass/volume of the tube. Soil nutrient concentrations were then converted to g/m^2 using bulk density measurements for each of the microsite locations. Additionally, mineralization tubes (PVC of 5-cm diameter and 7.5-cm depth) were placed in the same microsites ($n = 48$). Mineralization tubes were left in the field and sampled at the next time point (every 3–4 months); at the same time, we performed the inorganic N measurements described above. The difference between the initial N concentrations in the soil and those from soil inside the tube at the next sampling period was considered to represent net mineralization (change in NH_4-N and NO_3-N combined), net ammonification (change in NH_4-N), and net nitrification (change in NO_3-N). By calculating the sum of mineralization rates per day for each of the incubation periods, it was possible to calculate annual rates for each of the microsites.

For all soil samples, a subsample was sieved through a 2-mm screen to remove roots and large pebbles; soil was extracted for determination of inorganic N concentrations in the field plots. Soil extracts were transported to the laboratory, filtered using Whatman no. 42 filters, and analyzed for NO_3^- and NH_4^+ using an Alpkem Flow IV autoanalyzer (0.1 Corporation College Station, TX). In addition, extracts were analyzed for nitrite concentrations to assess the potential toxic effects of soil nitrite buildup. Nitrite concentrations in the soil were undetectable (data not shown).

We estimated plant primary production by changes in aerial plant cover at the peak of the growing season (January). A nondestructive estimate of primary production was needed because harvest methods would have created confounding effects on the ecosystem response variables (Greig-Smith 1983). We completed measurements of aerial cover prior to the beginning of the experiment by measuring transects with a line-intercept method. We marked two orthogonal diameters (approximately 12 m total distance in each plot) with steel rods for repeated measurements throughout the course of the experiment. Vegetative cover was identified at the species level, whereas standing dead cover was a single category. Measurements were taken at the peak of the growing season at the beginning of the experiments and for 2 years after the initial measurements. Changes in cover were evaluated using repeated-measures analysis of variance (ANOVA).

Decomposition was evaluated using litter from the dominant grass species, *Stipa speciosa*. We separated and used only the most recently senesced material (yellow), and 1 g of air-dried litter was placed in each of the fiberglass 2-mm mesh bags (10 × 10 cm). Subsamples of the original litter were dried in an oven at 70°C for determination of water content. We placed 12 litterbags in each of the 16 plots in bare soil, at a distance of at least 20 cm from any existing vegetation. These conditions were maintained throughout the experiment. At each time point, two litterbags were collected from each plot; all analysis was completed using the average of the two litterbags. We collected litterbags at six time points in September, January, and April of the 1st year, and September, January and May of the 2nd year. Ash-free dry mass was determined for all samples to correct for soil contamination from the field (Robertson and Paul 2000). Litter samples were analyzed using the acid detergent method for determination of fiber and lignin content (Van Soest 1963). Mass loss values at each date were arcsine-transformed before analysis of ANOVA (Sokal and Rohlf 1995). Dynamics of mass loss over time were determined by regressing the log of the proportion of

mass remaining over initial mass with time; slopes of these regressions (k constants) were compared for treatment effects using the Student t-test.

Foliar samples of the dominant grass (*Stipa speciosa*) and the dominant shrub (*Mulinum spinosum*) were taken from each of the plots at the peak of the growing season in the 2nd year (January) for analysis of natural abundance of the ratio of $^{15}N/^{14}N$. Samples were ground to a fine powder to pass a 40-μ mesh and sent for analysis to the University of Utah Stable Isotope Facility for Environmental Research in Salt Lake City. Values are expressed in parts per mil (‰), relative to the standard of atmospheric N, according to the following equation:

$$\delta^{15}N = [(R_{sample})/(R_{standard}) - 1] \times 1000 \quad (1)$$

where R = ratio of heavier to lighter isotope ($^{15}N/^{14}N$) for the sample and standard. Treatment effects were evaluated using a two-factor ANOVA, with post hoc comparisons completed using a Tukey post-hoc test.

We evaluated the potential preference of NO_3^- vs. NH_4^+ of the dominant shrub and grass species from the steppe site under controlled-climate conditions at the Faculty of Agronomy campus in Buenos Aires. *Stipa speciosa* plants and small individuals of *Mulinum spinosum* were collected from the field site in Patagonia in areas adjacent to the inhibition plots at the peak of the growing season (December) and placed in a greenhouse in Buenos Aires maintained at 18°C. After 2 weeks growing in their original soil substrate, *S. speciosa* culms and *M. spinosum* plants, with each individual having approximately 100 mg of green biomass, were removed from the soil. The roots were washed to remove any soil particles, and they were transferred to 30-ml glass vials covered with aluminum foil and filled with a nutrient solution without soil substrate. In addition, five blank vials were also added as procedural blanks. The nutrient solution was a standard 0.25 strength Johnson's solution, except that the N salts were altered to achieve a NO_3-N:NH_4-N ratio of 1:1 (Kronzucker and others 1999). The vials were filled to capacity, and the plants were carefully placed in the vials to avoid root damage. At this initial time point, 5 ml of solution was extracted from each vial to determine initial nutrient concentrations. At the end of the 5-day incubation period, we extracted 5 ml of solution again from each vial. The extracts were analyzed for NO_3^- and NH_4^+ using an Alpkem

Table 1. Mineral Soil Nitrogen (N) and NH_4:NO_3 Ratios for Sampling Dates during the 2-year Experimental Period

	NH_4-N (μg N/g dry soil)	NO_3-N (μg N/g dry soil)	NH_4/NO_3 Ratio
Year 1			
April			
Control	3.90 (1.09)	1.88 (0.55)	1.28 (0.61)
Inhibited	3.56 (0.33)	1.59 (0.78)	3.52 (1.50)[c]
October			
Control	2.82 (0.32)	0.99 (0.30)	2.11 (0.79)
Inhibited	3.23 (0.41)	0.57 (0.19)	5.63 (2.96)
December			
Control	2.78 (0.34)	1.18 (0.29)	1.41 (0.34)
Inhibited	3.58 (0.33)	0.41 (0.05)[a]	5.95 (2.96)
Year 2			
April			
Control	2.12 (0.16)	1.24 (0.29)	1.04 (0.27)
Inhibited	4.19 (0.38)[c]	0.67 (0.23)[a]	6.38 (2.76)[c]
October			
Control	4.66 (0.21)	0.60 (0.07)	4.28 (1.04)
Inhibited	6.58 (0.59)[b]	0.36 (0.06)[a]	12.07 (5.77)[c]
December			
Control	4.15 (0.40)	0.69 (0.05)	3.03 (0.58)
Inhibited	5.44 (0.59)	0.36 (0.10)[b]	12.90 (5.77)[b]

Parenthetical values represent 1 SE.
[a]*P < 0.05.*
[b]*P < 0.01.*
[c]*P < 0.001.*

Flow IV autoanalyzer. We calculated the difference between the initial and final NH_4^+ and NO_3^- concentrations, and the relative changes in concentration were tested using a two-factor ANOVA with species of inorganic N and plant species as factors. All statistical analyses were completed using Statistica® (Statsoft, Inc. Tulsa, software. A significance level of 5% was used in all cases.

RESULTS

Alterations of the N cycle were observed in both inorganic soil N concentrations and rates of N transformations. Inhibition of nitrification consistently resulted in higher soil NH_4^+ concentrations and lower soil NO_3^- concentrations and were significantly different in the 2nd year of the study ($P < 0.05$) (Table 1 and Figure 1). Net nitrification was significantly reduced in inhibited soils in the 2nd year ($P = 0.01$), whereas net mineralization was not affected over the course of the study ($P = 0.18$, data not shown). In the 1st year, there was a significant increase in net ammonification ($P < 0.05$), but this increase was not observed in
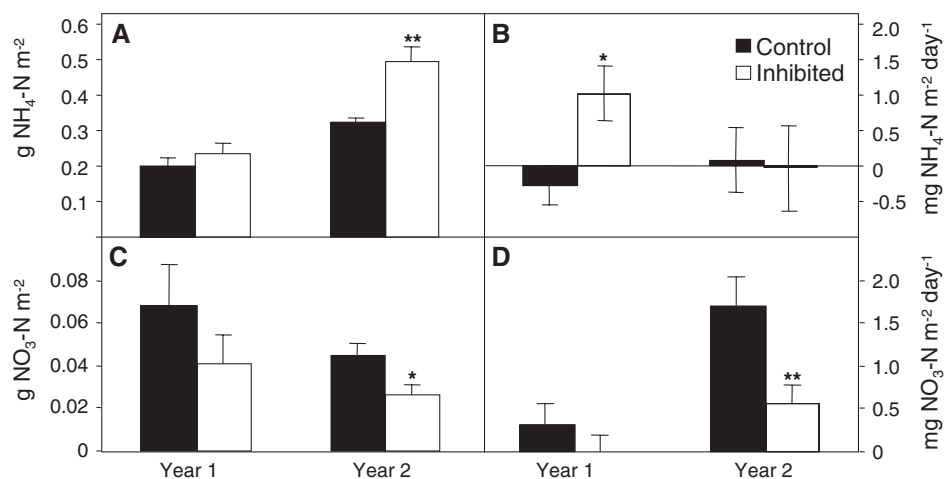
Figure 1. Effects of nitrification inhibition on soil nitrogen (N) dynamics over two growing seasons. **A** Ammonium (NH_4^+) concentrations in October. **B** Average annual net ammonification. **C** Nitrate (NO_3^-) concentrations in October. **D** Average annual net nitrification. Bars (±1 SE) represent means of all plots ($n = 8$ for each treatment, 0–5-cm depth), with each value representing the average of samples taken in three microsites in each plot (under shrub, next to grass tussock, and bare soil). Levels of significance are * $P < 0.05$ and ** $P < 0.01$ between control and inhibited plots.

the 2nd year. These results suggest that the action of the inhibitor was quite specific for nitrification; other soil N transformations did not appear to be inhibited. The contrasting effects of nitrification inhibition on soil NO_3^- and NH_4^+ resulted in significantly higher soil NH_4:NO_3 ratios and higher total soil mineral N at most dates, particularly in the 2nd year of the study (Table 1). In addition, the increase in NH_4^+ concentrations exceeded the decrease in NO_3^- concentrations, such that overall inorganic N availability increased (Figure 1).

The alteration of N pools and processes via nitrification inhibition translated to significant effects on the C cycle. Plant cover was significantly decreased in both years of the study in the nitrification-inhibited plots ($P < 0.05$) (Figure 2). As such, the reduction in aboveground net primary production was reduced by 4% in year 1 and 7% in year 2 of the experiment (Figure 2). Interestingly, when considered at a functional group level (grasses and shrubs), there was no significant effect on plant cover ($P > 0.05$), suggesting that both functional groups were affected similarly by alterations of the nitrogen cycle and that the cumulative effect on total cover was not due to differential effects on the different life forms. Finally, when considered at the species level, the effect of nitrification inhibition was not significant, although only marginally so ($P = 0.098$), suggesting again that a community-level response was observed due to changes in N cycling.

Significant differences were observed in the natural abundance of δ^{15}N in inhibited versus control
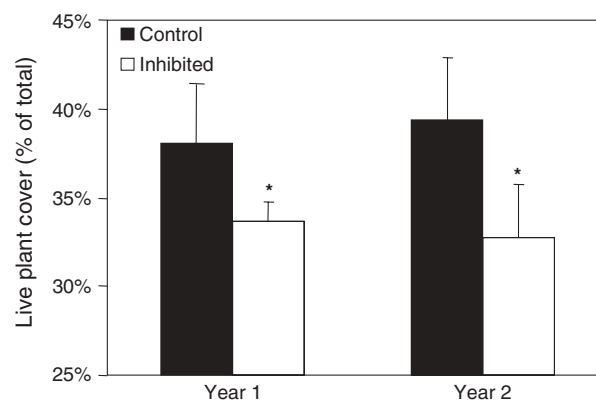

Figure 2. Live plant cover as a nondestructive estimate of primary production over two growing seasons, measured at the peak of the growing season (January). Asterisks indicate significant difference at $P < 0.05$.

plots, which could indicate a shift in the form of N used by the shrubs and grasses ($P < 0.05$) (Table 2). At the same time, foliar N concentrations were not significantly different between control and inhibited plots ($P = 0.54$), suggesting that N uptake per unit of biomass did not change. Both *Mulinum spinosum* and *Stipa speciosa* showed a preference for NO_3^- over NH_4^+ under controlled conditions. There was a significant decrease in the NO_3^- concentrations relative to NH_4^+ concentrations in the nutrient solution ($P < 0.001$ for both species) (Figure 3). Moreover, NH_4^+ concentrations actually increased due to higher water uptake over NH_4^+. There was no difference between plant species in degree of

Table 2. Foliar Percent Nitrogen (N) and Natural Abundance of δ^{15}N for Shrubs and Grasses in the Patagonian Steppe under Control and Nitrification-inhibited Conditions

	Grass	Shrub
Foliar N (%)		
Control	0.54 (0.03)^a	0.91 (0.09)^b
Inhibited	0.58 (0.09)^a	0.80 (0.04)^b
δ^{15}N (‰)		
Control	−1.83 (0.13)^a	−1.39 (0.14)^b
Inhibited	−1.24 (0.18)^{bc}	−1.00 (0.25)^c

Superscript letters indicate significant differences between means at P < 0.05 (1 SE in parentheses).
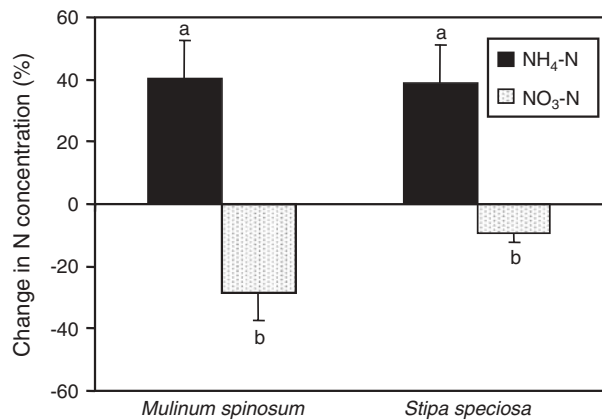
Figure 3. Preference for inorganic nitrogen (N) species in dominant plants of the Patagonian steppe. Changes in N concentration for nitrate (NO_3^-) and ammonium (NH_4^+) in hydroponic conditions for *Mulinum spinosum* and *Stipa speciosa*. Bars represent average (± 1 SE) for each plant species and form of inorganic N, $n = 10$ for each treatment. Different letters indicate significant differences at $P < 0.05$.

NO_3^- preference, although *M. spinosum* showed, on average, a greater reduction in NO_3:NH_4 ratios (43%) than *S. speciosa* (35%).

For decomposition, mass loss of litter at the soil surface was significantly reduced in litter from the nitrification-inhibition treatments at all dates except the 1.25 year pickup (Figure 4A). In addition, k constants for decomposition were significantly lower for the litter from the inhibited plots, with an overall reduction of 13% of litter decomposition with soil nitrification inhibition ($P < 0.0001$) (Figure 5). In contrast, there was no notable effect on patterns of N release from decomposing litter, with similar rates of N release in control and inhibited plots over the 2.5-year incubation period (Figure 4B). Significant changes occurred in the quality of the remaining litter, with
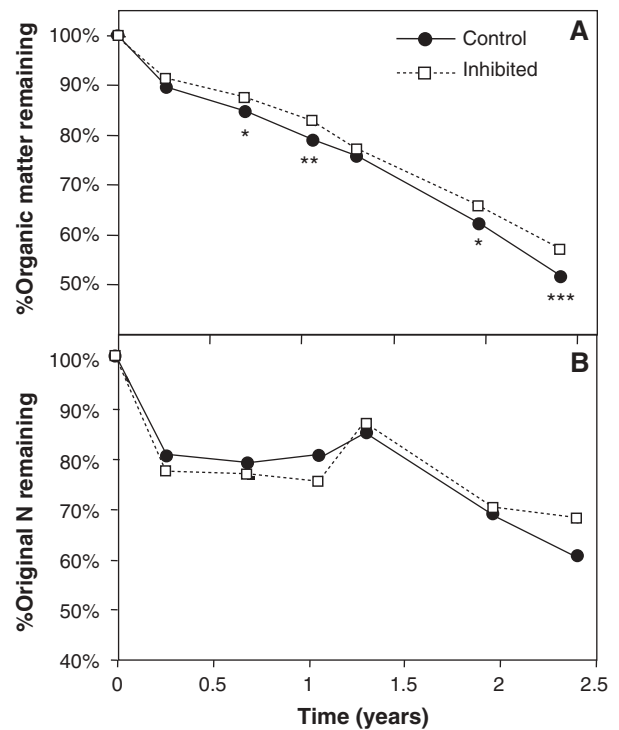

Figure 4. Mass loss and nitrogen (N) release of litter of *Stipa speciosa* in control and nitrification-inhibited plots ($n = 8$ for each treatment) after 2.5 years of field incubation. **A** Changes in organic matter over time, with percent remaining in litterbag. Asterisks indicate significant differences for each date (*$P = 0.05$, ** $P = 0.01$, *** $P = 0.003$). **B** Nitrogen release from decomposing litter over time in control and nitrification-inhibited plots. There were no significant differences at any of the sampling dates.
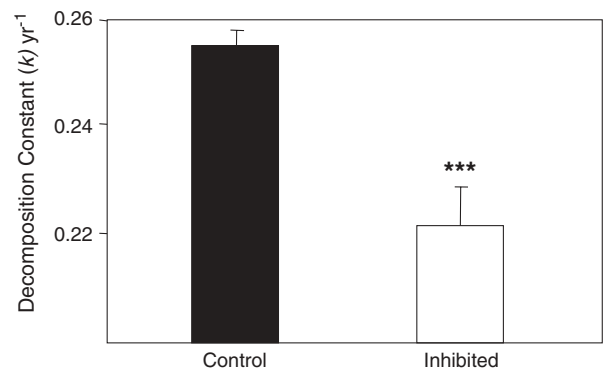

Figure 5. Aboveground response in decomposition to nitrification inhibition. Decomposition constants (k values) (± 1 SE) for entire incubation period (2.5 years) of *Stipa speciosa* litter. Asterisks indicate significant difference between treatments ($P < 0.001$).

a higher percentage of lignin in the remaining litter of the inhibited plots (4.68% versus 3.68% in uninhibited plots, $P = 0.02$).

DISCUSSION

The switch in the relative availability of NO_3^- versus NH_4^+ and the increase in NH_4^+ availability due to nitrification inhibition resulted in decreased aboveground plant cover and reduced litter decomposition, both of which represent direct effects on C cycling (Figures 2, 4, and 5). The observed reduction in plant cover, our proxy for aboveground net primary production, occurred despite an overall increase in N availability in the inhibited plots, and appears to be the result of changes in the soil $NH_4:NO_3$ ratio (Table 1). The lower mobility of NH_4^+ in most soils in comparison with NO_3^- (Paul and Clark 1996) and microbial competition for NH_4^+ (Jackson and others 1989) could effectively reduce the availability of N for plant uptake and enhance N limitation for C gain. In spite of the high energetic cost associated with the reduction of NO_3^- to synthesize organic compounds, dominant plants of the Patagonian steppe appear to prefer NO_3^- over NH_4^+ (Figure 3). Ammonium toxicity is usually invoked as the physiological basis for NO_3^- preference (Lambers and others 1998), and we hypothesize that in addition to decreased N mobility, it could be one of the mechanisms behind the observed reduction in plant cover in the treatments with high $NH_4:NO_3$ ratios. The net result of this shift in the form of available mineral N was a decrease in aboveground production by the vegetation. Soils of the Patagonian steppe, which are predominantly aerobic with a relatively high pH (López and others 2003), have approximately 25% of their mineral N in the form of NO_3^-; in contrast, less than 1% of the soil inorganic N is in NO_3^- form in forested sites at the same latitude (Austin and Sala 2002).

A switch in the N economy of the vegetation (from NO_3^- to NH_4^+) is further supported by the differences in $\delta^{15}N$ of both shrubs and grasses in the inhibited plots (Table 2). Although nitrification discriminates strongly for the lighter isotope of ^{14}N (Mariotti and others 1981), ammonification does not (Handley and Raven 1992; Handley and others 1999), such that NO_3^- values are generally more depleted in ^{15}N than NH_4^+ in the soil. Both grasses and shrubs had more enriched values for foliar $\delta^{15}N$ in inhibited plots than in control plots, suggesting that nitrification inhibition increased NH_4^+ uptake by plants. The costs associated with this switch may have increased the cost of N acquisition and assimilation, thereby resulting in reduced aboveground plant growth.

The decrease in aboveground litter decomposition is surprising, because the removal of nitrifying bacteria from the microbial community should not directly affect organic matter decomposition. This pattern could be explained by several mechanisms. Increased NH_4^+ and/or total inorganic N in the soil can inhibit the production of lignin- or cellulose-degrading enzymes and reduce litter decomposition (Magill and Aber 1998; Carreiro and others 2000). The reduction in lignin degradation and the significantly larger fraction of lignin remaining in the litter from the inhibited plots support this possibility for decreased decomposition. This reduction in lignolytic enzyme activity with increased mineral soil N was directly observed in a study of forest litter (Carreiro and others 2000); it has also been demonstrated indirectly in studies where high-lignin litters showed decreased decomposition after the addition of exogenous inorganic N (Magill and Aber 1998; Carreiro and others 2000; Hobbie 2000). These studies do not address the possibility of a differential response to NO_3 versus NH_4 concentrations in the soil, but they do suggest that an increase in total inorganic N, as observed in the inhibited plots in this study (Table 1), could contribute to a reduction in lignolytic enzyme activity and thereby a decrease in litter decomposition.

A second hypothesis is that of the microbial community may have been altered to effect a more "efficient" use of C substrate, the change being accomplished by a shift in the soil community from species with high nutrient-use efficiency (fungi) to those with low nutrient-use efficiency but lower energy requirements (bacteria) (Ågren and others 2001). Conceptually, this change would be similar to that observed in fertilization effects on aboveground biomass, with a shift in floristic composition to nitrophilious species that can take advantage of the increased N availability (for example, Aerts and Berendse 1988; Tilman 1993) and increase C gain per unit of energy.

By showing the relative importance of species of N (NH_4^+ versus NO_3^-) in controlling C turnover, our results indicate that the identity of the N species may be more important the quantity of N as a means of determining controls on ecosystem functioning and C cycling in the Patagonian Steppe. Nitrogen availability appears to be determined not only by pool size but by the relative proportions of NH_4^+ versus NO_3^- and by the timing of release and location of the available N in the soil profile, as shown recently in arctic ecosystems (McKane and others 2002). As N deposition due to human activities continues to increase, not only the amount but also the form of N will be an important factor in the disruption of N cycling. The important linkage with the C cycle highlights the

significant effects that may be exerted on C turn-over via subtle changes in the relative availability of forms of mineral N.

In addition, the inhibition of the activity of a soil functional group with low diversity exerted a major control on ecosystem functioning. This group, which contained just a few genera of bacteria and processed only a small fraction of the ecosystem energy flow, had a disproportionately large effect on the cycling of C. The substantive effects of nitrifying bacteria on ecosystem functioning occurred primarily through subtle changes in the species of soil inorganic N, whereas total inorganic pools were minimally affected or actually increased (Table 1). Our results highlight the inadequacy of assessing C–N interactions and the effects of biodiversity by examining only bulk C and N pools. In this case, the species of inorganic N affected C turnover in a way similar to that observed when increases in N availability affected the turnover of different soil organic matter fractions in a long-term fertilization experiment in Colorado (Neff and others 2002). It thus appears that interactions between C cycling and N cycling can occur at the level of the form of available N, or the specific fraction of soil organic matter. This process is illustrative of the variety of ways in which the interactions between C and N turnover can affect ecosystem functioning in terrestrial ecosystems.

ACKNOWLEDGEMENTS

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