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ECOSYSTEMS

Calcium Additions and Microbial Nitrogen Cycle Processes in a Northern Hardwood Forest

Peter M. Groffman,[1]* Melany C. Fisk,[2] Charles T. Driscoll,[3] Gene E. Likens,[1] Timothy J. Fahey,[4] Christopher Eagar,[5] and Linda H. Pardo[6]

[1]Institute of Ecosystem Studies, Box AB, Millbrook, New York 12545, USA; [2]Department of Biology, Appalachian State University, Boone, North Carolina 28608, USA; [3]Department of Civil and Environmental Engineering, Syracuse University, Syracuse, New York 13244, USA; [4]Department of Natural Resources, Cornell University, Ithaca, New York 14853, USA; [5]Northeastern Research Station, USDA Forest Service, PO Box 640, Durham, New Hampshire 03824, USA; [6]Northeastern Research Station, USDA Forest Service, PO Box 968, Burlington, Vermont 05402, USA

Abstract

Evaluating, and possibly ameliorating, the effects of base cation depletion in forest soils caused by acid deposition is an important topic in the northeastern United States. We added 850 kg Ca ha^{-1} as wollastonite ($CaSiO_3$) to an 11.8-ha watershed at the Hubbard Brook Experimental Forest (HBEF), a northern hardwood forest in New Hampshire, USA, in fall 1999 to replace calcium (Ca) leached from the ecosystem by acid deposition over the past 6 decades. Soil microbial biomass carbon (C) and nitrogen (N) concentrations, gross and potential net N mineralization and nitrification rates, soil solution and stream chemistry, soil:atmosphere trace gas (CO_2, N_2O, CH_4) fluxes, and foliar N concentrations have been monitored in the treated watershed and in reference areas at the HBEF before and since the Ca addition. We expected that rates of microbial C and N cycle processes would increase in response to the treatment. By 2000, soil pH was increased by a full unit in the Oie soil horizon, and by 2002 it was increased by nearly 0.5 units in the Oa soil horizon. However, there were declines in the N content of the microbial biomass, potential net and gross N mineralization rates, and soil inorganic N pools in the Oie horizon of the treated watershed. Stream, soil solution, and foliar concentrations of N showed no response to treatment. The lack of stimulation of N cycling by Ca addition suggests that microbes may not be stimulated by increased pH and Ca levels in the naturally acidic soils at the HBEF, or that other factors (for example, phosphorus, or Ca binding of labile organic matter) may constrain the capacity of microbes to respond to increased pH in the treated watershed. Possible fates for the approximately 10 kg N ha^{-1} decline in microbial and soil inorganic pools include components of the plant community that we did not measure (for example, seedlings, understory shrubs), increased fluxes of N_2 and/or N storage in soil organic matter. These results raise questions about the factors regulating microbial biomass and activity in northern hardwood forests that should be considered in the context of proposals to mitigate the depletion of nutrient cations in soil.

Key words: acid deposition; calcium; carbon; Hubbard Brook; microbial biomass; nitrification; nitrogen; phosphorus.

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*Corresponding author; e-mail: groffmanp@ecostudies.org

Introduction

Calcium (Ca) depletion has emerged as an issue in forest soil ecology over the last 10–20 years. Acid deposition has depleted labile pools of nutrient cations (Bailey and others 1996; Likens and others 1996, 1998; Fernandez and others 2003) and increased concentrations of aluminum (Al) in soil drainage waters (Cronan and Schofield 1979, 1990; Lawrence and others 1995; Tomlinson 2003). Loss of Ca has been associated with changes in the abundance or inorganic nutrition of biotic populations, especially sugar maple (*Acer saccharum*) (Long and others 1997; Driscoll and others 2001). Areas in the northeastern United States are of particular concern because they have shallow soils, dominated by minerals with low Ca content and slow weathering rates leading to small pools of biologically available Ca (Johnson and others 1981; April and Newton 1985; Landers and others 1988; Driscoll 1991; Eilers and Selle 1991; Kirchner 1992; Bailey and others 2005; Huntington 2005).

New concerns about soil Ca depletion have emerged as legislation to reduce acid deposition has been proposed (Driscoll and others 2001). Atmospheric deposition and streamwater concentrations of sulfate have declined in recent years, but the pH of forest streams in the northeastern United States has increased much less markedly, likely due to depletion of labile Ca pools from forest soils (Likens and others 1996, 1998, 2001; Chen and Driscoll 2005). It would therefore be helpful to increase our understanding of the factors regulating Ca dynamics in forest soils as decision makers consider amendments to the Clean Air Act and/or large-scale applications of Ca to forest soils to foster recovery from past acid deposition in the United States, as has been done in Europe (Zirlewagen and von Wilpert 2004).

The need to understand the role of acid deposition in the long-term depletion of Ca in soil and vegetation (Likens and others 1996, 1998; Gbondo-Tugbawa and Driscoll 2003) led to the initiation of a watershed-scale Ca addition experiment at the Hubbard Brook Experimental Forest (HBEF) in New Hampshire in 1999 (http://www.hubbardbrook.org). We treated an 11.8-ha watershed (Watershed 1, W1) with a Ca-silicate mineral (wollastonite) to restore the Ca that we estimate has been leached from the ecosystem by 60 years of acid deposition (Fiorentino and others 2003; Likens and others 2004; Peters and others 2004).

One of the main response variables of interest in the Ca addition study at the HBEF was soil microbial carbon (C) and nitrogen (N) cycle processes. Many previous studies (reviewed below) of responses to additions of base cations (mostly $CaCO_3$) and comparisons of naturally base-rich and base-poor sites have suggested that base saturation is a fundamental controller of microbial biomass and activity in forest soils. Any changes in C and N cycle processes induced by the Ca addition must be evaluated in terms of plant nutrition, C sequestration, and the water-quality and air-quality ecosystem services that are driven by these processes (Groffman and others 2004). There is particular concern that acceleration of N cycling by Ca additions could exacerbate N saturation problems caused by high rates of atmospheric N deposition in our region (Aber and others 1989; Driscoll and others 1996, 2001).

In this paper, we present 2 years of pretreatment and 4 years of posttreatment data on soil microbial biomass C and N concentrations, gross and potential net N mineralization and nitrification rates, soil solution and stream chemistry, foliar N concentrations, and soil:atmosphere trace gas (CO_2, N_2O, CH_4) fluxes from the Ca-treated watershed and from reference areas at the HBEF. Our objective was to look for evidence that C and N cycle processes are changing in response to the Ca addition in ways that would influence the services derived from northern hardwood forest ecosystems.

Methods

Site Description

The HBEF is located in the White Mountain National Forest in New Hampshire, USA (43°56′N, 71°45′W) (http://www.hubbardbrook.org). The northern hardwood forest vegetation is dominated by American beech (*Fagus grandiflora*), sugar maple (*Acer saccharum*), and yellow birch (*Betula alleghanieusis*). The forest was selectively cut in the 1880s and 1910s, and some of the older stands were damaged by a hurricane in 1938. Soils are shallow (75–100 cm), acidic (pH 3.9), Typic Haplorthods developed from unsorted basal tills.

The objective of the W1 manipulation at HBEF is to evaluate the role of Ca supply in regulating the structure and function of base-poor forest and aquatic ecosystems. The watershed has been continuously monitored for streamflow since 1956 and stream chemistry since 1963. Response variables for the experiment include stream chemistry, soil and soil water chemistry, forest floor mass and chemistry, composition and structure of the forest, phytosociology and nutrient status of the herbaceous layer, aquatic ecology, foliar chemistry, soil microbial activity, and tree growth and vigor.

In October 1999, wollastonite ($CaSiO_3$) was added to W1 at a rate of 850 kg Ca/ha in an attempt to increase the current base saturation of the soil from 10% to 19%. This latter value is thought to have been the base saturation of soil at the HBEF before the advent of acid deposition. To accomplish this manipulation, 56 metric tons of VANSIL-10, a commercial form of wollastonite, was crushed, pelletized with a lignin sulfonate binder (approximately 2% wet weight), and applied by helicopter. A total of 110 collectors were systematically positioned throughout W1 prior to the treatment to assess the spatial variability of the application, which was quite even (Peters and others 2004).

Soil Process Measurements

Sampling plots for soil measurements were established at four elevations in W1 and in the Bear Brook watershed, a reference area located just west of the biogeochemical reference watershed (W6) at HBEF as described by Fiorentino and others (2003). Both watersheds are located on south facing slopes (20%–30% slope) and have similar vegetation along an elevational gradient from 450 to 800 m above sea level. In both watersheds, five replicate treatment plots were established in four elevation zones corresponding to low hardwood (520–560 m), mid-hardwood (600–650 m), high hardwood (725–750 m), and spruce/fir/white birch (770–850 m) vegetation zones.

Two to eight soil samples were taken from each of the 40 sampling plots (4 elevations × 5 replicate plots × 2 watersheds) and composited by horizon (Oie, Oa, and the top 10 cm of the mineral soil) at each sample date. There were three sample dates (May, July, October) in 1998, 2000, and 2002 and one sample date (July) in 1999, 2001, and 2003, consistent with the long-term monitoring of soil microbial biomass and activity at the HBEF (Bohlen and others 2001).

Samples were stored at 4°C between sampling and analysis (less than 1 week). Soil samples were hand-sorted, mixed, and held at field moisture for all analyses. Soil moisture content was determined by drying at 60°C for 48 h. Soil pH was measured with a glass electrode in a 1:2 (Oa, mineral soil) or 1:4 (Oie) soil:solution (H_2O). Amounts of inorganic N (NO_3^- and NH_4^+) in soil were determined by extraction with 2 M KCl followed by colorimetric analysis with a Perstorp Flow Solutions 3000 flow injection analyzer.

Microbial biomass C and N content were measured using the chloroform fumigation-incubation method (Jenkinson and Powlson 1976). In this method, soils are fumigated to kill and lyse microbial cells in the sample. The fumigated samples are inoculated with fresh soil, and microorganisms from the fresh soil grow vigorously using the killed cells as substrate. The flushes of carbon dioxide (CO_2) and 2 M KCl extractable inorganic N (NH_4^+ and NO_3^-) released by the actively growing cells during a 10-day incubation at field moisture content are assumed to be directly proportional to the amount of C and N in the microbial biomass of the original sample. A proportionality constant (0.45) was used to calculate biomass C from the CO_2 flush. Carbon dioxide was measured by thermal conductivity gas chromatography. Inorganic N flush data were not corrected with a proportionality constant. Inorganic N as (NH_4^+ and NO_3^-) was measured colorimetrically as described above.

We also measured inorganic N and CO_2 production in unfumigated ''control'' samples. These incubations provided estimates of microbial respiration/respirable C and potential net N mineralization and nitrification. Microbial respiration was quantified from the amount of CO_2 evolved over the 10-day incubation. Potential net N mineralization and nitrification were quantified from the accumulation of NH_4^+ plus NO_3^-, and NO_3^- alone during the 10-day incubation.

Gross N transformations were measured using a ^{15}N pool dilution technique in short-term laboratory incubations of forest floor (Oe + Oa horizon) samples. Four replicate subsamples (20–25 g fresh weight) of each forest floor sample were pre-incubated in Mason jars for 12 h. Following pre-incubation, 99% enriched ^{15}N label was added to two replicates as $K^{15}NO_3$ (2 mg N kg^{-1} soil) and to two replicates as $^{15}NH_4Cl$ (2 mg N kg^{-1} soil). ^{15}N solution (approximately 1 ml, depending on the dry weight equivalent of soil) was added dropwise while soil was gently mixed by rotation of the jar. One replicate that received $^{15}NO^3$ and one replicate that received $^{15}NH^4$ were extracted in 2 M KCl at a 5:1 soil:solution mass ratio, 1 h after label addition. The second replicate subsamples were extracted in 2M KCl after 48 h incubation at 25–30°C in 1999 and at 16°C in 2000. The KCl extracts were analyzed for NH_4^+ and NO_2^- + NO_3^- with a continuous flow analyzer (Orion, Inc.). A diffusion procedure similar to that of Brooks and others (1989) was used to collect $^{15}NH_4^+$ and $^{15}NO_3^-$, and ^{15}N enrichment was determined at the Cornell Laboratory for Stable Isotope Analyses. Gross N mineralization, gross nitrification, and NH_4^+ and NO_3^- immobilization were calculated using the equations of Kirkham and Bartholomew (1955).

Soil:Atmosphere Trace Gas Fluxes

Trace gas fluxes were measured using an in situ chamber design identical to that used by Bowden and others (1990, 1991). Chambers (three per elevation per watershed) consisted of 287-mm diameter (ID) by 40-mm high polyvinyl chloride (PVC) cylinders, which were placed on permanently installed PVC base rings immediately prior to measurement. At sampling intervals of approximately 0, 10, 20, and 30 min after placement of the chamber on the base, 9-ml gas samples were collected from gas sampling ports in the center of the chamber top using fine-needle polypropylene syringes. Samples were transferred to evacuated glass vials, which were stored at room temperature prior to analysis by gas chromatography with electron capture (N_2O), thermal conductivity (CO_2), or flame ionization (CH_4) detection. Fluxes were calculated from the linear rate of change in gas concentration, the chamber internal volume, and soil surface area.

Soil Solution Chemistry

Zero-tension lysimeters (three replicates) of the design described by Johnson and others (2000) were installed at three depths (just below the Oa and Bh horizons and in the middle of the Bs horizon at approximately 5-, 10-, and 20-cm depth below the surface of the forest floor, respectively) in each of the four elevation zones of W1 in 1998. These lysimeters were closely paired with lysimeters installed in the reference watershed in 1983. Lysimeter samples were collected at monthly intervals. All soil water samples were stored at 4°C prior to analysis. Ammonium was analyzed with an autoanalyzer via phenate colorimetry (APHA 1981). Nitrate was analyzed by ion chromatography (Tabatabai and Dick 1983). Total nitrogen (TN) was analyzed by persulfate oxidation and analysis of NO_3^- on an autoanalyzer via hydrazine reduction (Ameel and others 1993). Dissolved organic N (DON) was calculated as the difference between TN and inorganic N ($NH_4^+ + NO_3^-$). Dissolved organic C (DOC) was measured by detection of CO_2 after persulfate digestion and ultraviolet-enhanced oxidation (McDowell and others 1987).

Foliar Chemistry

We collected leaf samples in the four elevation zones in both the treated and reference watersheds in mid-August each year. Leaves in the treated site were collected from the upper canopy of dominant and codominant trees using shotguns. Leaves in the reference watershed were collected from canopy trees by tree climbers. All leaf samples from an individual tree were pooled, oven-dried at 70°C, ground in a Wiley mill to pass a 2-mm screen, pulverized in a shatterbox, and stored in plastic bottles until analysis for total N. Foliar samples from the treated watershed were digested in a block digester in H_2SO_4, H_2O_2 and H_2SeO_3 (Isaac and Johnson 1976), and TN was determined on a TRAACS 800 autoanalyzer. Samples from the reference watershed were analyzed on a PDZ Europa ANCA-SL Elemental Analyzer at the Center for Stable Isotope Biogeochemistry at the University of California, Berkeley.

Stream Chemistry

Streamwater samples were collected at the base of the reference (W6) and treated (W1) watersheds in acid-washed, deionized water–rinsed polyethylene bottles; they were taken from a small waterfall above the stream gauging station to avoid contamination from ponded debris or weir construction materials (Buso and others 2000). The normal sampling interval was weekly, with more frequent samples taken at times of increased discharge. At low-flow summer (drought) conditions, samples were occasionally taken with a clean polyethylene syringe from undisturbed standing pools in the stream channel. Concentrations of NO_3^-, NH_4^+ and TN were analyzed as described above.

Statistical Analysis

Treated and reference watersheds and plots were compared using one-way analysis of variance, using the four elevation zones as replicates. Bonferroni (Dunn) *t*-tests were done to control for type I errors that arise when multiple one-way comparisons are made. All analyses were done with the Statistical Analysis System (SAS) software (SAS Institute, Cary, NC, USA).

As is common in watershed-scale studies, this is a pseudo-replicated design. A true evaluation of the response of the northern hardwood forests to Ca addition would require treatment (and comparison with a reference) of multiple watersheds in several locations. However, our analysis provides a very robust evaluation of the response to Ca addition at the HBEF in that significant differences only emerge when a response is consistent across all four elevation zones, which encompass a wide range of soil, vegetation, and microbial conditions (Johnson and others 2000; Bohlen and others 2001).

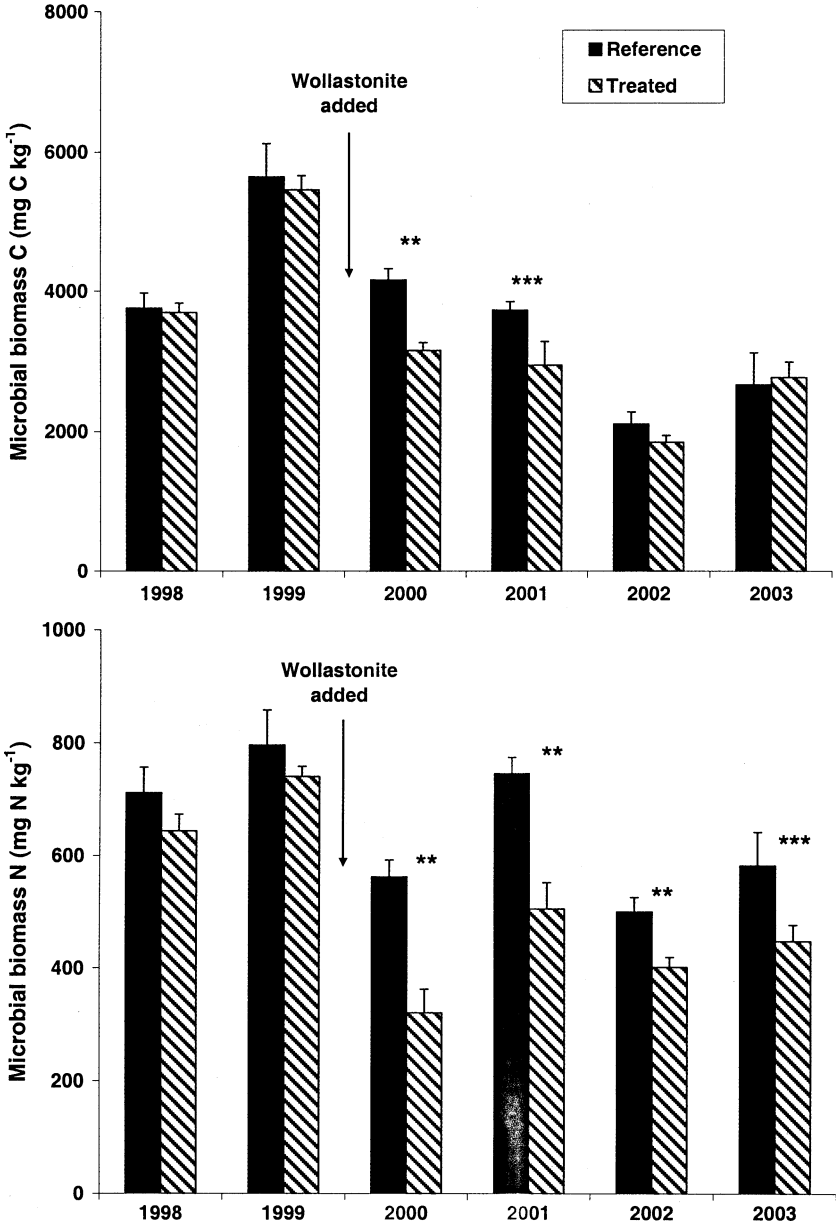

Figure 1. Microbial biomass carbon (C) (*top*) and nitrogen (N) (*bottom*) concentration in the Oie horizon of treated and reference watersheds before (1998, 1999) and after calcium (Ca) addition. Values for 1998, 2000, and 2002 are the mean of 20 samples taken in each watershed (five replicates at four elevations) at three sampling dates (May, July, October), $n = 60$. Values for 1999, 2001, and 2003 are the mean of samples taken only in July, $n = 20$. **, *** indicate statistically significant differences between watersheds at $P < 0.05$ and $P < 0.10$ respectively.

Results

The wollastonite addition caused significant increases in soil pH in the surface soil horizons. By 2000, the year after addition, pH in the Oie horizon was 5.45 in the treated watershed versus 4.29 in the reference watershed ($P < 0.001$). The pH differences were sustained into 2002 (4.98 versus 3.83, $P < 0.001$), 2003, and 2004 (4.75 versus 3.98, $P < 0.01$). The effect was less marked in the Oa horizon, but differences became significant over time (4.42 versus 4.06 in 2000, 4.22 versus 3.88 in 2002, $P < 0.10$; and 4.18 versus 3.82 in 2004, $P < 0.01$). In the mineral soil, there was no effect on pH by 2000 (4.33 in both watersheds) or 2002 (4.36 versus 4.26); but by 2004, differences were beginning to occur (4.25 versus 4.02, $P < 0.10$).

There was a transient decrease in soil microbial biomass C in the Oie horizon of the treated watershed in 2000 and 2001 (Figure 1 top) and a more persistent decrease in soil microbial biomass N, with significant decreases in biomass N in 2000, 2001, 2002, and 2003 (Figure 1 bottom). The recovery of biomass C in the treated watershed, coupled with the persistent decrease in biomass N, suggested that there has been a marked increase in the C:N ratio of the microbial biomass in the Oie horizon (6.2 in treated versus 4.6 in reference in 2003). There were no significant differences in microbial biomass C in the Oa or mineral soil

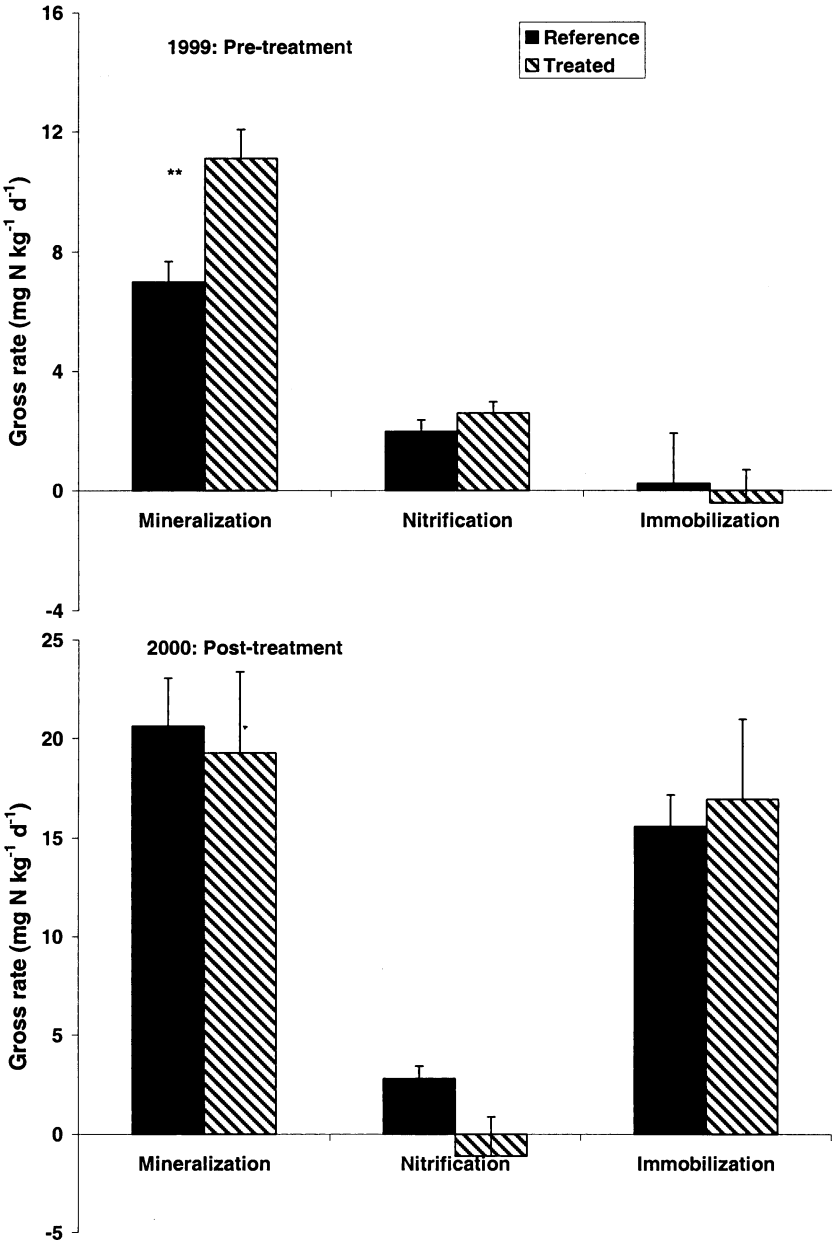

Figure 2. Gross rates of nitrogen (N) mineralization, nitrification, and immobilization in the Oie horizon of treated and reference watersheds before (1999) (*top*) and after (2000) (*bottom*) calcium (Ca) addition. Values are the mean of 16 samples taken in each watershed (four replicates at four elevations) in July. ** indicates a statistically significant difference between watersheds at $P < 0.05$.

horizons and only one significant difference in biomass N (a decrease in the treated watershed in 2000 in the Oa horizon). Concentrations of biomass C and N were markedly lower in these horizons than in the Oie horizon. Microbial biomass C ranged from 1200 to 3000 mg C kg^{-1} in the Oa horizon and from 400 to 950 mg C kg^{-1} in the mineral soil (data not shown). Microbial biomass N ranged from 130 to 400 mg N kg^{-1} in the Oa horizon and from 40 to 100 mg N kg^{-1} in the mineral soil (data not shown). Although concentrations of microbial biomass are relatively low in the Oa and mineral soil horizons, note that these horizons have greater mass than the Oie. Thus, approximately 50% of the mass of soil microbial biomass is in the mineral soil, with 25% in the Oie and 25% in the Oa at the HBEF (Bohlen and others 2001). The approximately 30% reduction in microbial biomass N that we observed in the treated watershed thus represents approximately 0.9 g N m^{-2}.

Consistent with an increase in microbial biomass C:N ratio (which suggests increased microbial demand for N), there was a decrease in gross (Figure 2) and potential net N mineralization rates in the Oie horizon of the treated watershed relative to the reference watershed (Figure 3 top). Gross mineralization was significantly higher ($P < 0.05$) in W1 than in the reference watershed before treatment,

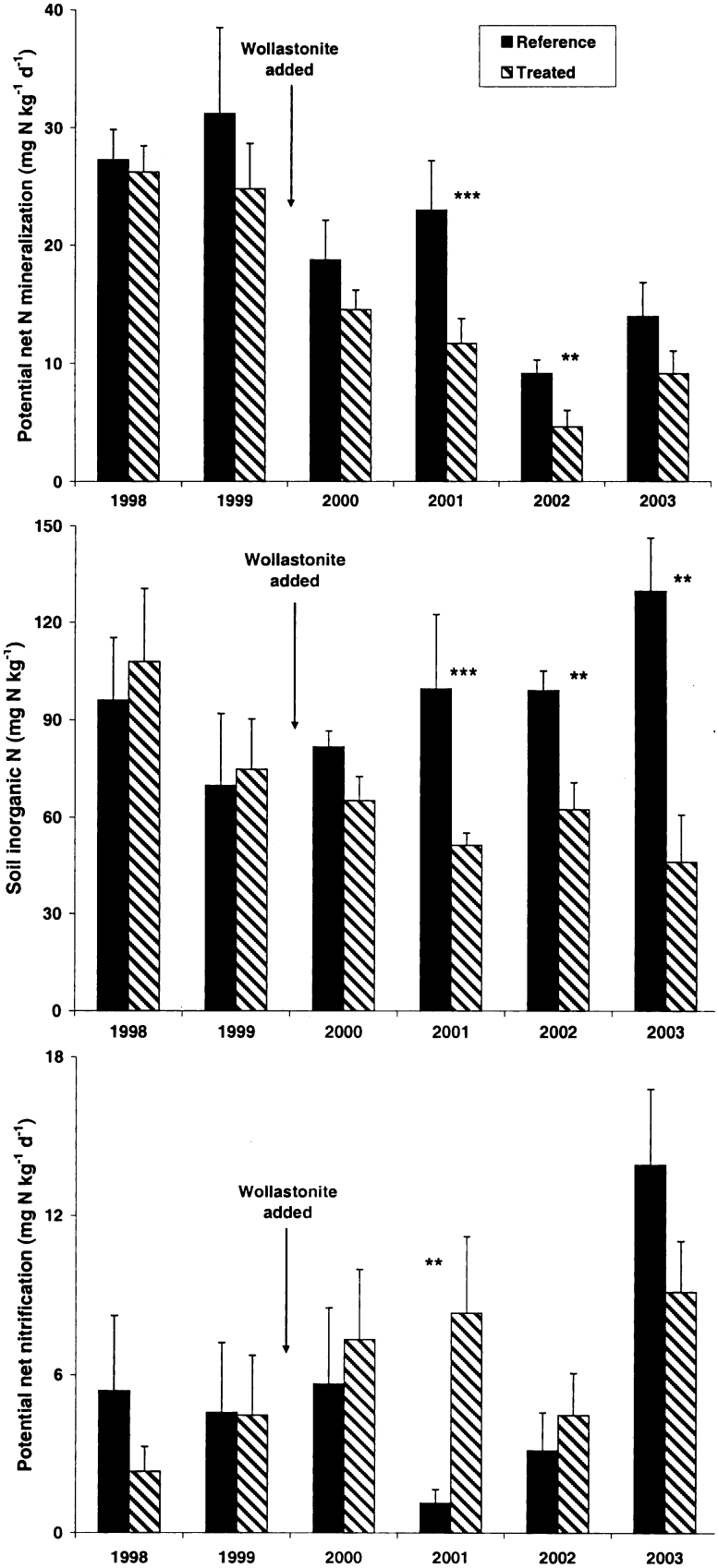

Figure 3. Potential net nitrogen (N) mineralization (*top*), soil inorganic N pools (*middle*), and potential net nitrification (*bottom*) concentration in the Oie horizon of treated and reference watersheds before (1998, 1999) and after calcium (Ca) addition. Values for 1998, 2000, and 2002 are the mean of 20 samples taken in each watershed (five replicates at four elevations) at three sampling dates (May, July, October), $n = 60$. Values for 1999, 2001, and 2003 are the mean of samples taken only in July, $n = 20$. ** indicates a statistically significant difference between watersheds at $P < 0.05$.

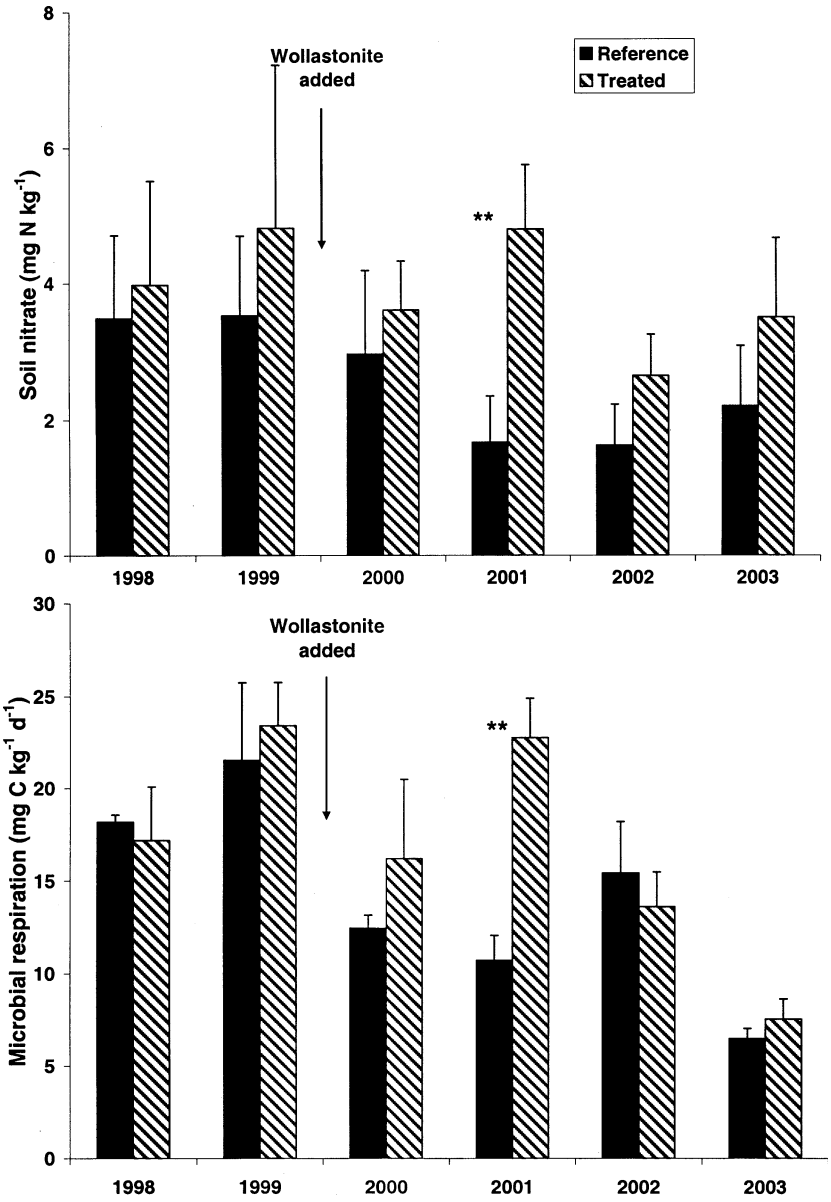

Figure 4. Soil nitrate pools (*top*) and microbial respiration (*bottom*) in the mineral soil of treated and reference watersheds before (1998, 1999) and after calcium (Ca) addition. Values for 1998, 2000, and 2002 are the mean of 20 samples taken in each watershed (five replicates at four elevations) at three sampling dates (May, July, October), $n = 60$. Values for 1999, 2001, and 2003 are the mean of samples taken only in July, $n = 20$. ** indicates a statistically significant difference between watersheds at $P < 0.05$.

and this difference disappeared after treatment. Net mineralization was significantly ($P < 0.05$) reduced in the Oie horizon in 2001 and 2002, but there was no effect in 2003 or in the Oa or mineral soil horizons. However, there were marked (and significant in 2001, 2002, and 2003) reductions in soil inorganic N levels in the Oie horizon (Figure 3 middle) in the treated watershed. Potential net nitrification showed an opposite pattern to mineralization, with a significant ($P < 0.05$) but transient increase in nitrification in the Oie horizon of the treated watershed in 2001 (Figure 3 bottom).

The transient increase in nitrification was consistent with higher concentrations of nitrate observed in the mineral soil horizon of the treated watershed in 2001 (Figure 4 top); that is, nitrate produced in the Oie may have leached into the mineral soil. However, the changes in nitrification and soil nitrate disappeared by 2002 and were not observed in 2003. Similar to nitrification and soil nitrate, there was a transient increase in microbial respiration in the mineral soil in 2001 (Figure 4 bottom) that may have been driven by leaching of respirable C produced as a by-product of the transient decline in microbial biomass C in the Oie in that year.

There was no effect of the treatment on soil:atmosphere fluxes of CO_2, N_2O, and CH_4 (Figure 5). There were patterns for increases in N_2O production and decreases in CH_4 uptake in the treated watershed, but these differences were not significant.

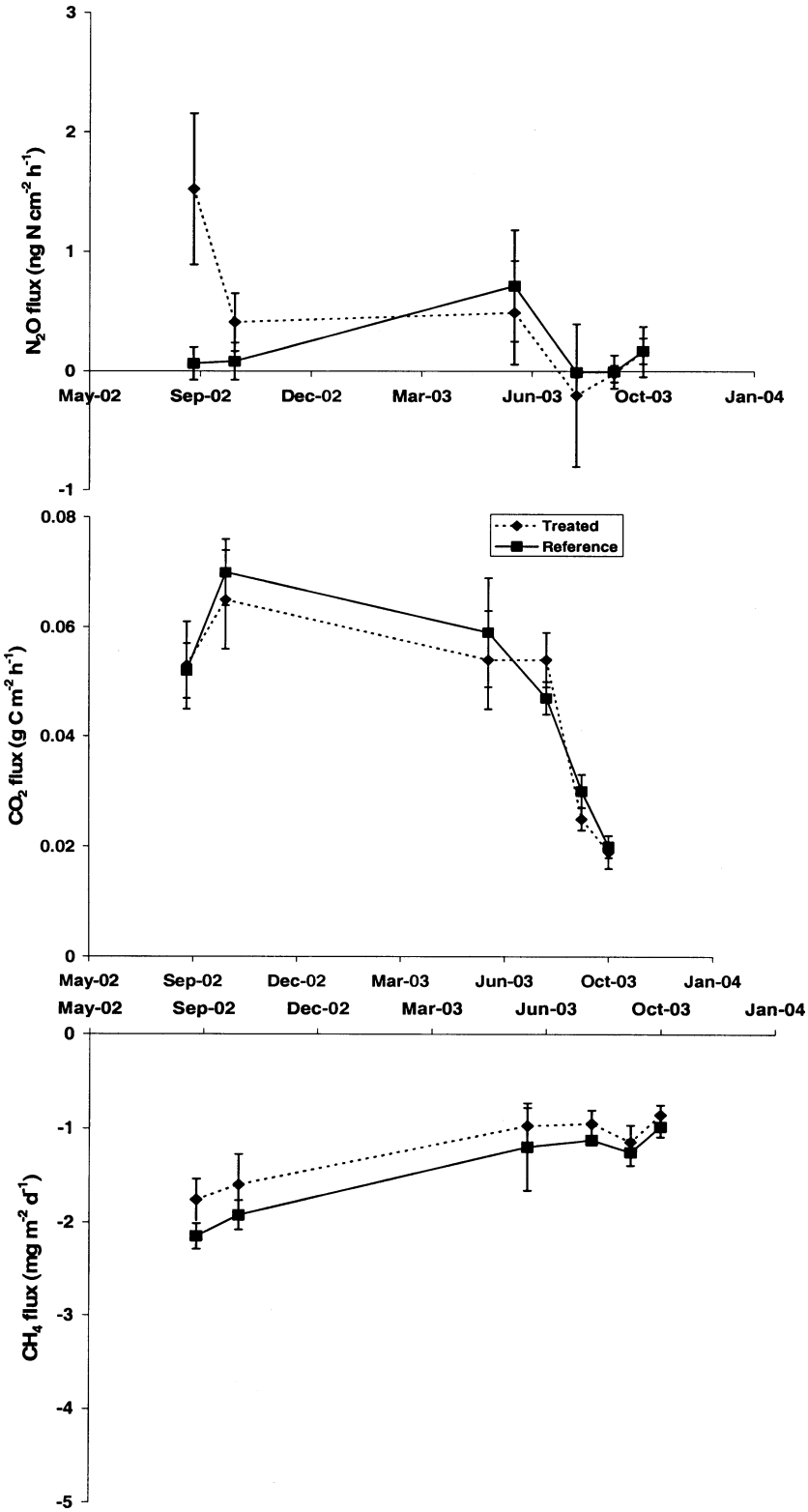

Figure 5. Soil:atmosphere flux of N_2O (*top*), CO_2 (*middle*), and CH_4 (*bottom*) in treated and reference watersheds from fall 2002 to fall 2003. Values are the mean of 12 chambers sampled in each watershed (three replicates at four elevations), $n = 12$.

There were no significant changes in soil solution nitrate, DON, or DOC concentrations after the treatment (Figure 6). Nitrate concentrations in stream water leaving W1 were higher than in W6 before treatment, with concentrations decreasing and converging after treatment (Figure 7). Treatment effects on stream nitrate concentrations were confounded by an ice storm that affected the HBEF

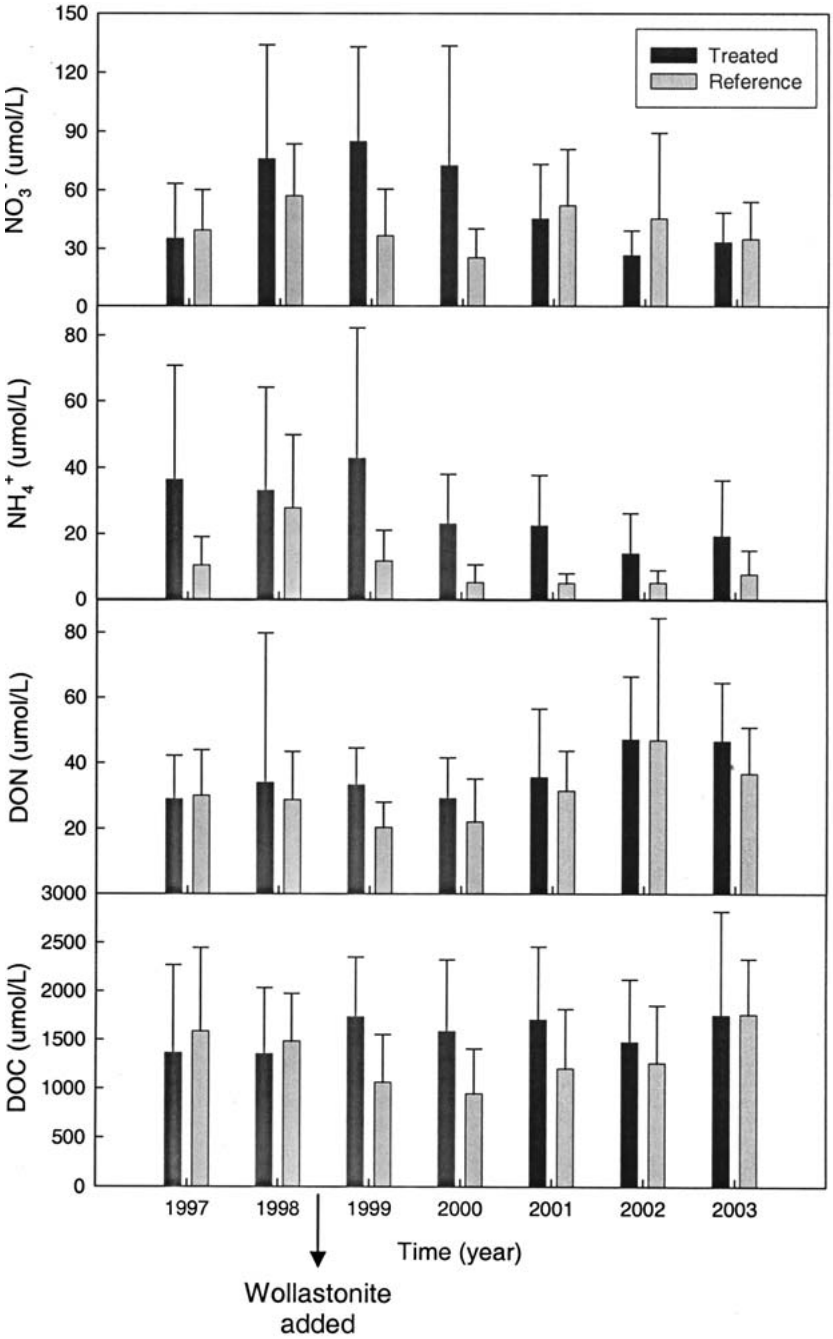

Figure 6. Nitrate (NO_3^-), ammonium (NH_4^+), dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) concentrations in Oa horizon lysimeter solutions collected before (1997–99) and after (2000–03) calcium (Ca) addition. Values are the mean (SE) of samples collected monthly from replicate lysimeters (13 treatment, eight reference) positioned longitudinally in each watershed.

in January 1998, and had a greater impact on W1 (Bernhardt and others 2003; Houlton and others 2003). Ammonium concentrations in streamwater were much lower than nitrate, with no differences between W1 and W6 (data not shown).

Although the 1998 ice storm may have affected stream nitrate concentration comparisons between the treatment and reference watersheds, it did not affect comparisons of soil and soil solution variables. Each of our sample plots (and their surrounding area) was surveyed for ice damage in the spring of 1998. The ice storm was restricted to a narrow elevation band (600–740 m); thus, some of our plots were strongly affected (for example, high-elevation areas) whereas others were not (for example, low-elevation areas). There were no consistent differences in response to Ca additions in ice-affected and nonaffected plots and elevations. For example, in 2001, microbial biomass N was reduced in the treated watershed relative to the reference watershed at both the low (no ice damage in either watershed) and high (extensive

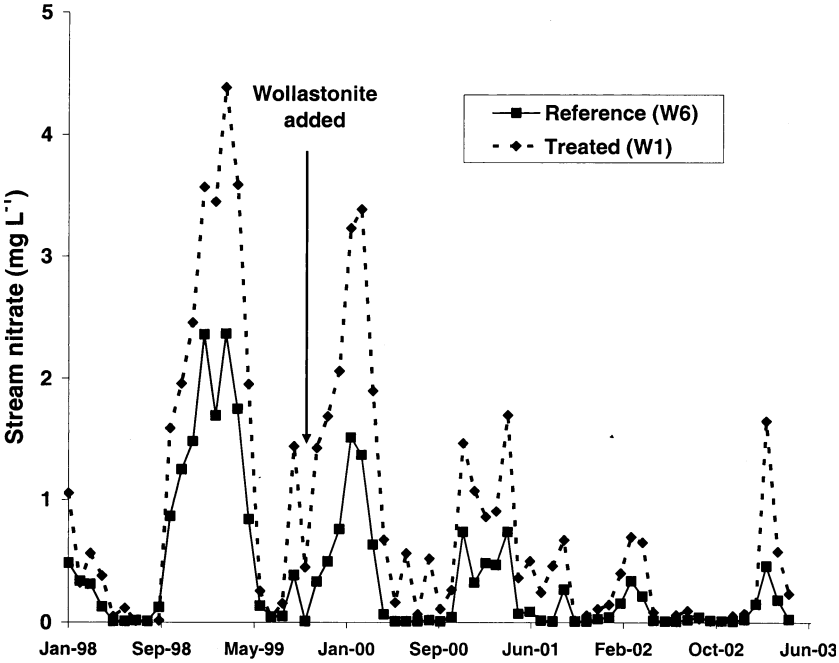

Figure 7. Mean monthly nitrate concentration in streams draining treated (W1) and reference (W6) watersheds before (January 1998–September 1999) and after (October 1999–May 2003) calcium addition. Values are the mean of weekly samples.

damage in both watersheds) elevations (673 ± 199 versus 487 ± 30 at the low elevation and 801 ± 200 versus 532 ± 83 at the high elevation). The lack of an effect of the ice storm on our results is consistent with Houlton and others (2003), who found that soil N cycling at the HBEF was not responsive to ice storm damage and that stream nitrate effects were driven by reductions in plant uptake.

Nitrogen concentrations in foliage showed no coherent response to treatment (Figure 8). There were patterns for relative increases in N concentration in American beech and decreases in concentration in yellow birch in the treated watershed. Sugar maple showed variable differences between the watersheds, with no pattern associated with the Ca treatment.

Discussion

The wollastonite addition produced significant alteration of the Ca and pH status of the treated watershed. By the year after addition, soil pH was increased by more than a full unit in the Oie horizon (approximately 5.5 versus approximately 4.3); and within 3 years, it was increased by more than 0.5 units in the Oa horizon. These soil pH effects were sustained through the study and were beginning to appear in the mineral soil as well. Streamwater pH increased nearly a full unit, and stream Ca concentrations more than doubled (Likens and others 2004; Peters and others 2004). Stream Al concentrations decreased by more than half, and acid-neutralizing capacity nearly doubled (G. E. Likens and C. T. Driscoll unpublished). Although these stream responses were initially caused by dissolution of wollastonite that fell directly into the stream channel (Likens and others 2004; Peters and others 2004), effects after 2000 were driven by the movement of Ca added to the watershed to the stream (Ash Dasch and others 2006). Soil solution chemistry in the Oa horizon was affected by the wollastonite addition; but consistent with the soil pH results, effects in the mineral soil were much less marked (Ash Dasch and others 2006). Distinct strontium isotope ($^{87}Sr/^{86}Sr$) and Ca/Sr signatures in the added wollastonite have been used to trace the movement of the added Ca into vegetation and show that over 60% of the Ca in understory wood fern (*Dryopteris spinulosa*) and over 30% of the Ca in overstory sugar maple comes from the wollastonite addition (Ash Dasch and others 2006).

Lack of Microbial Response

The lack of response of microbial N cycling processes that we observed is in contrast to previous studies of the responses of soil microorganisms to additions of base cations (mostly as $CaCO_3$) and comparisons of naturally base-rich and base-poor sites that suggest that base saturation is a fundamental controller of microbial biomass and activity in forest soils. Additions of base cations have been shown to increase the specific activity of microbial cells (Ivarson 1977; Adams and others 1978; Lohm and others 1984; Zelles and others 1978; Yavitt and

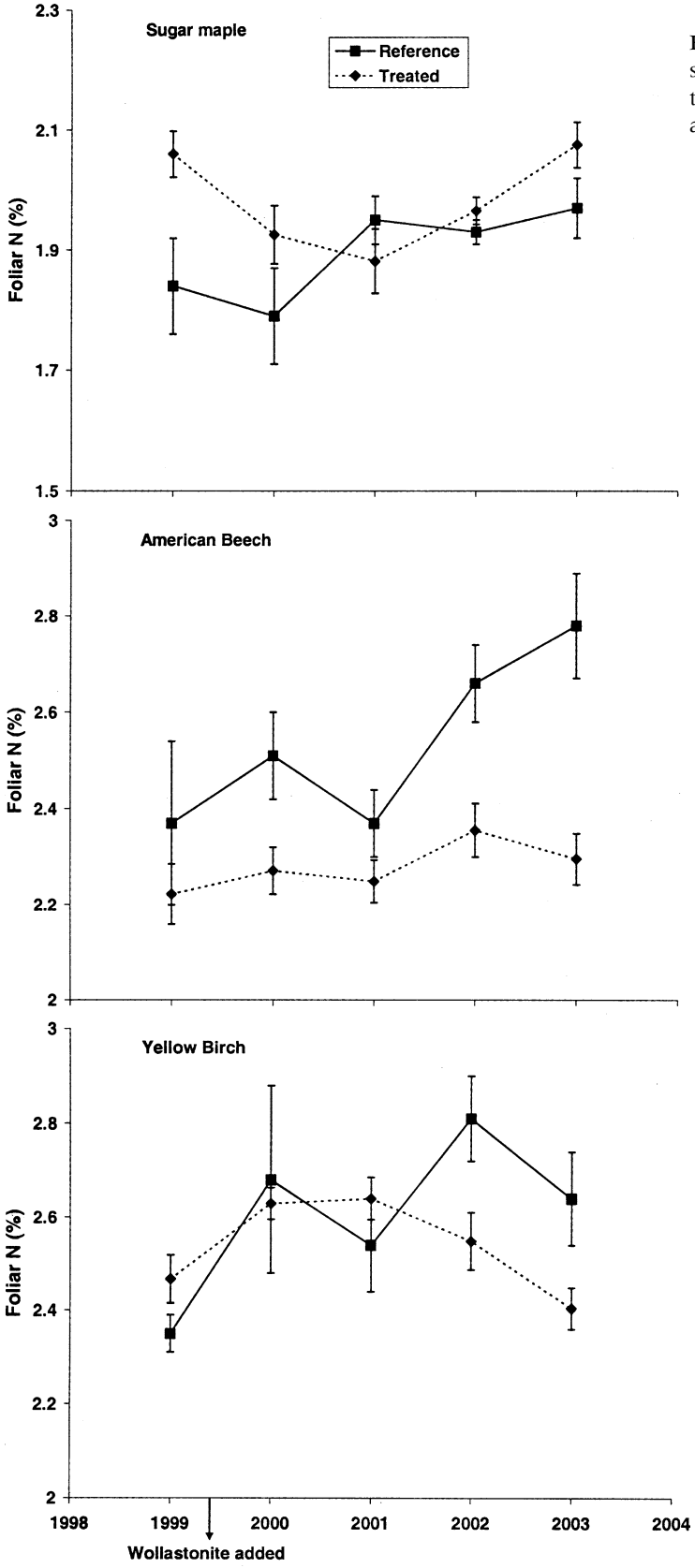

Figure 8. Foliar nitrogen (N) concentrations in sugar maple, American beech, and yellow birch in treated and reference watersheds before (1999) and after calcium (Ca) addition).

Newton 1990; Illmer and Schinner 1991; Neale and others 1997; Giesler and others 1998; Ste.-Marie and Paré 1999) and to alter microbial community composition (Adams and others 1978; Fritze 1991; Nodar and others 1992; Frostegard and others 1993). These changes, in combination with changes in the chemistry of recalcitrant soil organic matter, have been shown to increase microbial utilization of soil C pools (Persson and others 1989).

Although additions of base cations have clearly been shown to increase the rates of key soil microbial C processes in previous studies, effects on N dynamics have been more complex and difficult to predict. Increased processing of soil organic matter can increase gross rates of N mineralization and immobilization (Hart and others 1994), but it may reduce net N mineralization when immobilization is stimulated more than mineralization (Nömmik 1978; Nyborg and Hoyt 1978; Persson and others 1989; Simmons and others 1996). Thus, net effects are likely to be complex and variable. Even given this complexity, however, we expected an increase in nitrification, which has been shown to be highly sensitive to pH (De Boer and Kowalchuk 2001; Bäckman and Klemedttsson 2003; Clough and others 2004).

Why Didn't the Microbes Respond?

Our results raise fundamental questions about the factors regulating microbial biomass and activity in northern hardwood forest soils. The first issue that emerges is the possibility that microbial function is not physiologically responsive to pH within the range present at the HBEF. Northern hardwood forest soils are characterized by low pH (approximately 4.0), and microbial communities obviously function under these conditions. Several studies have suggested that microbes will not always increase in response to increases in pH (Parkin and others 1985; De Boer and others 1993; Ingerslev 1997; Rudebeck and Persson 1998; Blagodatskaya and Anderson 1999; Simek and Cooper 2001; Chapin and others 2003). In earlier experiments at the HBEF, nitrification was clearly vigorous after clear-cutting, even as streamwater pH declined by nearly 1 unit (Likens and others 1969).

These ideas about possible microbial indifference to pH increases, although plausible, are in stark contrast to the abundant literature showing that microbial processes respond positively to base cation additions and to pH increases of the magnitude that we have observed in response to the W1 treatment. We therefore suspect that microbes in HBEF soils are indeed sensitive to pH changes, but they have not responded to the increases in pH on W1 because they are limited by other factors. Two possible limiting factors suggested by previous research are phosphorus (P) availability and reductions in labile C availability due to Ca binding. The P hypothesis is supported by the transient increase in nitrification that we observed in 2001. This increase was accompanied by a transient increase in phosphorus availability, as described by Fiorentino and others (2003), who reported 1-year increases in resin-available P and decreases in foliar N:P ratios in canopy trees in W1. They suggested that the increase in pH stimulated mineralization of microbial P that may have catalyzed a series of changes in microbial biomass and activity in 2001. Enhanced P availability was short-lived, however, likely due to uptake of P by vegetation, which had high N:P ratios before treatment, suggestive of P deficiency (T. J. Fahey unpublished). These findings suggest that P limitation may be more important in the northern hardwood forest ecosystem at the HBEF than previously thought. Strong P limitation of microbial processes has been observed in moist tropical forests (Cleveland and others 2002), warm temperate forests (Gallardo and Schlesinger 1994), and temperate salt marsh sediments (Sundareshwar and others 2003).

Evidence that Ca binding of labile C can limit microbial response to increased pH comes from work published by Hobbie and others (2002), who found higher rates of C and N cycling in acidic tundra sites than in sites with higher pH, base cation availability, and organic matter quality. They suggested that the low rates of C and N cycling in the high-pH sites were associated with stabilization of soil organic matter by high concentrations of Ca, perhaps by formation of stable cation bridges among particulate and dissolved organic matter or between organic matter and mineral surfaces (Muneer and Oades 1989; Romkens and others 1996; Oste and others 2002). The importance of Ca as a regulator of C stabilization was shown by Paul and others (2003), who observed that soil C sequestration, but not plant biomass, was related to soil Ca content in a series of long-term afforestation plots in eastern North America. Increased organic matter stabilization at our site would be dependent on increases in exchangeable Ca exceeding any decreases in exchangeable Al or other multivalent cations, because all multivalent cations can participate in stabilization reactions with organic matter.

It is also possible that there have been significant changes in microbial community structure in response to the Ca addition but we have not seen a

functional response in C and N cycling because of P limitation and/or chemical interference with C availability.

Fungal:bacterial ratios have been shown to decrease with increases in soil pH (Badalucco and others 1992; Bååth and Anderson 2003). Other changes in community composition could be detected using more sophisticated molecular characterization techniques (for example, Tiedje and others 1999).

Why Did Soil Nitrogen Cycling Decrease and Where Did the Nitrogen Go?

Although the lack of stimulation of soil N cycling by $CaSiO_3$ addition is surprising, the declines in microbial biomass N and in inorganic N pools (approximately 10 kg N ha^{-1}) in soil and soil solution are downright puzzling. Possible fates for this N include plant uptake, storage in soil organic matter, and N_2 flux.

An increase in plant uptake in response to the Ca addition seems quite plausible. When Ca addition reduces acid and Al stresses on plants, their ability to compete with microbes for N could be increased (Long and others 1997; St. Clair and Lynch 2005). Over time, enhanced plant competitiveness for nutrients would result in less N in microbial biomass. Improved plant competitiveness over several cycles of microbial turnover could also explain the reductions in microbial biomass P observed by Fiorentino and others (2003). This explanation is consistent with recent studies showing that plants outcompete microbes for nutrients, especially over the long term (months to years) (Perakis and Hedin 2001; Zak and others 2004). However, this explanation is not supported by our foliar N data, which did not show any coherent increases in foliar N concentrations in the treated watershed. But note that the amount of N is small relative to, and well within the error term of, the pool of foliar N. It is also possible that the extra plant N uptake occurred in plant components or strata that we did not sample (for example, woody tissue, roots, seedlings, or understory trees and shrubs). Small increases in foliar or root biomass could also account for the extra N uptake.

It is quite possible that the N lost from the microbial biomass and inorganic N pools has shifted into the soil organic matter pool, a result that would be consistent with a Ca-induced increase in organic matter stabilization. This pool is vast, however, and it would be impossible to detect changes in this pool as small as 10 kg N ha^{-1} (Johnson 1995). It is also possible that the ''lost N'' left the ecosystem as N_2, the only major N gas that we did not measure. However, given that NO (P. M. Groffman unpublished) and N_2O fluxes did not respond to the treatment, it seems unlikely that N_2 flux was increased.

Conclusions

Not only did soil N cycle process not increase as expected, several lines of evidence suggest decreases in soil N cycling in response to Ca additions to soils at the HBEF. Despite increases in soil pH of between 0.5 and 1.0 unit, microbial biomass N, potential net N mineralization, and soil inorganic N levels all declined in response to the Ca addition; whereas other variables (microbial biomass C, soil solution chemistry, stream chemistry, foliar N, trace gas fluxes) showed no response over the first 4 years after treatment. Our findings clearly show that Ca additions have not led to increased N losses and/or symptoms of N saturation in the northern hardwood forest ecosystems at the HBEF.

The results suggest that our understanding of the influence of pH on microbial biomass and activity, and thus our ability to predict the response of northern hardwood forest ecosystems to $CaSiO_3$ inputs, is incomplete. There is a clear need for a better understanding of the multiple factors that influence microbial biomass and activity and plant:microbial interactions in these ecosystems as we assess, and possibly attempt to ameliorate, the effects of acid deposition. Before any efforts are made to add base cations to forests to replace those lost due to acid deposition, we need to consider the possibility that microbes may not be able to respond to increases in pH if other factors (for example, P), constrain their activity. We also need to consider the possibility that plant and microbial responses may be decoupled (that is, plants may be able to respond more readily than microbes to increases in pH). Decoupled responses could have major effects on plant:microbial competition for nutrients. An understanding of these responses is imperative if we hope to address problems of soil nutrient cation depletion effectively and to predict the effects of management efforts on ecosystem services related to forest production, water and air quality, and C sequestration.

It is important that we continue to track the long-term effects of the watershed-scale Ca addition. For example, if forest productivity is increased by the addition, there may be increased flow of C to fine roots and mycorrhizae, with important effects on uptake and dynamics of both Ca and N. Changes

in faunal communities may also be a key long-term response to the addition that could catalyze changes in litter decomposition, microbial communities, and N dynamics.

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