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Rapid abiotic transformation of nitrate in an acid forest soil

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Abstract. Nitrate immobilization into organic matter is thought to require catalysis by the enzymes of soil microorganisms. However, recent studies suggest that nitrate added to soil is immobilized rapidly and this process may include abiotic pathways. We amended living and sterilized soil with ^{15}N-labeled nitrate and nitrite to investigate biotic and abiotic immobilization. We report rapid transformation of nitrate in incubations of the O layer of forest soils that have been sterilized to prevent microbial activity and to denature microbial enzymes. Approximately 30, 40, and 60% of the ^{15}N-labeled nitrate added to live, irradiated, or autoclaved organic horizon soil disappeared from the extractable inorganic-N pool in less than 15 minutes. About 5% or less of the nitrate was recovered as insoluble organic N in live and sterilized soil, and the remainder was determined to be soluble organic N. Added ^{15}N-nitrite, however, was either lost to gaseous N or incorporated into an insoluble organic N form in both live and sterile organic soils. Hence, the fate and pathway of apparent abiotic nitrate immobilization differs from the better-known mechanisms of nitrite reactions with soil organic matter. Nitrate and nitrite added to live A-horizon soil was largely recovered in the form added, suggesting that rapid conversion of nitrate to soluble organic-N may be limited to C-rich organic horizons. The processes by which this temperate forest soil transforms added nitrate to soluble organic-N cannot be explained by established mechanisms, but appears to be due to abiotic processes in the organic horizon.

Introduction

In northeastern North America and throughout much of Europe, the input of nitrate to forest ecosystems by atmospheric deposition has increased as much as 5-to-20 fold due to fossil fuel burning (Schlesinger & Hartley 1992; Galloway et al. 1995). Initially, this enhanced N input may stimulate forest productivity. It appears that enhanced N input resulted in a modest terrestrial

sink of atmospheric carbon (Houghton et al. 1998, Nadelhoffer et al. 1999a), apparently because most of the N from atmospheric deposition remains in the soil rather than being taken up by plants (Nadelhoffer et al. 1999a, b). Ultimately, if N inputs exceed demand, limitations on the availability of other nutrients and cation leaching may result leading to forest decline. When nitrate inputs exceed the soil nitrate immobilization potential, a state of N-saturation is said to exist (Aber et al. 1989, 1998; Ågren & Bosatta 1998). Because nitrate immobilization is believed to be mediated biologically, N-saturation has been related to nitrate inputs, successional status of the vegetation, season, temperature, and availability of other nutrients (Aber et al. 1998). Our ability to model and to predict the consequences of atmospheric N deposition depends upon our understanding of the mechanisms of N cycling within forest ecosystems, including transformations of nitrate.

In most aggrading temperate and boreal forests, little nitrate is present in soils because N is conservatively and efficiently cycled within these ecosystems (Vitousek & Reiners 1975), and because gross rates of nitrate production via nitrification are generally matched by equal rates of plant uptake and microbial immobilization (Davidson et al. 1992; Stark & Hart 1997). Exogenous sources of nitrate appear to be retained primarily in organic forms. A ^{15}N labeling study at the Harvard Forest in central Massachusetts showed that only 4–32% of the applied nitrate label was recovered in tree biomass whereas 51–79% of the nitrate label was found in soil pools at the end of a single growing season (Nadelhoffer et al. 1999b). A similar result was found in an analysis of several other ^{15}N-labeling studies in New England and Europe (Tietema 1998; Nadelhoffer et al. 1999a). Furthermore, 10 years of fertilizer addition to plots within the Harvard forest at the rate of 150 kg ha^{-1} y^{-1} have suggested that this mid-successional mixed hardwood forest is fairly resilient to large increases in N availability, immobilizing $>$ 70% of the added nitrogen with at least half of the N retained in the forest floor (Magill et al. 1997; Magill et al. 2000). However, CO_2 respired as a consequence of organic carbon mineralization, often presumed to be necessary to fuel microbial N immobilization (Schimel 1988), has not been detected following N additions (Aber et al. 1998).

Soil microorganisms have been shown to immobilize nitrate in temperate forest soils (Schimel & Firestone 1989; Davidson et al. 1992; Stark & Hart 1997; Tietema & Wessel 1992; Zak et al. 1990), and were the presumed agents of nitrate uptake observed in New England forests (Nadelhoffer et al. 1995). However, recent studies of ^{15}N-nitrate added to incubations of Harvard Forest soils have revealed surprisingly rapid immobilization of the label – i.e., disappearance of 34–62% of ^{15}N-nitrate within 15 minutes of adding the label (Berntson & Aber 2000). The degree to which microbially – mediated

processes account for such a large and rapid decline in extractable nitrate is unclear because biotic and abiotic processes could not be assessed independently in these live soil incubations. Rapid nitrate immobilization coupled with the observation that fertilizer-N is immobilized without concomitant production of CO_2 has fueled speculation about abiotic and alternative biotic pathways leading to nitrate immobilization (Aber et al. 1998).

Abiotic reactions of nitrite with soil organic matter (SOM) have been demonstrated to form organically-bound N (Smith & Chalk 1980; Azhar et al. 1986; Thorn & Mikita 2000). It is possible that partial reduction of nitrate to nitrite by biological respiration or by reduced metals could be followed by abiological reactions of nitrite with SOM. Although there are no known pathways by which nitrate reacts directly with soil organic matter in abiotic reactions that result in immobilization, unknown chemical reactions in the complex matrix of soil cannot be ruled out.

We incubated O ($O_{e/a}$) and A-horizon soils from a hardwood stand at the Harvard Forest, Massachusetts, with ^{15}N as either nitrate (NO_3^-) or nitrite (NO_2^-) to measure N-immobilization in both sterilized and live soils. The objectives of the study were (i) to investigate the potential for nitrate and nitrite immobilization; (ii) to distinguish between biotic and abiotic immobilization; and (iii) to identify fates of immobilized N.

Materials and methods

Soil collection, characterization and sterilization

The experiment was conducted on O and A-horizon soils collected from the Harvard Forest, Petersham, MA. Soils were collected from a mixed hardwood stand that has been exposed to ambient levels of wet N deposition estimated to be 0.9 g N m^{-2} yr^{-1} with more than 50% as nitrate (Ollinger et al. 1993; Currie et al. 1996). Soils were sieved (< 2.0 mm), with roots removed by hand and then stored field moist at 4 °C prior to analysis. Organic C content was 250 g kg^{-1} for the O and 48 g kg^{-1} for the A-horizon. We estimated soil organic-N by submitting whole soil to combustion CHN analysis and subtracting inorganic-N from this value. Inorganic-N (nitrate, nitrite and ammonium) was determined colorimetrically using a 10:1 ratio of 0.5 m K_2SO_4 to dry soil extraction at 4 °C for 40 min.

Sterilization of soil was achieved either by autoclaving (2 times, 3d and 1d prior to ^{15}N amendment; 121 °C for 0.5 h) or by γ-irradiation (final dosage 4 Mrad) of separate soil samples. A comparison of the two sterilization methods was performed because neither is free of artifactual effects on soil properties (Ramsey & Bawden 1983; Lotrario et al. 1995). Irradiation at doses higher

than those used in this study have been shown to produce nitrite, and this sterilization method does not completely inhibit extracellular enzymatic activity (Cawse & Crawford 1967). Autoclaving increases soluble C and N fractions and also denatures intracellular and extracellular enzymes. The effects that each technique had on selected soil properties measured in the present study are reported in Table 1. Maintenance of sterility over the course of incubation was confirmed by elimination of CO_2 production and negative standard plate counts (i.e., no colony forming units) for soil dilutions (10^{-1} to 10^{-6}), plated on multiple nutrient media. Soluble soil C (DOC) was measured on acidified 10:1 water to soil extracts using a Shimadzu Model 5000A Total Organic C Analyzer.

Inorganic ^{15}N amendments

Initial soil nitrate and nitrite contents were below our limits of detection (using 10:1 extracts; limits of 0.4 and 0.2 μg N per gram soil, respectively). We added filter sterilized ^{15}N (99% atom enriched) nitrate or nitrite at the rate of 5 μg N per gram soil dry mass, dissolved in a solution that brought the soil to 50% of water holding capacity as determined on non-sterilized soils. Soil and label were mixed aseptically with a sterile glass rod, which was then enclosed with the soil in a 350 mL incubation jar. Each combination of soil treatment ('live' control, irradiated sterile, and autoclaved sterile), and N amendment (^{15}N-nitrate, ^{15}N-nitrite) was replicated three times. Jars were incubated for 24 h at 20 ° C. Sub-samples were taken immediately (< 5 min) and 24 h after ^{15}N addition for N pool fractionation.

At each sampling time a 4 g sub-sample of soil from each replicate was removed to a 50 mL centrifuge tube, closed and placed upright in a bath of 90% ethanol and dry ice. This freezing bath ceased microbiological activity and prepared samples for freeze drying in preparation for mass spectrometry. A second 4.0 g soil sub-sample was added to a 50 mL centrifuge tube containing 20 mL cold (4 °C) 0.5 m K_2SO_4 and shaken vigorously for 20 min at the refrigerated temperature. The suspension was centrifuged (> 25,000 g) to sediment soil and microorganisms into a pellet. The supernatant solution was removed by aspiration and then filtered (0.45 μm pore size). A second extraction of the soil pellet was performed as above. The soil light fraction (that material not pelleted under centrifugation) was returned to the pellet by removing it from the surface of the filter. This pellet was then freeze-dried and prepared for mass spectrometry, the resultant ^{15}N referred to hereafter as insoluble-N, as the K_2SO_4 extraction process did not remove it from the soil. For each replicate the two filtered extracts were combined (to achieve a final extractant to soil ratio of 10:1) and NO_3^-, NO_2^-, and NH_4^+ were determined colorimetrically < 1 d after extraction.

A 1.0 g soil sub-sample was dispersed in 0.2% sodium pyrophosphate solution (pH 6.0) and serially diluted to perform viable organism plate counts. Soil samples removed at the beginning and end of the 24 h incubation period were serially diluted to 10^{-1}, 10^{-3} and 10^{-6} dilutions and these dilutions were used to inoculate reduced-strength trypticase soy agar (TSA) and soil isolation agar culture plates (Martin 1975; Trolldenier 1996). Comparison of plate counts among the pretreatments is reported in Table 1.

Given a maximum time for removal, weighing, extraction, peroxide treatment, or freezing, no 'initial' soil sample was incubated with label for more than 15 minutes.

^{15}N pool enrichment determination

Total ^{15}N recovery after incubation was determined by submitting freeze-dried soil samples directly to isotope ratio mass spectrometry analysis. An ammonia diffusion method was used to trap inorganic-N on acidified filter disks to prepare them for isotope ratio mass spectrometry (Stark & Hart 1996). K_2SO_4 extracts were diffused sequentially to obtain NH_4^+ and then NO_2^-/NO_3^- on acidified disks using a modified (Sigman et al. 1997) procedure of Sørensen and Jensen (1991). ^{15}N standards and blanks were prepared and isotope diffusion bias correction calculations were performed as outlined in Stark and Hart (1996) for both inorganic-N samples. Soil N pool enrichment is reported as a percent of initial label recovered among the various N forms: total ^{15}N recovered, nitrate plus nitrite, ammonium and organic-N.

During the course of analysis it became evident that for samples incubated with ^{15}N as nitrate, the ^{15}N in inorganic pools (NO_2^-/NO_3^- and NH_4^+; pools **c** and **d** in Figure 1), and insoluble N in the pellet (pool **b**, Figure 1) did not sum to that ^{15}N recovered in the non-extracted soil (pool **a**, Figure 1), e.g., **a** > **b+c+d**. This was not the case for ^{15}N-nitrite incubated soils, where **a = b+c+d**. Mass balance calculations indicated that 30 to 60% of N was not accounted for in samples incubated with $^{15}NO_3^-$. The only non-measured soil pool of N was the organic N removed by the K_2SO_4 extraction, which we refer to as dissolved organic N (DON). We then utilized archived samples to measure the $DO^{15}N$ pool directly, which greatly improved the mass balance and confirmed the fate of $^{15}NO_3^-$. Samples were extracted with 0.5 m K_2SO_4 as before and the K_2SO_4 extracts were analyzed for NO_2^-/NO_3^-, NH_4^+, concentrations and ^{15}N enrichment as described above (Figure 1). Pool sizes and ^{15}N enrichment of these new extracts were compared to those of previous analyses of fresh soil and extracts in order to account for any changes brought about by freeze-drying and storage of samples. No differences due to freeze-drying or storage were detected. Dissolved organic nitrogen in K_2SO_4 soil extracts was measured by the persulfate oxidation method (D'Elia et al.

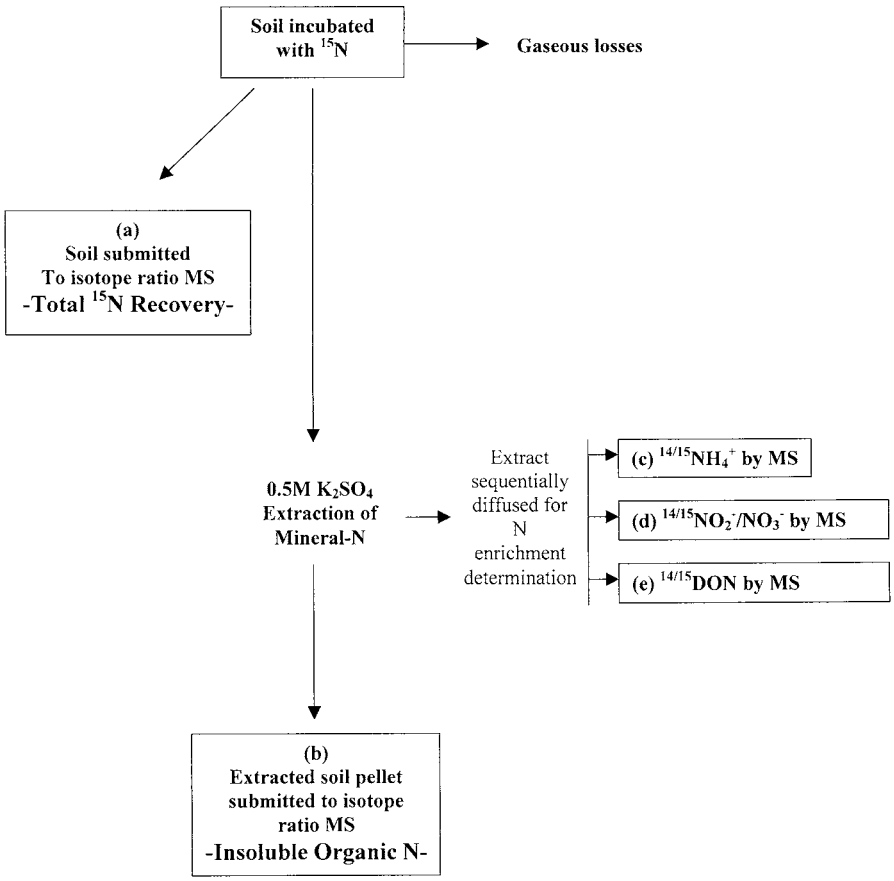

Figure 1. Procedure for determination of ^{15}N enrichment of soil N pools.

1976; Ameel et al. 1993). After oxidation of the DON and dissolved NH_4^+ to NO_3^-, we diffused this solution to acidified disks to determine directly the enrichment of the DON pool by mass spectrometry (MS) (pool **e**, Figure 1).

Results

Effects of sterilization treatment on soils

Autoclaving, and to a lesser degree γ-irradiation, increased the soluble or K_2SO_4-extractable amounts of organic-C and ammonium in both O and A soils (Table 1). Soluble organic carbon in the O soil, reported as DOC, increased from $<$ 0.2 g C kg^{-1} soil in control soil to 1.5 g C kg^{-1}in irra-

Table 1. Soil properties and effects of sterilization techniques on soil chemistry

Treatment	pH	DOC$^{\alpha}$ (g/kg)	NO_3^- $^{\beta}$ (mg/kg)	NH_4^+ (mg/kg)	Organic N (mg/kg)	Plate counts$^{\delta}$ CFU/g
O-horizon						
Control	3.9	0.16 (0.02)	nd	16 (0.37)	10,800 (415)	3.2×10^5
Irradiated	4.0	1.58 (0.02)	nd	95 (5.14)	11, 400 (274)	ng
Autoclaved	3.8	5.38 (0.45)	nd	119 (6.67)	12,000 (1013)	ng
A-horizon						
Control	4.3	0.06 (0.01)	nd$^+$	1.41 (0.64)	2,400 (88)	1.1×10^5
Irradiated	5.0	0.89 (0.01)	nd	18.07 (0.14)	2,400 (50)	ng
Autoclaved	5.0	0.96 (0.04)	nd	43.17 (0.94)	2,460 (149)	ng

$^{\alpha}$Water extractable organic carbon.
$^{\beta}NO_3^-$ = (NO_3^- + NO_2^-); nd = not detected, limits of detection 0.04 mg N/L in a 10:1 solution-to-soil 0.5M K_2SO_4 extraction.
$^{\delta}10^{-1}$ and 10^{-6} serial dilutions of 10:1 water-to-soil slurries plated on TSA and Soil Isolation Agar and incubated for 5 d at 20 °C. CFU = Colony Forming Units at day 5 on a per g soil dry mass basis average calculated from plates with 30–300 cfu's; ng = no growth observed.

diated and > 5 g C kg^{-1} in autoclaved soils. Nitrate and nitrite remained undetectable (prior to ^{15}N addition). An increase in soluble ammonium was observed, presumably from physico-chemical mineralization of the organic-N pool. The pH of A-horizon soil was slightly increased in irradiated soil and autoclaved soil; no differences were detected in O soil (Table 1).

Fate of inorganic ^{15}N added to soil

A-horizon soil. Nearly 100% of ^{15}N added as either NO_2^- or NO_3^- to live control A-horizon soil was recovered in the form added immediately after addition (Table 2). In contrast, immediate losses of added $^{15}NO_2^-$ were observed in the autoclaved and irradiated A-horizon soil. Losses of $^{15}NO_2^-$increased during the 24-hour incubation in all three pretreatments (Table 2), and this was attributed to gaseous loss as the ^{15}N was not recovered in whole soil submitted to isotope ratio MS. Losses could have been due to microbial or abiotic reduction of NO_2^- to N gases (Firestone & Davidson 1989), which were not measured in this study.

The recovery of $^{15}NO_3^-$ also declined somewhat over 24 hours in both control and autoclaved A-horizon soil, but the difference between control and sterile soils could not be evaluated statistically due to a loss of two of three replicates (Table 2). About 12% of added nitrate was recovered as organic-N in control soils. Recovery of ^{15}N, added as either NO_2^- or NO_3^-, in organic-N

Table 2. Fate of ^{15}N-nitrate, nitrite in A-horizon soil: % ^{15}N recovered in the form added immediately after addition and after 24 h

Soil pretreatment	$^{15}NO_2^-$ amended		$^{15}NO_3^-$ amended	
	t = 15 min.	t = 24 h	t = 15 min.	tv = 24 h
Control	92.4 ± 4.0	22.7 ± 1.9	101.6 ± 16.0	82.2 ± ND
Irradiated	40.5 ± 11.5	7.4 ± 0.6	79.3 ± 7.0	75.8 ± 2.2
Autoclaved	14.6 ± 9.9	3.9 ± 1.7	90.1 ± 16.0	64.4 ± 12.0

% Recovery of ^{15}N reported as a mean of 3 samples ± SD.
ND = SD not determined; 1 of 3 samples was determined.

or NH_4^+ pools of A-horizon soil was less than 2% in irradiated and autoclaved soils (data not shown).

O-horizon soil: nitrite addition. Total recoveries of 73% to 85% of added NO_2^- were observed in O-horizon control soils (Figure 2). Almost half (40 to 48%) of the ^{15}N was recovered in the extracted pellet, suggesting substantial interaction between $^{15}NO_2^-$ and soil organic matter producing an insoluble organic-N product (Figure 2). The immobilization of NO_2^- in O-horizon soil occurred within minutes of addition to both sterile and live samples, and no significant changes occurred over the course of the live soil incubation (Figure 2(a)). Up to 2.5% of ^{15}N-NO_2^- was recovered as NH_4^+ in live soil and less in sterilized soil (Figure 2).

Total recovery of ^{15}N in NO_2^--amended irradiated and autoclaved O-horizon soil was $< 50\%$ and $< 25\%$, respectively (Figure 2(b), (c)). The reduced recovery (as compared to live soil) was detectable in samples taken immediately after $^{15}NO_2^-$ addition. In contrast to $^{15}NO_2^-$ amended A-horizon soils, recovery in the O horizon did not decrease significantly during the 24h incubation. As was the case for the A-horizon soils, the rapid disappearance of NO_2^- added to O horizon soil is attributed to gaseous loss. Greater than half of the recoverable $^{15}NO_2^-$ in sterilized soil was in the insoluble organic pool of the extracted pellet, and the remaining ^{15}N was recovered largely as nitrite (Figures 2(b), 2(c)). The sum of ^{15}N recovered as insoluble organic-N plus that remaining in the NO_2^- pool was roughly equal to that recovered when the non-extracted whole soil was submitted directly for ^{15}N analysis (Figure 2).

O-horizon: nitrate addition. Recovery of 100–120% of NO_3^- immediately after addition to organic horizon soil was calculated for all treatments when non-extracted soils were submitted directly to isotope ratio mass spectrometry (Figure 3). Recoveries $> 100\%$ could be due to analytical errors or to an actual amendment rate of NO_3^- somewhat greater than the target of 5

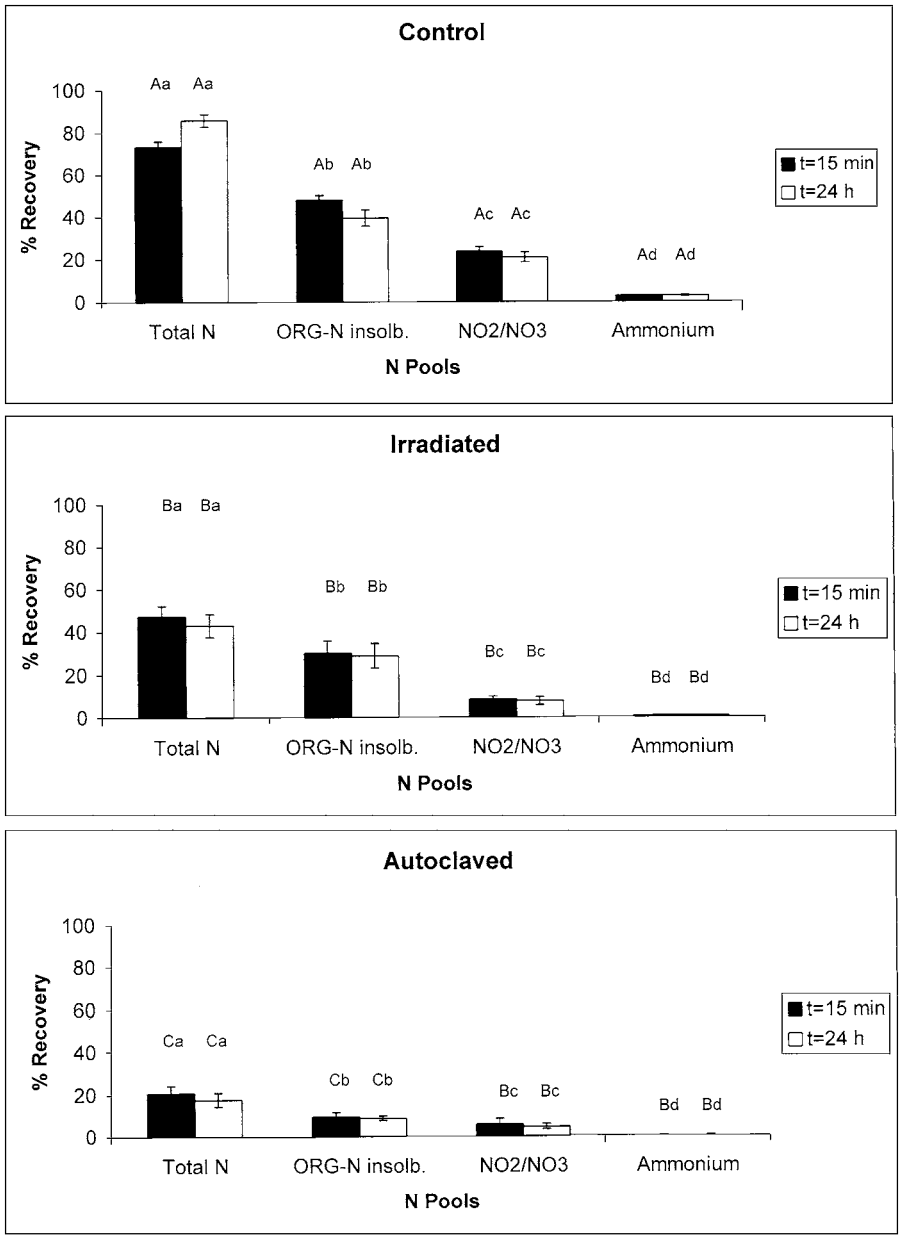

Figure 2. Percent recovery of ^{15}N in O-horizon soil N pools reported for (a) control, (b) gamma irradiated and (c) autoclaved soils amended with $^{15}NO_2^-$ at the rate of 5 μg ^{15}N per gram soil dry mass (n = 3; $\pm$ SD). Total N = soil ^{15}N recovered in direct mass spec analysis of non-extracted soil. Org-N Insolb. = ^{15}N remaining in soil pellet after K_2SO_4 extraction. Columns with different upper case letters indicate significant difference within-pool, between soil treatments at $\alpha = 0.05$ level. Columns with different lower case letters indicate significant difference between soil N pools within a soil treatment at $\alpha = 0.05$ level.

μg N g^{-1}. Less than 1% of ^{15}N was recovered as NH_4^+. About 60% of the added $^{15}NO_3^-$ was recovered as NO_3^- with no significant change from initial to final sampling for all soil pretreatments. About 5–8% of the label was recovered as insoluble organic-N in the extracted pellet. Greater than 80% of the remaining ^{15}N was recovered as soluble organic N (DON) in persulfate digests of K_2SO_4 extracts of the O horizon soils. Roughly 30, 40 and 55% of added $^{15}NO_3^-$ to control, irradiated and autoclaved soils was recovered in this DON fraction.

Discussion

Recent reports of an appreciable capacity for nitrate immobilization in forest soils subjected to high NO_3^- deposition and excess N by experimental fertilization are difficult to explain solely by biological mechanisms (Aber et al. 1998; Magill et al. 1997; Berntson & Aber 2000). In order to examine microbial and abiotic N immobilization, we chose two independent methods to sterilize soils. Although NO_3^- is the form of N in atmospheric deposition that we are studying, we also included studies of NO_2^- immobilization to see if NO_3^- and NO_2^- have similar fates. Similar fates might indicate that biological reduction of NO_3^- to NO_2^- is followed by subsequent abiotic reactions of NO_2^- with soil organic matter (Azhar et al. 1986).

Fate of ^{15}N-nitrite added to soil

We observed a high potential for reaction of NO_2^- with viable O and A horizon soils. Up to 77% of $^{15}NO_2^-$ added to A-horizon soil was lost, presumably to gaseous-N, within 24 h of addition. Gaseous loss of N was exacerbated by sterilization; both irradiation and autoclaving of soils prior to NO_2^- addition increased N loss markedly. Rapid production of large pulses of NO and N_2O have been observed when nitrite was added to sterilized soils (Davidson 1992), and spontaneous self-decomposition or dismutation of nitrous acid (HNO_2^-) to NO and/or NO_2 has been reported for acid soils with pH values similar to those used in this study (Chalk & Smith 1983, Schwartz & White 1983). Of the label that was not lost to gaseous processes, nearly all was recovered as NO_2^- in A horizon soils (Table 2), but up to 50% was recovered as insoluble organic-N immediately upon addition to O-horizon soils (Figure 2). Hence, reaction with organic matter appears to compete well with gaseous loss processes in soil with higher organic matter. These findings are consistent with investigations showing increased NO_2^- fixation capacity in soils with high organic matter content and pH less than 5 (Nelson & Bremner 1969, 1970).

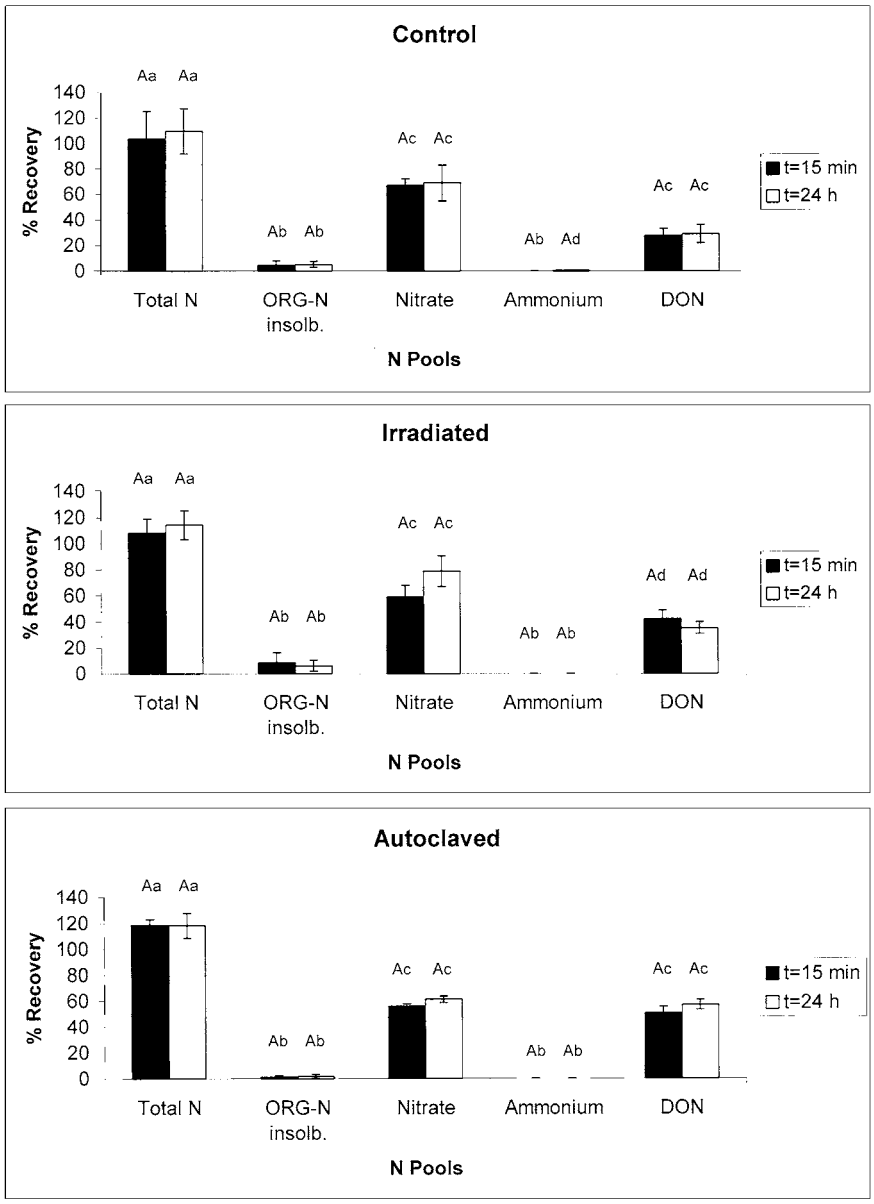

Figure 3. Percent recovery of ^{15}N in O-horizon soil N pools reported for (a) control, (b) gamma irradiated and (c) autoclaved soils amended with $^{15}NO_3^-$ at the rate of 5 μg ^{15}N per gram soil dry mass (n = 3; $\pm$ SD). Total N = soil ^{15}N recovered in direct mass spec analysis of non-extracted soil. Org-N Insolb. = ^{15}N remaining in soil pellet after K_2SO_4 extraction; DON = ^{15}N recovered after persulfate oxidation of K_2SO_4 extracts. Columns with different upper case letters indicate significant difference within pool, between soil treatments at α = 0.05 level. Columns with different lower case letters indicate significant.

The rapidity of insoluble organic-^{15}N formation in both control and sterilized O-horizon soil indicates predominately abiotic nitrite immobilization. Furthermore, while overall recovery of $^{15}NO_2^-$ in O-horizon soil was drastically affected by sterilization, the *relative* recovery of ^{15}N in the insoluble organic-N pool as compared to other N-pools was constant across all treatments: about half of total ^{15}N recovered. In other words, the total ^{15}N recovered ('a' in Figure 1) was accounted for mostly in the insoluble organic N pool (fraction 'b') and the remainder in the soluble inorganic fractions NH_4^+ and NO_2^-/NO_3^- (fractions 'c' and 'd'). Thus for $^{15}NO_2^-$-amended O-horizon soil: **a = b+c+d**, regardless of initial gaseous losses (cf. Figures 1, 2).

Fate of ^{15}N-nitrate added to soil

The amount of $^{15}NO_3^-$ remaining in O-horizon soil as solid phase organic-N in the extracted soil pellet was much smaller than that observed for $^{15}NO_2^-$ amended soil. Immobilization of $^{15}NO_3^-$ into insoluble organic matter after 24 hr incubation was less than 6% of added ^{15}N in the control O soils (Figure 3). This result contrasts to reports of detectable NO_3^- immobilization during 24-hour incubation of other forest soils (Davidson et al. 1992, 1991; Stark & Hart 1997; Tietema & Wessel 1992; Zak et al. 1990). In many of those studies, initial (15 minute) NO_3^- pool sizes and enrichments were determined to correct for small initial losses that would cause errors in the estimates of gross immobilization during 24 hour incubations (Davidson et al. 1991). In the Harvard Forest soils, however, 30–60% of the added $^{15}NO_3^-$ disappeared from the extractable inorganic-N pool within 15 minutes in this study and did not appear in the solid phase organic-N. Similar rapid losses of $^{15}NO_3^-$ were observed by Berntson & Aber (2000), indicating that a different process of $^{15}NO_3^-$ immobilization is at work at the Harvard Forest compared to other studies. In this case, the initial rapid loss of $^{15}NO_3^-$ is the *signal* of this important immobilization process rather than the '*noise*' that was corrected for in previous studies.

Our results reveal that a K_2SO_4-soluble, organic-N product was the dominant fate of the lost $^{15}NO_3^-$. Including DON resulted in good mass balance of ^{15}N recovery (**a = b+c+d+e**, cf. Figures 1 and 3). The amount of $^{15}NO_3^-$ that moved into a soluble organic-N pool immediately after addition to soil was 28, 43 and 50% of ^{15}N added to control, irradiated and autoclaved soils, respectively (Figure 3). The amount of ^{15}N recovered as DON in live, irradiated, and autoclaved soil is the same order of ranking of DOC concentrations: 0.2, 1.6, and 5.4 g kg^{-1}, respectively (Table 1). Although sterilization methods may create important artifacts by increasing DOC concentrations, this variability provides a clue about the mechanism of rapid immobilization of NO_3^-; the formation of this DON is correlated with the presence of

DOC. This relationship may also explain why the conversion of NO_3^- to DON appears to be more important in organic horizons than in mineral horizons.

Implications for Ecosystem processes of N immobilization

The incorporation of NO_2^- into an insoluble, organic component and the fact that NO_3^- was immobilized into DON suggests that, under the experimental conditions and concentrations, the pathways of immobilization of these two nitrogen oxides were different. When NO_2^- is added to acid soil in a relatively concentrated aliquot, either NO production or organic-N formation can result (Blackmer & Cerrato 1986; Chalk & Smith 1983; Davidson 1992; Nelson 1982; Nelson & Bremner 1969; Thorn & Mikita 2000). In contrast, abiotic reduction of NO_3^- to NO_2^- by reduced metals in the soil might lead to lower localized concentrations of NO_2^- which could promote its binding to soluble organics (Azhar et al. 1986). Indeed, DOC extracted from the O horizon of Harvard Forest has been shown to promote the reductive dissolution of Fe(III) oxyhydroxide (abiotically) at pH 4 (Chorover & Amistadi, 2001). Products of this reaction include Fe(II), which could then catalyze the reduction of nitrate to more reactive species. The quantity and quality of DOC, the availability of reducing agents, and the concentration of NO_3^- may thus affect the transformation of NO_3^- into DON or reactive N intermediates, but the precise reactions and the mechanisms remain unknown.

A significant, rapid, soluble-organic fate for nitrate in acid forest soils could have important implications under enhanced NO_3^- deposition. In this laboratory study, about one-third of the nitrate applied to live O-horizon material was immediately converted to DON. In field studies, DON has been shown to be the major component of N exported from a number of forested watersheds (Chorover et al. 1994; Hedin et al. 1995) including the Harvard Forest (Currie et al. 1996). DON leaching from superficial organic horizon soils has been positively correlated to inorganic-N amendments at the Harvard Forest site (Currie et al. 1996). Upon addition of nitrate, the microbially-mediated immobilization of N into organic matter may be largely bypassed, undermining the utility of traditional microbial immobilization-remobilization and turnover models to estimate the fate and residence time of N in this forest ecosystem. DON that leaches from the organic horizon to mineral horizons may be largely stabilized there, leading to accumulation of organic-N in soil, perhaps making it less available to plants than the N that cycles in microbial mineralization-immobilization pathways. More study is clearly now needed on identifying the reactions by which NO_3^- could be converted, apparently abiotically, to DON.

Acknowledgements

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