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Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA

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Abstract

Nitrogen retention in soil organic matter (SOM) is a key process influencing the accumulation and loss of N in forest ecosystems, but the rates and mechanisms of inorganic N retention in soils are not well understood. The primary objectives of this study were to compare ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) immobilization among soils developed under different tree species in the Catskill Mountains of New York State, and to determine the relative roles of biotic or abiotic processes in soil N retention. A laboratory experiment was performed, where ^{15}N was added as NH_4^+, NO_2^-, or NO_3^- to live and mercury-treated O horizon soils from three tree species (American beech, northern red oak, sugar maple), and ^{15}N recoveries were determined in the SOM pool. Mercuric chloride was used to treat soils as this chemical inhibits microbial metabolism without significantly altering the chemistry of SOM. The recovery of ^{15}N in SOM was almost always greater for NH_4^+ (mean 20%) and NO_2^- (47%) than for NO_3^- (10%). Ammonium immobilization occurred primarily by biotic processes, with mean recoveries in live soils increasing from 9% at 15 min to 53% after 28 days of incubation. The incorporation of NO_2^- into SOM occurred rapidly (<15 min) via abiotic processes. Abiotic immobilization of NO_2^- (mean recovery 58%) was significantly greater than abiotic immobilization of NH_4^+ (7%) or NO_3^- (7%). The incorporation of NO_2^- into SOM did not vary significantly among tree species, so this mechanism likely does not contribute to differences in soil NO_3^- dynamics among species. As over 30% of the $^{15}NO_2^-$ label was recovered in SOM within 15 min in live soils, and the products of NO_2^- incorporation into SOM remained relatively stable throughout the 28-day incubation, our results suggest that NO_2^- incorporation into SOM may be an important mechanism of N retention in forest soils. The importance of NO_2^- immobilization for N retention in field soils, however, will depend on the competition between incorporation into SOM and nitrification for transiently available NO_2^-. Further research is required to determine the importance of this process in field environments.

Keywords: atmospheric nitrogen deposition, nitrogen excess, nitrogen retention, northern hardwood forest, soil organic matter, soil sterilization

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Introduction

Anthropogenic activity has considerably altered the global nitrogen (N) cycle and has resulted in increased rates of atmospheric N deposition in industrialized regions of the world (Vitousek *et al*., 1997). Over the long term, chronic atmospheric deposition of N to forest ecosystems can result in the accumulation of soil N and a condition of N excess characterized by greater N production than biological N consumption (Ågren & Bosatta, 1988; Aber *et al*., 1998). Results of N excess potentially include lower soil C : N ratios, increased production of nitrate (NO_3^-) (Tietema, 1998), as well as the acidification of soils and surface waters (van Breemen *et al*., 1987; Fenn *et al*., 1996).

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Concern over N excess has stimulated study of the sinks for N inputs to forest ecosystems (Magill *et al.*, 1997). Field experiments at the plot scale utilizing inorganic N isotopes enriched in ^{15}N have demonstrated that incorporation into soil organic matter (SOM) is the dominant fate of N additions to forest ecosystems of the eastern US and Europe (Nadelhoffer *et al.*, 1995; 1999a, b). In addition to biologically mediated processes that result in the retention of N in SOM, abiotic mechanisms can also incorporate ammonium (NH_4^+) and nitrite (NO_2^-) into SOM (Bremner & Fúhr, 1966; Johnson *et al.*, 2000; Dail *et al.*, 2001; Barrett *et al.*, 2002). It is not currently believed that nitrate (NO_3^-) can be incorporated directly into SOM via abiotic processes (Dail *et al.*, 2001; Davidson *et al.*, 2003).

Although NO_2^- concentrations (range 0.0002–0.0008 $\mu mol\,g^{-1}$; Venterea *et al.*, 2003a) are generally low relative to other forms of inorganic N in soils of northern hardwood forests (ranges for NH_4^+ and NO_3^- in soils of the Catskill Mountains are ~1.1–1.4 and 0.1–0.9 $\mu mol\,g^{-1}$, respectively; Lovett *et al.*, in press), and while rates of atmospheric NO_2^- deposition are likely to be negligible relative to atmospheric inputs of NO_3^- and NH_4^+, NO_2^- is an important intermediate in the nitrification and denitrification processes (Venterea & Rolston, 2000b), with the production of NO_2^- being the first step in the generation of NO_3^- via autotrophic nitrification. With an average total soil mass of 71 $Mg\,ha^{-1}$ for the Oe and Oa horizons in the Catskill Mountains (Templer, 2001), and an average gross nitrification rate of 0.2 $\mu mol\,g^{-1}\,day^{-1}$ measured in forest floors of the Catskills (Verchot *et al.*, 2001), the flux of N cycling through the NO_2^- pool in just the Oe and Oa horizons (~5200 $mol\,ha^{-1}\,yr^{-1}$) is approximately 650% of the rate of total atmospheric N deposition in the Catskills (~800 $mol\,ha^{-1}\,yr^{-1}$; Ollinger *et al.*, 1993; Lovett & Rueth, 1999). Reactions involving NO_2^-, therefore, can significantly affect the fate of inorganic N in soils (Venterea & Rolston, 2000a).

In a previous paper, we found that $^{15}NO_2^-$ added to live forest floor soils from the Catskill Mountains was incorporated rapidly into SOM, at time scales of 1 day or shorter, and that incorporation into SOM was the dominant fate for the $^{15}NO_2^-$ tracer (Fitzhugh *et al.*, 2003). However, the design of our previous experiment did not allow us to determine if this incorporation occurred via biotic or abiotic processes, nor did it allow us to compare the magnitude of NO_2^- incorporation into SOM with the magnitude of incorporation of the other inorganic N forms (NH_4^+, NO_3^-). Here, we report the results of a laboratory experiment where ^{15}N was added as NH_4^+, NO_2^-, and NO_3^- to live and mercury (Hg)-treated soils sampled from monospecific plots of three tree species: American beech (*Fagus grandifolia* L.), northern red oak (*Quercus rubra* L.), and sugar maple (*Acer saccharum* Marsh.) from the Catskill Mountains in New York State. The rates of litter decomposition and N cycling can vary significantly among these species, with decomposition rates and nitrification tending to be greatest under maple, intermediate under beech, and lowest under oak (Pastor & Post, 1986; Berg & McClaugherty, 1987; Finzi *et al.*, 1998; Lovett & Rueth, 1999; Verchot *et al.*, 2001; Lovett *et al.*, in press). The objectives of this study were to determine the recovery of ^{15}N in the SOM pool and to compare recoveries among the different forms of labeled N and among tree species. Treating the soil with Hg allowed us to separate biotic and abiotic mechanisms of N immobilization. We hypothesized that: (i) immobilization of $^{15}NH_4^+$ and $^{15}NO_3^-$ would occur predominantly via biotic processes, (ii) $^{15}NO_2^-$ would move quickly (<15 min) into the SOM pool via abiotic processes, (iii) abiotic immobilization of NO_2^- would be greater than NH_4^+ and NO_3^-, and (iv) recovery of $^{15}NO_2^-$ in SOM would be greater under red oak and beech compared with maple, as leaf litter of oak and beech is high in lignin (Lovett *et al.*, in press) and would be expected to produce more lignin-derived phenolics in the soil which would enhance the incorporation of NO_2^- into SOM.

Materials and methods

Site description

The Catskill Mountains, located in southeastern New York State, encompass an area of ~5000 km^2 that receives among the greatest rates of atmospheric N deposition in the northeastern US (~800 $mol\,ha\,yr^{-1}$; Ollinger *et al.*, 1993; Lovett & Rueth, 1999). The dominant constituents of bedrock in the Catskill Mountains are Devonian age conglomerates, shales, and flat-lying sandstones (Stoddard & Murdoch, 1991) overlain by glacial till of variable depth (Rich, 1934). Thin Inceptisols that are moderate to highly acidic and generally stony and well-drained are the primary soils in the Catskill Mountains (Stoddard & Murdoch, 1991; Lovett *et al.*, 2000). Cool summers and cold winters characterize the climate. The mean annual temperature is 4.3 °C (January mean = – 8.5 °C, July mean = 16.7 °C) and the mean annual precipitation is 153 cm, approximately 20% as snow, at the Slide Mountain weather station (elevation of 808 m) in the central Catskills. Sugar maple, American beech, yellow birch (*Betula alleghaniensis*), and northern red oak are the dominant tree species of the Catskills (McIntosh, 1972).

Field techniques

Plots dominated by sugar maple, American beech, and red oak were selected in the central Catskills. Each plot was 3 m in radius and included two or three canopy dominant trees of the target species. These small plots were located within a 6 m radius plot of nearly monospecific composition to minimize the edge effects from neighboring trees of other species. Two plots were sampled per species. These plots were chosen because they are representative of soil N dynamics for the respective species within the Catskill Mountains, as determined by measurements of potential net N mineralization and nitrification as part of a larger study (Table 1; Lovett *et al*., in press). Litter (Oi horizon) was removed from the soil surface and then samples of the forest floor (Oe and Oa horizons) were obtained using a tulip bulb corer. The depth of the combined Oe and Oa horizons ranged from 1 to 8 cm. Three samples were taken from each plot, with each sample being a composite of two to three subsamples taken randomly within the plot, thus there were six composite samples per species. The pH of the Oe and Oa horizons measured in deionized water at a 2 : 1 ratio (water : soil) varied from 3.4 to 3.9, organic matter contents measured by loss on ignition varied from 42% to 84%, and soil C : N varied between 21 and 29.

Laboratory methods

The experimental design was completely balanced and had four factors. The factors were tree species with three levels (beech, maple, oak), form of ^{15}N added to the soil with three levels (NH_4^+, NO_2^-, NO_3^-), microbial status with two levels (live, Hg treatment), and time after ^{15}N addition with three levels (15 min, 1 day, 28 days). Each soil sample received ^{15}N as $^{15}NH_4^+$, $^{15}NO_2^-$, or $^{15}NO_3^-$. The ^{15}N was delivered either in a solution of deionized water (live soils) or in a solution of 5% mercuric chloride (Hg-treated soils). Mercuric chloride ($HgCl_2$) was used as the sterilizing agent because it is superior to alternative sterilizing methods (i.e., autoclaving, irradiation, fumigation) in two aspects critical to this study: (i) $HgCl_2$ is more likely to inhibit microbial metabolism throughout a 28-day incubation than alternative methods of sterilization because soils previously sterilized by alternative methods may become contaminated with microorganisms during the incubation period (Wolf & Skipper, 1994), and (ii) among sterilization techniques, $HgCl_2$ produces the fewest changes in soil chemical and physical properties, with no significant effects on nutrients (Wolf *et al*., 1989). Our rate of addition (33 800 mg $HgCl_2$ kg^{-1} of dry soil) was greater than the rates recommended by Wolf & Skipper (1994) of 500–20 000 mg $HgCl_2$ kg^{-1} of dry soil to achieve effective inhibition of microbial metabolism.

Soils were passed through an 8 mm sieve. Five subsamples from each composite sample were dried in an oven at 65 °C to determine the gravimetric soil moisture content, which varied from 0.8 to 4.1 among samples. For each soil sample ($n = 18$), a mass of soil at field moisture equivalent to 14.8 g dry soil was added to a 230 mL glass Mason jar. Ten milliliters of 394 μmol ^{15}N L^{-1} (99% atom enriched) was added to each soil sample as $^{15}NH_4Cl$, $Na^{15}NO_2$, or $Na^{15}NO_3$ in a solution of deionized water or 5% $HgCl_2$ and mixed thoroughly into the soil. These additions were equivalent to 0.267 μmol ^{15}N (g dry soil)$^{-1}$. Using an average total soil mass of 71 Mg ha^{-1} for the Oe and Oa horizons in the Catskill Mountains (Templer, 2001), our rate of ^{15}N addition is equivalent to 19 mol N ha^{-1}, ~2% of the annual rate of atmospheric N deposition in the Catskills. Immediately after addition of the label, the jars were covered with an airtight lid fitted with a butyl rubber septum and incubated at 20 °C. After 15 min, 1, and 28 days, the headspace of one-third of the jars was sampled for CO_2 using a syringe to test the effectiveness of the sterilizing agent. Following the sampling at 1 day, all jars were opened for 15 min at weekly intervals to aerate the soils and to ensure that the live soils did not become anaerobic. At the same time intervals of the CO_2 sampling (15 min, 1, and 28 days following ^{15}N addition), 10 g of wet soil was sampled from each jar and placed in a specimen cup. To extract exchangeable N, 50 mL of 2 N KCl was added to

Table 1 Comparison of rates of potential net mineralization and nitrification between single species plots for forest floors sampled during the current study (plots 1 and 2) and single species plots located throughout the Catskill Mountains (all single species plots)

	All plots ($n = 6$)				
Species	Minimum	Maximum	Median	Plot 1	Plot 2
Net N mineralization (μmol g^{-1} day^{-1})					
Beech	0.26	0.56	0.48	0.32	0.56
Maple	0.34	0.52	0.41	0.42	0.41
Oak	0.22	0.65	0.24	0.25	0.22
Net nitrification (μmol g^{-1} day^{-1})					
Beech	0.03	0.32	0.19	0.09	0.32
Maple	0.16	0.35	0.22	0.26	0.19
Oak	0.00	0.29	0.00	0.00	0.01

Soils were incubated in the laboratory at a moisture content 60% of the field capacity. Methods of analysis are given in Lovett *et al*. (in press).

each specimen cup, shaken twice during the first hour after the KCl was added, and allowed to stand overnight. The following day, the KCl was decanted from the specimen cup, and an additional 50 mL of KCl was again added to the soil. After thoroughly shaking the specimen cup, the soil plus extract was poured onto a funnel fitted with a Whatman 41 filter. The filtrate from the two 50 mL KCl extractions (total of 100 mL) was used to analyze soil-extractable NH_4^+ and $(NO_2^- + NO_3^-)$ concentrations. The soil remaining on the filter was placed in an oven at 65 °C to dry, pulverized, and analyzed for ^{15}N. All ^{15}N analyses were performed on a Europa Scientific Integra, a continuous flow Isotope Ratio Mass Spectrometer (IRMS) integrated with on-line combustion, at the University of California at Davis under the supervision of D. Harris. The percentage of the ^{15}N recovered in the soil samples following KCl extraction at a given time (15 min, 1, 28 days) was calculated relative to the ^{15}N label originally added to the sample at time 0. The ^{15}N recovered in Hg-treated soil was interpreted to be soil organic N resulting from abiotic incorporation of N into SOM, while the ^{15}N recovered in live soils was interpreted to quantify soil organic N and microbial biomass (i.e., N that had been taken up by microbes as well as N that had been incorporated into SOM by biotic and abiotic processes).

Following approximately 2 weeks of incubation, the rate of NO production was measured in all live soil samples and in one-third of the Hg-treated soils (the same soil samples that were measured for CO_2). The instantaneous rates of NO production in the incubating soils were determined by sealing each jar with a specially fitted lid attached to a dynamic flow-through system, which allowed for the continuous delivery of a humidified air stream through the jar prior to entering a chemiluminescent NO_x ($NO + NO_2$) analyzer (Unisearch Models LMA-3 and -3D). The rates of NO production on a dry soil mass basis were calculated from the difference between NO concentration in air upstream and downstream of the soil, the air flow rate, and the dry soil mass, as previously described (Venterea & Rolston, 2000a).

Extractable NH_4^+ and $(NO_2^- + NO_3^-)$ concentrations were determined using phenate colorimetry and cadmium reduction, respectively, on an Alpkem autoanalyzer for the live soils only. The net N mineralization was calculated as the accumulation of extractable NH_4^+ and $(NO_2^- + NO_3^-)$ during the 28-day incubation, and the net nitrification was calculated similarly using extractable $(NO_2^- + NO_3^-)$ concentrations. Extractable NH_4^+ and $(NO_2^- + NO_3^-)$ concentrations were not determined on Hg-treated soils because of concerns of handling liquid hazardous waste contaminated with $HgCl_2$. Concentrations of CO_2 sampled from the headspace of the jars were determined on a gas chromatograph equipped with a thermal conductivity detector.

Statistical analyses

The soil ^{15}N recoveries, extractable soil NH_4^+ and $(NO_2^- + NO_3^-)$ concentrations, net N mineralization and nitrification, and NO production of the three subsamples were averaged for each plot ($n = 6$) prior to statistical analyses. A four-way analysis of variance (ANOVA) was performed to test for the effects of tree species, microbial status, form of ^{15}N added, and time of incubation on the mean soil ^{15}N recoveries. A similar four-way ANOVA was conducted on mean CO_2 concentration. A three-way ANOVA was performed to test for the effects of species, form of ^{15}N added, and time of incubation on mean extractable soil NH_4^+ and $(NO_2^- + NO_3^-)$ concentrations of live soils. Two-way ANOVA was carried out on mean net N mineralization and nitrification for live soils to test for the effects of species and form of ^{15}N added. For those samples where NO production was measured in both live and Hg-treated soils, three-way ANOVA was used to test for the effects of species, microbial status, and form of ^{15}N added on mean NO production. As NO production was measured in every live soil sample, a two-way ANOVA was conducted to test for the effects of species and form of ^{15}N added on mean NO production in live soils. If effects for species, form of ^{15}N added, or time of incubation were significant for the above analyses, means were separated using Tukey's test on the least squares means.

Results

Effects of Hg treatment and tree species on CO_2 evolution

Addition of 10 mL 5% $HgCl_2$ had no effect on mean headspace CO_2 concentrations after 15 min of incubations, but resulted in significantly ($P < 0.0001$) lower mean CO_2 concentrations in the headspace of Hg-treated soils compared with live soils after 1 and 28 days of incubation (Fig. 1a), with mean Hg-treated headspace CO_2 being 52% and 39% of concentrations in live soils after 1 and 28 days, respectively. Within each tree species, the mean headspace CO_2 concentrations were significantly lower ($P < 0.05$) in Hg-treated than live soils (Fig. 1b). Additionally, tree species significantly affected the mean CO_2 concentrations in live soils ($P < 0.05$), with beech and oak being greater than maple (Fig. 1b).

The insignificant effect of Hg treatment on headspace CO_2 after 15 min of incubation may reflect insufficient

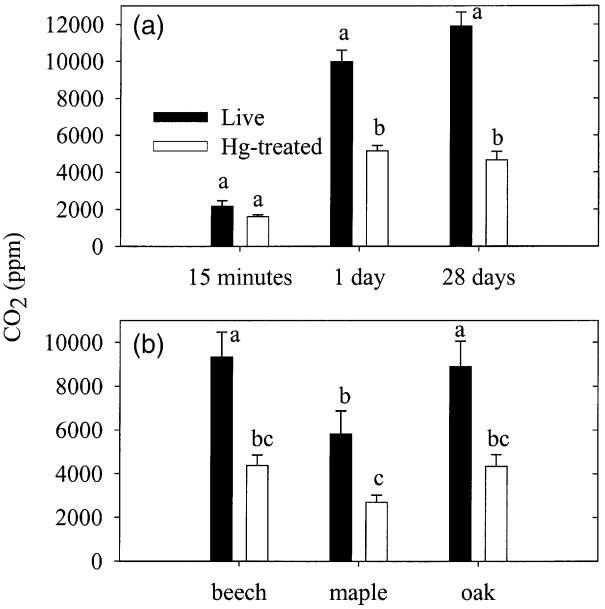

Fig. 1 Comparison of mean headspace carbon dioxide (CO_2) concentrations between Hg-treated and live soils (a) for each sampling time and (b) among tree species. In panel (a), means within a sampling time with different letters are significantly different ($P<0.05$) as determined by Tukey's test. In panel (b), means with different letters are significantly different ($P<0.05$) as determined by Tukey's test. Error bars are +1 SE.

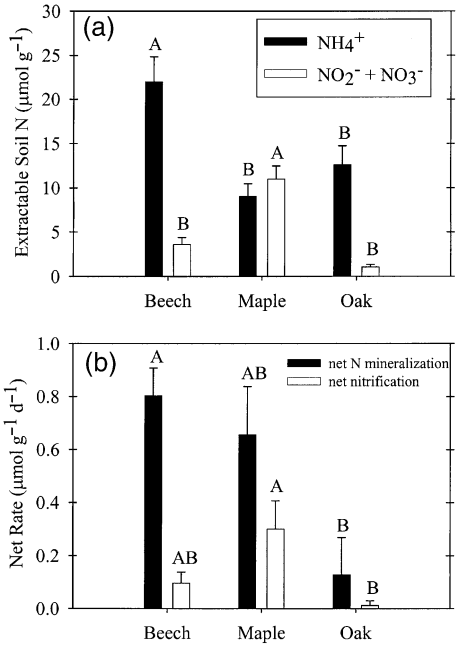

Fig. 2 Comparison among tree species for live soils of (a) mean extractable soil ammonium (NH_4^+) and nitrite plus nitrate ($NO_2^- + NO_3^-$) concentrations and (b) mean net rates of nitrogen mineralization and nitrification. Species with different letters within an assay have significantly different mean values as determined by Tukey's test. Error bars are +1 SE.

time for differences in headspace CO_2 to develop between live and Hg-treated soils and/or that Hg-treated soils were not sterile after this relatively short time from addition of the $HgCl_2$. While there was a significant effect of Hg treatment on headspace CO_2 after 1 and 28 days of incubation, the CO_2 concentrations in Hg-treated soils suggested that microbial respiration was not completely eliminated (Fig. 1). The hydrophobic nature of the forest floor SOM in our samples may have inhibited the diffusion of the $HgCl_2$ into all the soil pores.

Effects of tree species on extractable soil N concentrations and net N dynamics

There were significant effects of tree species on mean extractable soil NH_4^+ concentrations ($P = 0.0003$), with beech being greater than maple and oak, as well as on mean extractable ($NO_2^- + NO_3^-$) ($P<0.0001$), with maple being greater than beech and oak (Fig. 2a). Additionally, there were significant effects of tree species on the rates of net N mineralization ($P = 0.016$), with beech being greater than oak, as well as on net nitrification ($P = 0.028$), with maple being greater than oak (Fig. 2b).

Effects of microbial status and tree species on NO production

The mean NO production was significantly ($P = 0.0001$) greater in live than Hg-treated soils (Fig. 3a). For live soils, there was a significant tree species effect on mean NO production ($P<0.0001$), with maple being greater than beech and oak (Fig. 3b). The extremely low rates of NO production in our Hg-treated soils (Fig. 3a) suggested that the Hg treatment was effective in eliminating the activity of microorganisms involved in nitrification. The mean rates of NO production in live soils were considerable, being 11%, 22%, and 44% of the mean rates of net nitrification under beech, maple, and oak, respectively. Assuming a constant rate of NO production during the incubations, a total quantity of NO produced varied from 2.2 μmol for oak soils to 27 μmol for maple, significant in comparison with the quantity of ^{15}N added to the soils (4.0 μmol).

Effects on recovery of ^{15}N in soils

All factors and most of their interactions had statistically significant ($P<0.05$) effects on mean soil ^{15}N

recoveries, defined as the proportion of added ^{15}N recovered in the non-extractable soil pool (Table 2). Fifteen minutes after addition, recoveries of ^{15}N in the soil matrix were greatest for soils that received $^{15}NO_2^-$, and the quantity recovered did not vary between live and Hg-treated soils within a species (Fig. 4a). At 15 min, the mean soil ^{15}N recovery was 52.5±4.0% (mean±SE) for soils that received $^{15}NO_2^-$, but only 6.5±0.5% for soils that received $^{15}NH_4^+$ and $^{15}NO_3^-$. One day after ^{15}N addition to the soil, soil ^{15}N recovery was greatest in Hg-treated soils that received $^{15}NO_2^-$ (Fig. 4b). After 1 day, live soils under oak that received $^{15}NH_4^+$ exhibited relatively large recoveries and were significantly greater than the mean recovery after 15 min (Fig. 4b).

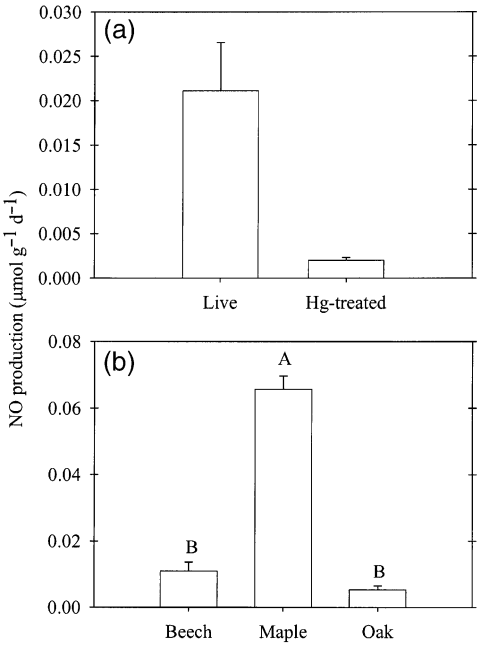

Fig. 3 Comparison of mean rates of nitric oxide (NO) production (a) between live and Hg-treated soils ($P = 0.0001$) and (b) among tree species for live soils ($P < 0.0001$). Species with different letters have significantly different mean gross NO production rates as determined by Tukey's test. Error bars are +1 SE.

Twenty-eight days after the ^{15}N addition, recovery was greatest for live soils under oak that received $^{15}NH_4^+$ (Fig. 4c). The mean recoveries in Hg-treated beech and oak soils were significantly greater for $^{15}NO_2^-$ than for $^{15}NH_4^+$ and $^{15}NO_3^-$ 28 days following the addition. The recovery of $^{15}NO_2^-$ in live soils was not significantly different than for Hg-treated soils for any of the species 28 days following the addition. Recovery 28 days following the addition was significantly greater in live than Hg-treated soils for beech soils that received $^{15}NH_4^+$ as well as for oak soils that received $^{15}NH_4^+$ or $^{15}NO_3^-$. The mean recovery was significantly greater at 28 days than 15 min for live beech soils that received $^{15}NH_4^+$ as well as for live oak soils that received $^{15}NH_4^+$ or $^{15}NO_3^-$. The mean recovery was significantly lower at 28 days than 15 min for Hg-treated maple soils that received $^{15}NO_2^-$. Recoveries of ^{15}N in Hg-treated soils that received $^{15}NH_4^+$ and $^{15}NO_3^-$ were relatively small throughout the incubation. The recovery of ^{15}N in SOM was not related to gravimetric soil moisture or organic matter content ($P > 0.05$).

Table 2 Results of four-way analysis of variance for soil ^{15}N recovery

Source	DF	*F* value	*P*
Species	2	19.0	<0.0001
Microbial status	1	7.42	0.0087
N	2	240	<0.0001
Time	2	512	0.0004
Species*microbial status	2	13.4	<0.0001
Species*N	4	4.42	0.0037
Microbial status*N	2	88.9	<0.0001
species*time	4	4.29	0.0044
Microbial status*time	2	23.0	<0.0001
N*time	4	18.8	<0.0001
Species*microbial status*N	4	2.51	0.0524
Species*microbial status*time	4	2.02	0.1040
Species*N*time	8	0.68	0.7068
Microbial status*N*time	4	8.43	<0.0001
Species*microbial status*N*time	8	2.43	0.0256

Form of ^{15}N added is represented by N.

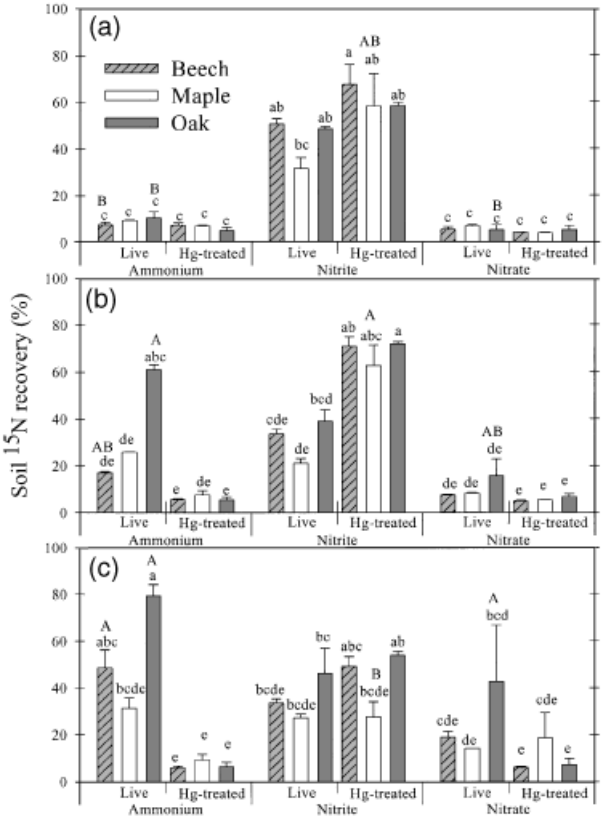

Fig. 4 Comparison of mean soil ^{15}N recoveries at (a) 15 min, (b) 1 day, and (c) 28 days after the ^{15}N label was added to the soil. Mean values with different lowercase letters within a panel are significantly different ($P < 0.05$). Mean values with different uppercase letters within each species-microbial status-form of ^{15}N addition combination ($n = 18$) are significantly different ($P < 0.05$) over time. Pairwise comparisons were performed with Tukey's test using the mean square error of the species-microbial status-form of ^{15}N addition-time interaction as the error term.

Discussion

Comparison of soil ^{15}N recovery among different forms of inorganic N addition

Identification of the mechanisms resulting in the rapid incorporation of inorganic N into SOM has become an important goal in research on N dynamics in temperate ecosystems (Berntson & Aber, 2000; Johnson *et al*., 2000; Dail *et al*., 2001; Barrett *et al*., 2002). To the best of our knowledge, our current study is the first experiment to compare the incorporation of N into soils among all the forms of inorganic N cycling in forest soils. Previous work by our research group determined that incorporation into SOM was the dominant fate of $^{15}NO_2^-$ added to forest soils in the Catskill Mountains and that this incorporation occurred rapidly, at time scales of 1 day or shorter (Fitzhugh *et al*., 2003). However, the design of our previous experiment did not allow us to determine if the incorporation of the $^{15}NO_2^-$ label occurred in the form added or if the incorporation occurred after the NO_2^- underwent dissimilatory reduction to NH_4^+ or oxidation to NO_3^-. Additionally, the design of our previous experiment did not allow us to discern whether the incorporation of the $^{15}NO_2^-$ occurred via biotic or abiotic mechanisms. Our current experiment inhibited microbial metabolism by adding $HgCl_2$, which should eliminate the microbial conversion of NO_2^- to the other inorganic N forms prior to incorporation into SOM. Although the Hg treatment was not completely effective in eliminating microbial respiration (Fig. 1), the extremely low rates of NO production in our Hg-treated soils (Fig. 3a) suggested that the Hg treatment was effective in suppressing nitrification. As over 50% of the $^{15}NO_2^-$ label was recovered in SOM only 15 min following addition to Hg-treated soils, our results indicate that abiotic mechanisms can result in the rapid incorporation of NO_2^- into SOM in acidic forest soils of the Catskill Mountains. The lower recovery of $^{15}NO_2^-$ in live than Hg-treated soils at all times, while not always statistically significant, suggested that nitrification can compete effectively with abiotic reactions for available NO_2^- in live soils. The recovery of $^{15}NO_2^-$ in live soils exhibited a positive relationship with soil C : N ($R^2 = 0.90$, $P < 0.05$, $N = 6$) and a negative correlation with net nitrification ($R^2 = 0.79$, $P < 0.05$, $N = 6$), suggesting that relatively high rates of nitrification in live soils with low C : N ratios consumed some of the available NO_2^- that would otherwise have been incorporated into SOM. However, the substantial recovery of $^{15}NO_2^-$ in live soils and stability throughout the experiment suggest that abiotic sinks could act as effective retention mechanisms for inorganic N, at least during the period of incubation used for this experiment. The recovery of $^{15}NO_2^-$ was significantly greater than recovery of $^{15}NH_4^+$ and $^{15}NO_3^-$ in live soils under beech and oak 15 min following addition, demonstrating that incorporation of NO_2^- into SOM plus microbial biomass is greater than the incorporation of NH_4^+ or NO_3^- at the time scale of minutes.

In addition to being incorporated into SOM, NO_2^- can be transformed to NO via abiotic mechanisms; this aqueous disproportionation reaction may also produce NO_3^- (Van Cleemput & Baert, 1976). The gas NO can subsequently be converted to nitrogen dioxide (NO_2), which can then also be converted to NO_3^- via abiotic chemical reactions (Van Cleemput & Baert, 1976). The design of the current experiment did not allow quantification of the transformation of NO_2^- into these products, but it was likely that the production of NO

accounted for a significant portion of the added $^{15}NO_2^-$. Fitzhugh *et al.* (2003), using live soils from the same plots as the current experiment, measured the recovery of $^{15}NO_2^-$ in the SOM, microbial biomass, extractable inorganic, N_2, and N_2O pools and found that between 8% and 64% of the $^{15}NO_2^-$ was not recovered; the production of NO was estimated and likely explained the fate of the unrecovered $^{15}NO_2^-$.

The low recoveries of ^{15}N in Hg-treated soils that received $^{15}NH_4^+$ or $^{15}NO_3^-$ suggested that abiotic incorporation of these inorganic N forms into SOM is a relatively minor process relative to incorporation of NO_2^- in acidic forest soils of the Catskill Mountains. The recovery of $^{15}NH_4^+$ in live soils, however, was significant beginning 1 day after the addition; this immobilization was likely microbially mediated as $^{15}NH_4^+$ recovery in Hg-treated soils was small throughout the experiment. The recovery of $^{15}NO_3^-$ in live soils only became appreciable 28 days after the addition and was always smaller than the recoveries of $^{15}NH_4^+$ and $^{15}NO_2^-$.

In O horizon soils at Harvard Forest in Massachusetts, between ~40% and 50% of $^{15}NO_2^-$ addition was recovered as SOM in live soils and between ~10% and 30% as SOM in sterilized soils (Dail *et al.*, 2001). In contrast with our results, Dail *et al.* (2001) observed lower recoveries of $^{15}NO_2^-$ in SOM in sterilized than live O horizon soils, a result that suggested that NO_2^- can be incorporated into SOM via both abiotic and biotic processes. Dail *et al.* (2001), however, used irradiation and autoclaving to sterilize their soils, methods of sterilization that, in contrast with $HgCl_2$, can create significant changes in the chemistry of SOM (Wolf & Skipper, 1994).

The role of the incorporation of NO_2^- into SOM as a mechanism of N retention in forest ecosystems has been studied relatively little. Evidence that the incorporation of NO_2^- into SOM is a potentially important N sink in forest ecosystems receiving elevated inputs of atmospheric N is provided by results from our current experiment, as well as those of Dail *et al.* (2001) and Fitzhugh *et al.* (2003). The incorporation of NO_2^- into SOM is likely to be most significant in soils where the production of NH_4^+ exceeds the sinks for NH_4^+ of: (i) abiotic fixation and exchange reactions onto negatively charged surfaces of soil colloids, (ii) plant and microbial uptake, and (iii) leaching (i.e., sinks other than nitrification). Because heterotrophs and plants are generally believed to be superior competitors for available NH_4^+ than nitrifiers (Zak *et al.*, 1990), NO_2^- incorporation into SOM is most likely to be an important N sink in systems where the supply of NH_4^+ exceeds the uptake of NH_4^+ by heterotrophs and plants. As an example, along an N deposition gradient in Europe, gross nitrification was evident in two forest ecosystems characterized by N excess, but not in two N-limited systems (Tietema, 1998). Berntson & Aber (2000) suggested that relative to N-limited ecosystems, microbial immobilization becomes less significant and abiotic immobilization more important in systems characterized by N excess. In ecosystems where accumulation of N in soils results in greater NH_4^+ production than consumption by heterotrophs and plants, the incorporation of NO_2^- into SOM may constrain the generation and leaching of NO_3^-. However, the significance of NO_2^- incorporation into SOM as a mechanism for N retention will depend ultimately on how effectively it competes with other NO_2^- sinks in field soils (i.e., production of N gases, oxidation to NO_3^-, and dissimilatory reduction to NH_4^+).

Davidson *et al.* (2003) recently proposed a ferrous wheel hypothesis of N retention in temperate forest ecosystems, whereby iron (Fe) oxidation drives the reduction of NO_3^- to NO_2^-; NO_2^- in turn reacts with dissolved organic matter (DOM) to form dissolved organic nitrogen (DON), which is less mobile and more readily adsorbed in soils than NO_3^- and thus more easily retained within the ecosystem. As a complement to the ferrous wheel hypothesis, we suggest that nitrification is also a significant source for NO_2^- and our calculations demonstrate that the quantity of N flowing through the NO_2^- pool during nitrification is considerable. While Davidson *et al.* (2003) convincingly demonstrate that NO_2^- can react with DOM to form DON, we suggest that direct reactions between NO_2^- and solid-phase organic matter may also be important mechanisms of N retention in temperate forest ecosystems. Thus, there are multiple pathways that can produce NO_2^- and multiple reactions by which NO_2^- can react with organic matter to form stable organic N.

There are two ways in which our experimental design may have overestimated the importance of the incorporation of NO_2^- into SOM. First, the quantity of added NO_2^- was equivalent to 0.267 μmol ^{15}N (g dry soil^{-1}). Given a range in extractable NO_2^- concentrations from 0.0002 to 0.0008 μmol g^{-1} in soils of northern hardwood forests (Venterea *et al.*, 2003a), our addition of $^{15}NO_2^-$ caused a 300–1300-fold increase in the extractable NO_2^- pool. This substantial increase could overwhelm the biological capacity for oxidation to NO_3^- or reduction to NH_4^+ and/or enhance incorporation into SOM if the kinetics of this abiotic mechanism are substrate limited. Nelson & Bremner (1969) observed that increasing the amount of NO_2^- added to soils from 0.7 to 70 mmol g^{-1} did not affect the percentage recovery of N in SOM; note, however, that these authors added over three orders of magnitude more NO_2^- than was added in our study. Bancroft *et al.*

(1979) found that a NO_2^- addition of 0.07 μmol g^{-1} inhibited CO_2 evolution and O_2 utilization for 6 h but that an NO_2^- addition of 0.36 μmol g^{-1}, similar to the amount added in our experiment, stimulated CO_2 evolution after 1 day incubation. As the form of ^{15}N addition did not affect the mean headspace CO_2 concentration of live soils in the current experiment ($P = 0.87$), addition of NO_2^- did not appear to inhibit microbial activity. Second, if populations of NH_4^+- and NO_2^--oxidizing bacteria are found in close proximity in field soils, then it is possible that NO_2^- would be oxidized before becoming available for incorporation into SOM. However, Underhill & Prosser (1987) found evidence that NH_4^+- and NO_2^--oxidizing bacteria tend to grow on negatively and positively charged soil surfaces, respectively, reflecting the charge of the surfaces to which their primary substrate is adsorbed. Therefore, diffusion of NO_2^- over some distance is required before microbial utilization, allowing the opportunity for abiotic reactions with SOM to occur, despite low soil NO_2^- concentrations relative to NH_4^+ and NO_3^- (Venterea & Rolston, 2000a). Clearly, more research is needed to better understand the incorporation of NO_2^- in field environments.

Addition of the $^{15}NH_4^+$ label ranged from 18% to 30% of the extractable soil NH_4^+ pool in our soils and may have slightly enhanced microbial immobilization in live soils. Addition of the $^{15}NO_3^-$ label ranged from a 11-fold increase in the extractable soil NO_3^- pool under oak, which likely stimulated microbial NO_3^- immobilization to 26% of the extractable soil NO_3^- pool under one of the maple plots, which may have slightly stimulated microbial immobilization in live soils.

We observed negligible recovery of $^{15}NO_3^-$ in Hg-treated SOM, similar to the results of Dail *et al*. (2001). Berntson & Aber (2000) found that $^{15}NO_3^-$ added to O horizon soils from Harvard Forest was rapidly removed from solution and concluded that rapid immobilization of NO_3^- was an important mechanism of N retention in forest soils. Dail *et al*. (2001) found that between 30% and 55% of $^{15}NO_3^-$ added to Harvard Forest O horizon soils was recovered as DON within 15 min after addition. As our soils were extracted with KCl prior to the determination of soil ^{15}N recovery, DON was not included in our estimate of $^{15}NO_3^-$ immobilization, and therefore we cannot directly address the mechanism of NO_3^- incorporation into organic matter suggested by Dail *et al*. (2001).

Johnson *et al*. (2000) found that abiotic immobilization of NH_4^+ varied between 6% and 90% of total NH_4^+ immobilization. We observed similar results with $^{15}NH_4^+$ recovery in Hg-treated soils varying between 8% and 96% of the NH_4^+ immobilization in live soils. The percentage recovery of $^{15}NH_4^+$ in Hg-treated relative to live soils tended to exhibit an inverse relationship with time after addition, as NH_4^+ recoveries were similar between Hg-treated and live soils at 15 min, but recovery of $^{15}NH_4^+$ was lower in Hg-treated than live soils after 28 days. This pattern suggested a relatively slow, microbially mediated incorporation of NH_4^+ into SOM.

Tree species and the incorporation of ^{15}N into soils

The role of canopy tree species in influencing soil N dynamics has emerged as a focus of research on the biogeochemistry of forest ecosystems (Finzi *et al*., 1998; van Breemen & Finzi, 1998; Lovett & Rueth, 1999; Verchot *et al*., 2001; Venterea *et al*., 2003b; Lovett *et al*., in press). While oak and maple soils had similar extractable soil NH_4^+ concentrations, oak had significantly lower extractable soil ($NO_2^- + NO_3^-$) concentrations and net nitrification rates than maple (Fig. 2). As NO is a byproduct of nitrification, the significantly lower rates of NO production in oak than maple soils (Fig. 3) suggest that rates of gross nitrification were lower in oak than maple soils. Similarly, using isotope pool dilution, Verchot *et al*. (2001) observed significantly greater rates of gross nitrification in forest floor soils under maple than oak in the Catskills. Thus, NH_4^+ was produced readily in oak soils, but some mechanism(s) inhibited its oxidation to NO_3^-. While incorporation of NO_2^- into SOM is one mechanism that could decrease the production of NO_3^-, there was no statistically significant effect of tree species on the recovery of $^{15}NO_2^-$ within live or Hg-treated soils (Fig. 4). Based on the results from our experiment, we reject our hypothesis that incorporation of NO_2^- into SOM contributes to differences in net nitrification and extractable soil NO_3^- concentrations between oak and maple. Interestingly, NH_4^+ immobilization in live soils was significantly greater in oak than maple soils at 1 and 28 days (Fig. 4). As stated earlier, the small recoveries of NH_4^+ in Hg-treated soils suggest that NH_4^+ immobilization in live soils was dominantly microbially mediated. Greater microbial demand for NH_4^+ in oak than maple soils could contribute to differences in net nitrification between these species as the result of less intense competition for available NH_4^+ between heterotrophs and nitrifiers in maple soils. This greater demand for NH_4^+ in oak soils could be driven by labile C. The significantly greater concentrations of CO_2 in the headspace of oak than maple soils support this argument that high labile C in oak soils drives greater microbial NH_4^+ uptake (Fig. 1). However, using isotope pool dilution, Verchot *et al*. (2001) found that microbial uptake of NH_4^+ was not significantly different between maple and oak stands in O horizon soils from the

Catskill Mountains. In addition, a higher microbial demand for NH_4^+ might be expected to result in lower extractable NH_4^+ concentrations, but our results do not show significantly lower extractable NH_4^+ in oak soils compared with maple and beech (Fig 3a). Thus, the mechanisms producing lower nitrification rates in oak soils are still unclear.

Conclusions

We found that incorporation of NO_2^- into SOM occurred rapidly at the time scale of minutes via abiotic processes and that abiotic incorporation of NO_2^- into SOM was significantly greater than abiotic immobilization of NH_4^+ or NO_3^-. These results suggest that the role of NO_2^- in N retention should be considered in models of the forest N cycle. However, confirmation is first needed that NO_2^- produced by NH_4^+-oxidizing bacteria in the field undergoes the same fate as the $^{15}NO_2^-$ added to soil samples in our laboratory experiment. Field studies that examine competition for NO_2^- between abiotic immobilization and oxidation to NO_3^- would be helpful for the inclusion of NO_2^- immobilization in models. Abiotic NO_2^- immobilization did not appear to contribute to differences in NO_3^- production among species. The roles of labile C and microbial demand for NH_4^+ in differences in nitrification among species should be explored in future experiments.

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