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Forest Ecology and Management 196 (2004) 129–142
Forest Ecology and Management
www.elsevier.com/locate/foreco

Gross nitrogen process rates in temperate forest soils exhibiting symptoms of nitrogen saturation

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Abstract

In order to examine how fundamental soil N cycling processes are affected by elevated N inputs to temperate forest ecosystems, we made concurrent laboratory measurements of gross rates of nitrogen (N) mineralization, nitrification, nitrate (NO_3^-) consumption, ammonium (NH_4^+) immobilization, nitrous oxide (N_2O) and nitric oxide (NO) production, and NO consumption in soils from the Harvard Forest Chronic N Amendment Study. Gross nitrification rates varied with N addition rate in a manner that was entirely consistent with patterns of NO_3^- leaching and NO emissions that have been previously observed in field studies. Gross nitrification was elevated above controls in soils from a pine stand receiving 5 and 15 g N m^{-2} per year, and in soils from a hardwood stand receiving 15 g N m^{-2} per year. Gross nitrification tended to increase with decreasing soil pH, suggesting that acid-tolerant nitrifying bacteria predominate in these soils. Different patterns of inorganic N consumption in the two stands may provide some clues to understanding the more rapid onset of N saturation that has been historically observed in the pine stand. Absolute rates of NH_4^+ immobilization, and rates of NO_3^- consumption per unit of available NO_3^-, each tended to decrease with increasing N addition in the hardwood stand, but did not vary significantly in the pine stand. Gross NO production rates increased in a manner that was consistent with nitrification rate increases, and represented up to 19% of gross nitrification. Production of N_2O was generally $\leq$15% of NO production and <1% of gross nitrification. Consumption of NO represented $\geq$96% of gross NO production and may have contributed up to 25% of total NO_3^- production.

Keywords: Nitrogen deposition; Nitrification; N trace gas emissions; Soil acidity

1. Introduction

Many forests in Europe and North America receive elevated levels of atmospheric nitrogen (N) deposition deriving from fossil fuel combustion, fertilizer application, and activities associated with animal agricultural (Galloway et al., 1995; Skiba et al., 1999). Temperate forests that receive persistent N inputs may undergo a sequence of responses leading to ''N saturation'' (Aber et al., 1998). A critical feature of N saturated ecosystems is increased export of N in various forms. The Chronic N Amendment Study at Harvard Forest (HF) in Massachusetts, USA is one of

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doi:10.1016/j.foreco.2004.03.016

the most intensive studies of N deposition in temperate forests. Since 1988, a variety of plant, soil, and ecosystem responses to N additions have been monitored in two adjacent stands comprised of mixed hardwood and red pine, respectively (Magill et al., 2000, 2004).

Leaching of nitrate (NO_3^-) has been a key response variable in the Chronic N Amendment Study and other similar studies (e.g., Wright and Rasmussen, 1998). Tietema (1998) found that soils from two of three coniferous forests in Europe exhibiting increased NO_3^- export had higher gross nitrification rates compared to N-limited forests, and that gross NO_3^- immobilization rates were not detectable in any of the soils examined. While net nitrification rates have increased at HF (Magill et al., 2000; Venterea et al., 2003), it is not clear to what extent this trend reflects increased gross nitrification, decreased gross NO_3^- consumption, or a combination of both. It is also not clear if changes in gross N mineralization and/or ammonium (NH_4^+) immobilization may be affecting the availability of NH_4^+ to nitrifying organisms and thereby influencing nitrification.

Increased N oxide gas emissions from soils have also been proposed as an important response in forests undergoing N saturation (Aber et al., 1988). Recent findings have shown that nitric oxide (NO) emission rates have increased and represent up to 8% of N inputs in the Chronic N Study at HF (Venterea et al., 2003). Nitric oxide is highly reactive once emitted to the atmosphere, acting as a precursor to nitrogen dioxide and nitric acid, and also playing a central role in regulating tropospheric ozone (O_3) (Crutzen, 1979). Soil NO emissions can significantly influence local O_3 levels (Stohl et al., 1996), and therefore may contribute to the negative effects of O_3 on proximal forest vegetation (Reich et al., 1983). Much less is known about NO transformation in the soil compared to its reactivity in the atmosphere (Conrad, 1995). Recent experiments with agricultural soils have indicated that high rates of NO production and subsequent subsurface consumption may affect NO_3^- production and distribution in the soil profile (Venterea and Rolston, 2002). Emissions of nitrous oxide (N_2O), an important greenhouse gas and regulator of stratospheric O_3, have also shown some response to experimental N additions at HF and other forests (Venterea et al., 2003; Klemedtsson et al., 1997).

The objective of the current study was to improve our understanding of fundamental N transformation processes occurring in soils at the Chronic N Amendment Study. We made concurrent measurements of gross rates of N mineralization, nitrification, inorganic N consumption, and N oxide gas production and consumption in soils from organic and mineral horizons. We also conducted field experiments and modeling exercises to examine the potential importance of NO production and consumption in regulating soil NO_3^- production.

2. Methods

2.1. Site description

Experiments were conducted at the Chronic N Amendment Study at HF in central Massachusetts, USA (42°30′N, 72°10′W), which is part of the National Science Foundation Long-term Ecological Research network. Since 1988, two adjacent forested areas have been amended with ammonium nitrate (NH_4NO_3) to simulate atmospheric N deposition in excess of background wet plus dry deposition of ~0.8 g N m^{-2} per year. The current measurements were made in three of the four treatment plots within each of the hardwood and pine stands, i.e.: (i) control plots (no N additions), (ii) low-N plots (+5 g N m^{-2} per year), and (iii) high-N plots (+15 g N m^{-2} per year). Additional site and experimental details are presented in Magill et al. (2000, 2004).

2.2. Soil sampling

Soil samples were collected on 31 May and 1 June 2001 from three interior sub-plots within each of the control, low-N, and high-N plots of each stand. Prior to sampling, the upper layer (O_i horizon, approximately 5–20 mm thick) of the forest floor was removed from the sampling area. A section of polyvinyl-chloride (PVC) plastic pipe (50 mm ID × 200 mm long) was inserted into the soil to a depth of 150 mm. Each core sample was separated by organic ($O_e + O_a$) and mineral horizons, and delivered to the laboratory for processing within 1–3 days of collection. All samples were sieved (6 mm), homogenized by manual shaking in polyethylene bags, and

stored in sealed, inflated bags at room temperature for approximately 24 h prior to testing. Samples were dried at 105 °C (mineral horizon soil) or 65 °C (organic horizon soil) for determination of gravimetric soil-water content.

2.3. Nitrification, N mineralization and substrate consumption

Each homogenized sample was divided into two sub-samples (60–100 g each). One sub-sample was amended with 2 ml of ammonium sulfate solution (45 μg N ml^{-1}) enriched with 99% ^{15}N (Isotec,[1] Miamisburg, OH) for determination of gross rates of N mineralization and NH_4^+ consumption, and the other sub-sample was amended with 2 ml of potassium nitrate solution (45 μg N ml^{-1}) enriched with 99% ^{15}N (Isotec) for determination of gross rates of nitrification and NO_3^- consumption using ^{15}N dilution techniques (Hart et al., 1994). After spreading each sample in a thin layer (~5–10 mm deep) in a plastic dish, ^{15}N solutions were added as a mist using a syringe with a fine-tipped needle. The matrix was mixed for 1–2 min with a stainless steel spatula. Samples were then further divided into four portions (15–25 g each), two of which were extracted in 2N KCl at a soil:solution mass ratio of 5:1, and two of which were transferred to 250 ml glass jars with screw-on lids for incubation at 20 °C. Initial soil extractions were done 10–30 min after the addition of the ^{15}N solutions, and final extractions (per above) were done after 3 days of incubation. The KCl extracts were filtered (Whatman no. 42, Clifton, NJ), amended with 1–2 drops of chloroform per 50 ml, and stored at 4 °C prior to determination of NH_4^+-N and nitrite plus nitrate (NO_2^--N + NO_3-N) concentrations using an automated colorimetric analyzer (Perstorp Analytical, Silver Spring, MD).

Diffusion techniques (Brooks et al., 1989; Stark and Hart, 1996) were used to transfer dissolved inorganic N species from portions of the remaining extracts to glass fiber disks for subsequent determination of ^{15}N content of the initial and final NH_4^+ pools (for gross mineralization and NH_4^+ consumption) and NO_3^- pools (for gross nitrification and NO_3^- consumption). The at.% ^{15}N content of the disks were determined by isotope ratio mass spectrometry at the Stable Isotope Facility, University of California, Davis. Gross N transformation rates were calculated using published equations (Hart et al., 1994). Initial inorganic N pool sizes used in these calculations were obtained from analysis of KCl extracts taken 10–30 min after adding the ^{15}N-labeled substrates (Davidson et al., 1991).

Following Hart et al. (1994), we use the term *consumption* to describe the sum of all processes that result in a decrease in extractable soil NH_4^+ or NO_3^- during the course of incubation. Previous experiments using sub-samples of the same samples used in the current experiments indicated that NO_3^- production was inhibited in the presence of 30–40 Pa of acetylene (Venterea et al., 2003), indicating that autotrophic nitrifying bacteria were primarily responsible for NO_3^- production in these soils. Therefore, we calculated gross rates of non-autotrophic NH_4^+ consumption as the difference between the total gross NH_4^+ consumption and gross nitrification rates. Following Davidson et al. (1991), we refer to this derived process rate as the gross NH_4^+ *immobilization* rate to distinguish this quantity from the total gross NH_4^+ consumption rate.

The $^{15}NH_4^+$ and $^{15}NO_3^-$ additions to these soils increased the soil NH_4^+ and NO_3^-concentrations by approximately 2–3 μg N g^{-1}, which represented <10 to >100% of the initial ambient concentrations (data reported in Venterea et al., 2003). Since inorganic N addition may stimulate consumption (Hart et al., 1994), the rates should be interpreted with this in mind. As a way of compensating for any differential stimulation arising from the added $^{15}NO_3^-$ or due to varying levels of ambient NO_3^-, we also calculated NO_3^- consumption rates per unit of extractable NO_3^-. Assuming that soil inorganic N levels were at steady state, NH_4^+ and NO_3^- pool turnover times were calculated by dividing each KCl-extractable substrate concentration by gross mineralization and gross nitrification rates, respectively (Verchot et al., 2001). Because experimental applications of ^{15}N-enriched tracer were made to the control and low-N plots in both stands in 1988 (Nadelhoffer et al., 1999), we determined the extant at.% ^{15}N in the soil NH_4^+ pool in our samples prior to spiking with ^{15}N solutions. The at.% ^{15}N values in soils from the control and low-N

[1] Mention of product names is for the convenience of the reader and implies no endorsement on the part of the authors, their respective institutions, or the USDA.

plots were slightly elevated (plot mean = 0.380–0.391%) above the high-N plots (mean = 0.378–0.379%). However, use of the measured values instead of the assumed value of 0.37% in the rate calculations (Hart et al., 1994) had negligible effects (<2%) on calculated rates.

2.4. N oxide gas production and consumption—laboratory measurements

Rates of N_2O production in soils being incubated for gross nitrification rate were determined during the final day of incubation. Lids on the incubation jars were first removed for 10–20 min to allow the jar headspace to equilibrate with ambient (room) air. Jars were sealed with screw-caps fitted with butyl rubber septa and incubated for 18–28 h. Samples (9 ml) of jar headspace were removed with a polyethylene syringe and transferred to previously evacuated 9 ml glass vials, which were subsequently analyzed for N_2O using gas chromatography with electron capture detection. Rates of N_2O production on a dry soil basis ($\mu g\ N\ g^{-1}\ h^{-1}$) were calculated from the change in headspace N_2O concentration compared to the mean N_2O concentration measured in replicate samples of room air taken during the headspace-equilibration period.

Rates of NO production and consumption in the soils being incubated for gross nitrification rate were determined immediately after collecting headspace samples for N_2O analysis. Each jar was sealed with a specially fitted lid attached to a dynamic flow-through system which allowed for the continuous delivery of humidified, NO_x-free air through the jar prior to entering a chemiluminescent NO_x analyzer (LMA-3D, Unisearch, Ontario, Canada) (Venterea and Rolston, 2000). Rates of net NO production (P_{net}) on a dry soil mass basis ($\mu g\ N\ g^{-1}\ h^{-1}$) were calculated from the difference between NO concentration in the air upstream and downstream of the soil, the air flow rate (approximately $0.06\ m^3\ h^{-1}$), and the dry soil mass. Net NO production rates were measured at varying NO concentrations ([NO]) over the range of <600 to $1.1 \times 10^6\ \mu g\ N\ m^{-3}$ (<1–2000 parts per billion volume [ppbv]) by blending NO gas standards (Scott-Marrin, Riverside, CA) with humidified, NO_x-free air and supplying the mixture as influent. The data obtained allowed for calculation of the gross NO production rate (P_{NO}, $\mu g\ N\ g^{-1}\ h^{-1}$) and consumption rate coefficient (k_c) according to

$$P_{net} = P_{NO} - k_c[NO] \quad (1)$$

where k_c is the NO consumption rate per unit of gas-phase NO concentration ($\mu g\ N\ g^{-1}\ h^{-1}$ per $\mu g\ N\ m^{-3}$, or $m^3\ g^{-1}\ h^{-1}$), as previously described (Remde et al., 1989; Venterea and Rolston, 2000).

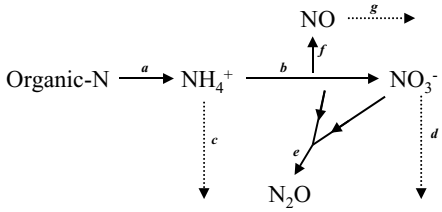

Fig. 1. Nitrogen transformation rates measured in incubating soils from Harvard Forest Chronic N Amendment Study plots: (a) N mineralization, (b) nitrification, (c) ammonium (NH_4^+) immobilization, (d) nitrate (NO_3^-) consumption, (e) sum of nitrification- and denitrification-derived nitrous oxide (N_2O) production, (f) nitric oxide production, and (g) NO consumption. Processes indicated by dashed lines have multiple potential end products that were not measured separately from each other.

The above procedures allowed for concurrent determination of multiple N transformation rates as illustrated in Fig. 1. A previous study (Venterea et al., 2003) demonstrated that NO production in sub-samples of these same samples was completely inhibited in the presence of acetylene (30–40 Pa). Therefore, NO production can be attributed to autotrophic nitrification (Klemedtsson et al., 1988). Possible sources of N_2O production include nitrification and denitrification (Firestone and Davidson, 1989). The contributions of each of these processes to the total N_2O production rate were not separately determined in this or the previous study.

2.5. Subsurface NO production and surface NO flux-field measurements

On 31 May and 1 June 2001, measurements of soil-to-atmosphere flux of NO and subsurface NO production rates in individual soil horizons were made in the hardwood high N and low-N plots. At three sub-plot locations within each plot, a thin-walled stainless steel flux chamber (180 mm ID × 110 mm high × 0.7 mm wall thickness) (Hutchinson and Mosier, 1981) was inserted to a depth of 20 mm immediately prior to

measuring NO flux using closed-chamber dynamic gas flux methods (Venterea et al., 2003). Immediately after each flux measurement, the top layer of the organic (O_i) horizon within the entire flux measurement area was removed, weighed, and transferred to the dynamic flow-through system (described above) set up in the field for measurement of NO production rate. A soil core sample was then taken from the center of the flux measurement area using a PVC pipe (50 mm ID) inserted to 150 mm below the lower boundary of the O_i horizon. Based on visual and tactile inspection, the soil core was divided into O_e, O_a, and mineral horizons, and the thickness (L, m) and mass of each horizon were determined prior to transfer of the samples to polyethylene bags. A sub-sample (15–30 g) of each horizon was then transferred to the field system for NO production rate measurement. Soil temperatures at 20 mm intervals to 150 mm were measured using temperature probes (Fisher Scientific) prior to soil sampling. The individual horizon samples were brought to the laboratory for determination of k_c at 20 °C. Field P_{net} measurements were converted to P_{NO} values using Eq. (1) with k_c values adjusted for field soil temperature (8–12 °C) using k_c values measured at 20 °C and a Q_{10} factor of 2.0 (Venterea and Rolston, 2002). Gravimetric water content (θ, g H_2O g^{-1} soil) was determined per above, which allowed for calculation of dry soil bulk density (ρ, g m^{-3}). At each location, the NO production rate ($P_{A,i}$) contributed by each horizon (i) expressed on an areal basis (μg N m^{-2} h^{-1}) was determined from $P_{A,i} = P_{NO,i}\rho_i L_i$. The total subsurface NO production rate over the sampled depth was calculated as $\sum_{i=1}^{4} P_{A,i}$. Total subsurface NO consumption was calculated as the difference between total subsurface production and surface flux measured immediately prior to soil sampling.

2.6. Diffusion-reaction modeling

A previously developed model (Venterea and Rolston, 2002) was used to simulate NO subsurface transport and transformation in the field. The model consists of a one-dimensional diffusion-reaction equation given by

$$-\frac{d}{dz}\left(D_s \frac{d[NO]}{dz}\right) = \rho P_{NO} - \rho k_c [NO] - S_g \qquad (2)$$

where [NO] is the gas-phase NO concentration (μg N m^{-3} gas), S_g a gas-phase sink term (μg N m^{-3} soil h^{-1}) describing the chemical oxidation of NO by molecular oxygen (Venterea and Rolston, 2002; Atkinson et al., 1997), z the soil depth (m), and D_s the soil gas diffusion coefficient (m^3 gas m^{-1} soil h^{-1}). Values of D_s were calculated using the Buckingham–Burdine–Campbell equation (Moldrup et al., 1999) which estimates D_s as a function of the diffusion coefficient of NO in free air at a given temperature (Bird et al., 1960), soil volumetric air content (ε), total porosity (ϕ), and the Campbell soil-water retention parameter (b). For each plot (low N and high N) within the hardwood stand, mean values of the measured parameters (P_{NO}, k_c, θ, ρ) for each horizon were used in solving Eq. (2). In the absence of independent data for this soil, b was varied over the range of 1–10 and ϕ was varied over the range of 0.45–0.75 in order to examine the sensitivity of simulation results to these parameters. Values of ε were calculated from assumed values of ϕ and measured values of θ and ρ. Solution of Eq. (2) allowed for calculation of total subsurface NO consumption integrated over the simulated soil depth (0.15–0.16 m) as a function of assumed values of b and ϕ as previously described (Venterea and Rolston, 2002).

2.7. Statistics

Within each stand, the effect of N addition was evaluated using one-way analysis of variance (ANOVA) and least significant differences (LSD) multiple comparison, with the level of N addition as the main factor and sub-plot measurements considered as treatment replicates, consistent with previous data analysis at the HF chronic N study (Magill et al., 2000; Rainey et al., 1999; Aber et al., 1993). All references to statistically significant differences and all reported linear regression results indicate significance at the $P < 0.05$ level unless otherwise noted. Linear regression, ANOVA, and t-tests were performed using Statgraphics Plus 5.1 (Manugistics, Rockville, MD).

3. Results

3.1. Gross N mineralization, nitrification and consumption

Gross N mineralization (N_{min}) rates did not differ significantly in the N-amended plots compared to

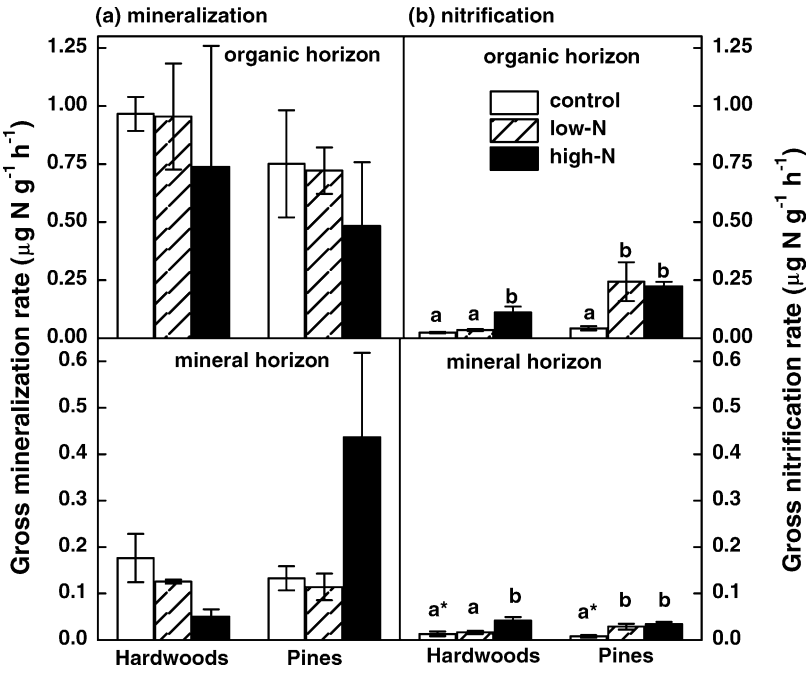

Fig. 2. Rates of (a) gross N mineralization, and (b) gross nitrification in incubating organic horizon soils (upper plates) and mineral horizon soils (lower plates) from Chronic N Amendments Study plots sampled May–June 2001 (mean ± S.E.). Within each forest and soil type, bars with different letter designation are significantly different based on least significant differences ANOVA ($P < 0.05$). Asterisk indicates that the mean value is not significantly different from zero.

control plots in either stand (Fig. 2a). There was some evidence for a decrease in N mineralization with N addition in the hardwood mineral horizon soils, where the mean N_{min} rate under each treatment was negatively correlated with annual N input rate ($r^2 = 0.99$, $P = 0.048$, $n = 3$). Gross nitrification rates were significantly elevated compared to controls in soils from the low-N and high-N plots in the pine stand, and in the high N plot in the hardwood stand (Fig. 2b).

There was a consistent trend of increasing NO_3^- consumption with level of N-addition (Table 1). Rates of NO_3^- consumption were positively correlated with substrate (KCl-extractable NO_3^-) concentrations in the pine ($r^2 = 0.66–0.85$) and hardwood stands ($r^2 = 0.53–0.88$). As previously reported, KCl-extractable NO_3^- concentrations were elevated in the high N plots in both stands and the low-N plot in the pine stand compared to control plots in June 2001 (Venterea et al., 2003). Therefore, as an attempt to correct for any substrate stimulation effects, the absolute gross NO_3^- consumption rates were normalized by dividing by the mean of the initial and post-incubation KCl-extractable NO_3^- concentrations. In contrast to absolute rates, NO_3^- consumption rates per unit of available NO_3^- in the hardwood organic horizon soils tended to decrease with the level of N addition (Fig. 3), and this difference was marginally significant ($P = 0.081$). There was also a significant negative correlation between the mean NO_3^- consumption rate per unit of available NO_3^- under each treatment and the annual N deposition rate in hardwood organic horizon soil ($r^2 = 0.99$, $P = 0.026$, $n = 3$). In contrast, NO_3^- consumption per unit of available NO_3^- in the pine stand did not vary across treatments in organic horizon soils (Fig. 3). No differences were evident in mineral horizon soils (not shown).

Rates of NH_4^+ immobilization in organic horizon soil were lower in both N-amended plots compared to the control in the hardwood stand only (Table 1). Rates of NH_4^+ immobilization were not significantly correlated with soil NH_4^+ concentrations or gross N mineralization rates in either stand. In the hardwood stand, the mean total NH_4^+ consumption rate (i.e.,

Table 1
Rates (μg N g^{-1} h^{-1}) of NH_4^+ immobilization and NO_3^- consumption in soil samples collected in June 2001[a,**]

	Gross NH_4^+ immobilization[b,**]		Gross NO_3^- consumption	
	Mineral horizon	Organic horizon	Mineral horizon	Organic horizon
Hardwoods				
Control	0.185 (0.057)	0.905 (0.075) b[**]	0.011 (0.009)	0.032 (0.005) a[**]
Low N	0.078 (0.025)	0.417 (0.130) a[**]	0.014 (0.004)	0.041 (0.004) a[**]
High N	0.082 (0.051)	0.070 (0.029) a[**]	0.041 (0.015)	0.084 (0.001) b[**]
Pines				
Control	0.108 (0.023)	0.674 (0.223)	0.008 (0.003) a[**]	0.045 (0.018) a[*]
Low N	0.055 (0.017)	0.498 (0.077)	0.029 (0.006) a[**]	0.224 (0.077) ab[*]
High N	0.094 (0.028)	1.22 (0.281)	0.061 (0.011) b[**]	0.303 (0.096) b[*]

[a] Mean values ($n = 3$, standard error in parentheses). Values with different letter designations are significantly different based on LSD comparisons within each stand and soil type.
[b] Calculated as the difference between gross NH_4^+ consumption and gross nitrification rate.
[**] $P < 0.05$.
[*] $P < 0.10$.

gross nitrification plus gross NH_4^+ immobilization), was significantly higher ($P = 0.003$) in soils from the control plot (0.93 μg g^{-1} h^{-1}) compared to both the low-N (0.45 μg g^{-1} h^{-1}) and high-N plots (0.19 μg g^{-1} h^{-1}). In contrast, total NH_4^+ consumption did not vary significantly across treatments in the pine stand, but tended to be higher ($P = 0.094$) in the high-N plots (1.5 μg g^{-1} h^{-1}) compared to the control and low-N plots (0.72–0.74 μg g^{-1} h^{-1}). In the hardwood stand, as a percentage of total NH_4^+ consumption, gross nitrification represented on average 64% in the high-N organic horizon soils, compared to only 8 and 3% in the low-N and control soils, respectively. In the pine stand, gross nitrification represented on average 17, 33 and 6.5% in the high-N, low-N and control organic horizon soils, respectively.

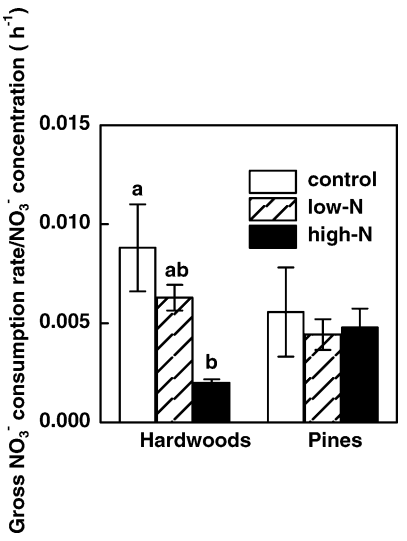

Fig. 3. Rates of NO_3^- consumption per unit concentration of NO_3^- in incubating organic horizon soils from Chronic N Amendment Study plots sampled May–June 2001 (mean ± S.E.). Within each forest, bars with different letter designation are significantly different based on least significant differences ANOVA ($P < 0.10$).

Our recoveries of added $^{15}NO_3^-$ in extractions done 10–30 min after addition were 37–65% in soils from the hardwood stand, and 30–52% in soils from the pine stand, with no significant differences in recovery between treatments. Recoveries of added NH_4^+ were 80–96%, except in mineral horizon soils in the pine stand (61–68%). Pool turnover times tended to increase with the level of N addition (Table 2).

3.2. N oxide gas production and consumption—laboratory measurements

In organic soils from the high-N plots in both stands, and in mineral horizon soil from the low-N plot in the pine stand, the gross NO production rate (P_{NO}) was significantly elevated above the respective controls (Fig. 4a). As a percentage of gross nitrification, P_{NO} values ranged from 1.2 to 19%. Gross NO production rates were positively correlated with gross nitrification rate ($r^2 = 0.68$) and NO_3^- turnover time ($r^2 = 0.72$) in organic horizon soils from the hardwood

Table 2
Inorganic N pool turnover times (days) in soil samples collected in June 2001[a,**]

	NH_4^+		NO_3^-	
	Mineral horizon	Organic horizon	Mineral horizon	Organic horizon
Hardwoods				
Control	2.2 (0.54) a[*]	1.1 (0.27)	4.7 (3.3) a[**]	0.20 (0.01) a[**]
Low N	2.9 (0.04) ab[*]	2.7 (0.43)	2.5 (0.76) a[**]	1.2 (0.49) a[**]
High N	7.5 (2.0) b[*]	7.8 (4.2)	13 (1.3) b[**]	7.9 (0.69) b[**]
Pines				
Control	15 (14)	3.6 (0.23) a[**]	13 (10)	1.6 (1.0) a[**]
Low N	2.0 (0.62)	2.4 (0.29) a[**]	9.4 (0.17)	4.9 (0.43) b[**]
High N	0.31 (0.29)	5.6 (0.69) b[**]	18 (1.6)	7.2 (1.5) b[**]

[a] Mean values are shown (standard error in parentheses). Values with different letter designation are significantly different based on LSD comparisons within each forest and soil type.
[**] $P < 0.05$.
[*] $P < 0.10$.

stand, and with gross nitrification rate ($r^2 = 0.44$) in mineral horizon soils from the pine stand.

Mean rates of N_2O production in soils from the N-treated plots were generally $\leq$1% of gross nitrification rates and $\leq$15% of P_{NO} values (Fig. 4b). Production of N_2O tended to increase with level of N addition, although not consistently. Rates of N_2O production were positively correlated with KCl-extractable NO_3^- concentration in hardwood organic horizon soil ($r^2 = 0.76$), with gross nitrification rate in hardwood mineral horizon soil ($r^2 = 0.58$) and pine organic horizon soil ($r^2 = 0.70$), with NO_3^- consumption rate in pine organic ($r^2 = 0.90$) and hardwood mineral horizon soil ($r^2 = 0.78$), and with NO_3^- turnover time in hardwood ($r^2 = 0.79$) and pine organic horizon soil ($r^2 = 0.37$, $P = 0.11$).

Mineral horizon soils from the pine high-N plot displayed significantly lower NO consumption rates than the control or low-N plots (Fig. 4c). Measured rates of NO consumption were used to calculate the half-life ($t_{1/2}$) of NO in the soil matrix from $t_{1/2} = -\ln(0.5)\varepsilon/(k_c\rho)$. Using the range of ρ values measured in the hardwood organic horizon (2×10^5 to 6×10^5 g m^{-3}) and ε estimates of 0.2–0.8 m^3 gas m^{-3} soil, calculated $t_{1/2}$ values ranged from 2 to 20 s.

3.3. Subsurface NO production and surface NO flux—field measurements

Rates of subsurface NO production in individual soil horizons were generally more than 10 times higher in the hardwood high-N plot compared to the low-N plot (Fig. 5a). Under both the low and high N treatments, surface NO flux represented a small proportion (<5%) of the total NO produced in the top 0.16 m of the soil profile (Fig. 5b). In other words, NO consumption calculated from the difference between subsurface production and surface flux represented 96–98% of total subsurface production.

Nitric oxide consumption rates predicted by the diffusion-reaction model were similar to those calculated from field measurements of NO flux and subsurface production (Fig. 6). Increased total porosity and increased values of the Campbell soil-water retention parameter (b) each resulted in increased simulated rates of NO gaseous diffusion, which corresponded with decreased simulated NO consumption rates. Decreased NO consumption predicted at higher diffusion rates follows from the lower NO residence time in the subsurface, which allows less time for NO to react in the soil prior to reaching the soil–atmosphere interface. Areal gross nitrification rates estimated from the laboratory measurements and the field soil profile information (bulk density and horizon depths) were 1600 and 6800 μg N m^{-2} h^{-1} in the low-N and high-N plots, respectively. As a percentage of gross nitrification, calculated and simulated NO consumption rates therefore represented approximately 8 and 25% of gross nitrification rates in the low-N and high-N plots, respectively (Fig. 6).

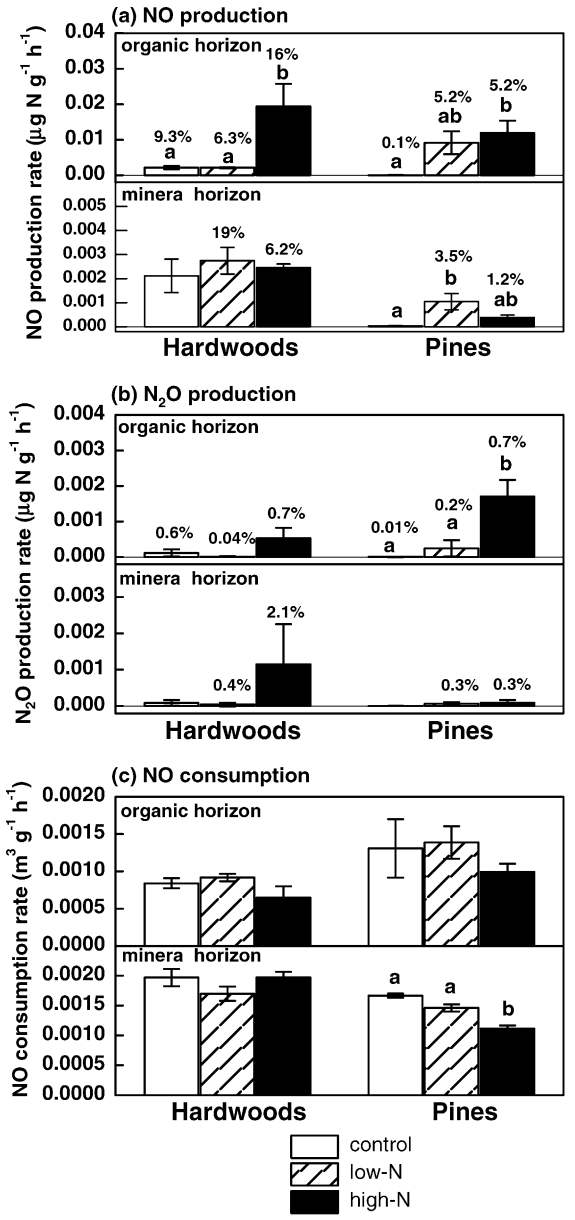

Fig. 4. Mean rates of (a) NO production, (b) N_2O production, and (c) NO consumption in organic horizon soils (upper plates) and mineral horizon soils (lower plates) from Chronic N Amendment Study plots sampled May–June 2001 (±S.E., $n = 3$). Within each forest and soil type, bars with different letter designations are significantly different based on least significant differences ANOVA ($P < 0.05$). Numbers above bars are mean production rates as percentage of gross nitrification rate. Percentages are not shown in cases where mean nitrification rates are not significantly different from zero (see Fig. 2).

4. Discussion

4.1. Gross NO_3^- production and consumption

Gross nitrification rates were consistently elevated in both organic and mineral horizon soils in only those plots at the Harvard Forest Chronic N Amendment Study which have displayed increased NO_3^- leaching and elevated NO emissions (Magill et al., 2000, 2004; Venterea et al., 2003). This consistent trend strongly suggests that enhanced gross nitrification is the fundamental process driving other symptoms of N saturation in the high-N plots in both stands and in the pine low-N plot. These results are consistent with Tietema (1998), who found that organic horizon soils sampled from two European coniferous forests exhibiting significant NO_3^- leaching displayed elevated gross nitrification compared to soils from two other forests which had no measurable NO_3^- losses. Gross nitrification rates measured by Tietema (1998) were in the range of 0.16–0.28 μg N g^{-1} h^{-1}, which is nearly identical to the range observed here (Fig. 2b).

There was no evidence that a suppression in the *absolute* rates of gross NO_3^- consumption is contributing to increased NO_3^- leaching, i.e., NO_3^- consumption rates actually tended to increase with increasing level of N input (Table 1). However, when NO_3^- consumption rates were normalized with respect to the available NO_3^- (Fig. 3), the data indicate that, in soils from the N-amended hardwood plots, NO_3^- consumption rates have not increased sufficiently to keep pace with the increasing supply of NO_3^-. The response shown in Fig. 3 for the hardwood soils is consistent with enzyme-mediated kinetic models (e.g., Michaelis–Menten), wherein the reaction (consumption) rate increases at a decreasing rate with increasing substrate (NO_3^-) concentration, until eventually the reaction rate approaches a maximum as the substrate concentration approaches a ‘‘saturation’’ level (Pauling, 1970). This does suggest that in the hardwood stand, NO_3^- consumption kinetics have responded to N additions and may be important in controlling NO_3^- leaching. In contrast, there was no evidence that either the absolute (Table 1) or normalized (Fig. 3) rates of NO_3^- consumption have decreased as a result of chronic N inputs to the pine stand.

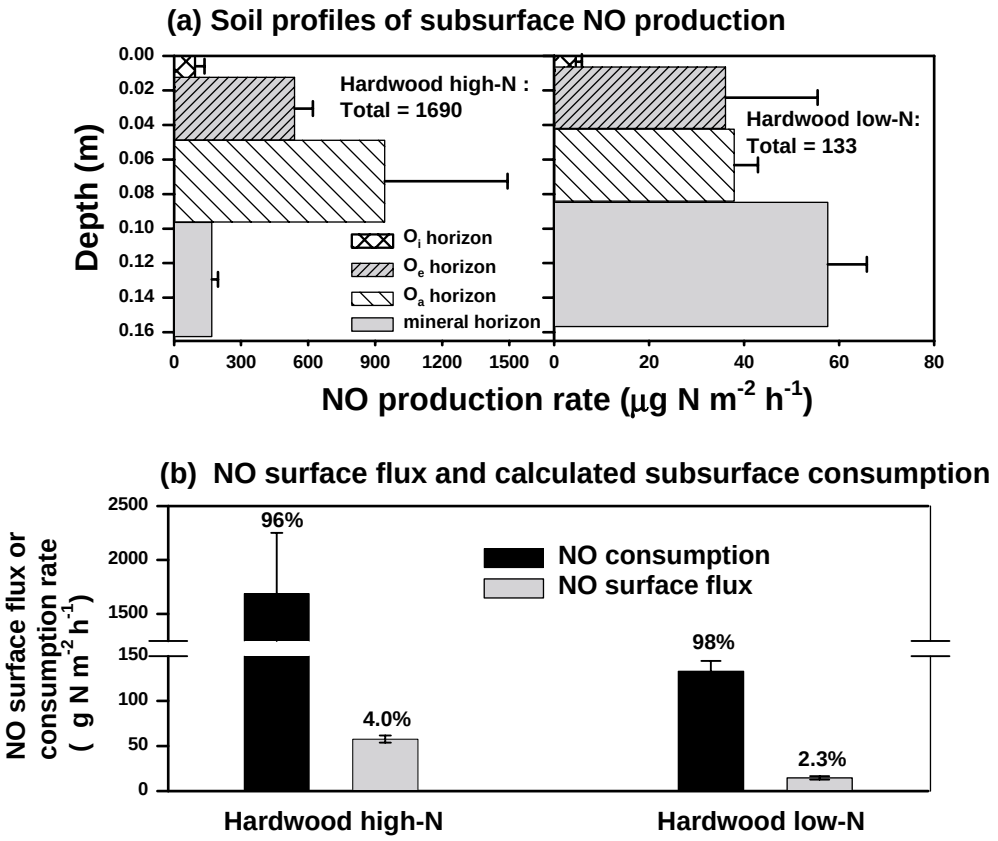

Fig. 5. (a) Mean subsurface NO production rates contributed by individual soil horizons from the hardwood high-N, and low-N plots obtained in the field immediately after measuring NO surface flux at the same location, and (b) subsurface NO consumption (left vertical axis) and surface NO flux (right vertical axis) at the same locations. In (a), the vertical width of each bar represents the relative mean thickness of each horizon. In (b), values above bars are mean NO consumption rates and surface fluxes as percentage of total subsurface NO production. Mean ± S.E., $n = 3$.

The current findings are consistent with Berntson and Aber (2000), who found lower absolute rates of NO_3^- consumption in soils sampled in September 1997 from the hardwood high-N plots compared to the control plots, but no treatment differences in the pine soils (using the 0.25 h incubation starting time in Fig. 2 of Berntson and Aber (2000), which is most comparable to the current methods). Since KCl-extractable NO_3^- concentrations were not reported by Berntson and Aber (2000), direct comparisons with respect to the normalized NO_3^- consumption rates cannot be made.

Berntson and Aber (2000) achieved recoveries of $\leq$50% of the $^{15}NO_3^-$ applied to soils from the Chronic N Amendment plots that were extracted 15 min after $^{15}NO_3^-$ application. These data, together with the findings of Davidson et al. (2003) and Dail et al. (2001), provided evidence that much of the applied $^{15}NO_3^-$ was rapidly immobilized and further suggested that NO_3^- produced via nitrification may also be rapidly immobilized via abiotic pathways. Our recoveries of applied $^{15}NO_3^-$ in soils extracted 10–30 min after N addition were very similar to those of Berntson and Aber (2000). Thus, while our experiments were not designed to accurately quantify the consumption of $^{15}NO_3^-$ occurring during the first 30 min after addition, our results are in support of a rapid process mediating at least part of the NO_3^- consumption in these soils. We found no significant differences in $^{15}NO_3^-$ recoveries between treatments which might suggest that N inputs are affecting rates of this rapid immobilization process. The absolute

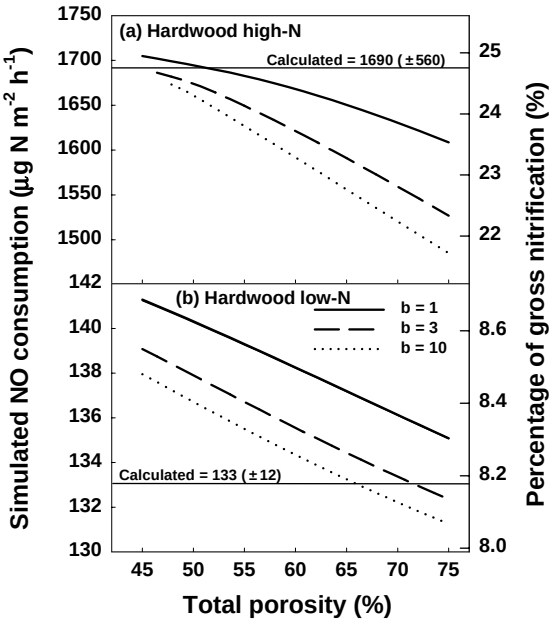

Fig. 6. Simulated rates of NO consumption on an areal basis (left-hand axis) and as a percentage of areal gross nitrification rates (right-hand axis) in hardwood (a) high-N and (b) low-N plots predicted from diffusion-reaction model (Eq. (2)) as a function of soil total porosity and the Campbell soil-water retention parameter (b). The mean NO consumption rates calculated from field data shown in Fig. 5 are indicated by horizontal lines for comparison.

rates of NO_3^- consumption found here in mineral horizon soils (0.003–0.04 μg N g^{-1} h^{-1}) are consistent with rates displayed by the majority of samples analyzed by Stark and Hart (1997) from several forests in the western US.

4.2. Differential forest response to chronic N inputs

There is yet no definitive explanation for why the N-amended pine plots have displayed more rapid onset of N saturation characteristics following the initiation of experimental N inputs compared to the hardwood plots. After 12 years of N treatments, increased NO_3^- leaching, NO emissions, and net nitrification were evident in both the high- and low-N plots in the pine stand, but only in the high-N plot in the hardwood stand (Magill et al., 2000, 2004; Venterea et al., 2003). While the current data do not elucidate underlying mechanisms for the differential forest response, they may provide clues and possibly eliminate some hypotheses. Two distinct differences in the patterns of N consumption between the pine and hardwood soils were evident: (1) NO_3^- consumption per unit of KCl-extractable NO_3^- decreased with N addition in the hardwood soils, but did not vary in the pine soils, and (2) NH_4^+ immobilization (i.e., non-autotrophic NH_4^+ consumption) decreased with N addition in the hardwood soils, but not in the pine soils.

If we assume the above findings, and other data reported here, reflect historical differences observed between the two stands, we can first conclude from: (1) above that the more rapid onset of NO_3^- leaching in the pine stand was *not* a result of a suppression in NO_3^- consumption arising from the N additions, or lower rates of NO_3^- consumption as compared to the hardwood stand. Perhaps more revealing, result (2) above is consistent with the idea that chronic N inputs to the hardwood stand resulted in a reduction in the NH_4^+-assimilation capacity of the heterotrophic microbial community, which in turn favored the nitrifying microbial community in their competition for available NH_4^+. The lack of such a decrease in heterotrophic NH_4^+ assimilation capacity in the pine soils as a prerequisite for enhanced nitrification suggests that the competition between autotrophs and heterotrophs for available NH_4^+ is not as important of a constraint on nitrification in the pine soils. A comparison of nitrification rates in the two control plots indicates that both net nitrification (Magill et al., 2000; Venterea et al., 2003) and gross nitrification (current data, organic horizon, $P = 0.126$) tend to be higher in the pine soils than the hardwood soils. These results suggest that the pine soils were better poised for nitrification increases, possibly due to differences in the composition of the microbial communities in hardwood and pine soils.

However, the current data do not identify the specific fundamental constraints on gross nitrification in the pine system, nor do they indicate whether the differences between pine and hardwood systems derive primarily from tree species effects or from other effects related to differential land use history (Magill et al., 2004). The determination of fundamental constraints on nitrification in forest soils influenced by different dominant tree species has proven to be a challenging research objective. While some studies (e.g., Melillo et al., 1982; Baldwin et al., 1983) have suggested that litter quality indices such as soil C:N ratio, foliar N content, lignin:N ratio, or

phenolic content may regulate soil N cycling and/or nitrification, a recent study found no strong relationships between these variables and net nitrification rates in soils from single-species plots in the Catskills of New York State (Lovett et al., 2004). Furthermore, it is not known how litter quality effects, or other constraints, may be altered in response to chronic N inputs.

While the hardwood low-N plot has yet to demonstrate elevated NO_3^- leaching or NO emissions, the significant ($P < 0.05$) decrease in NH_4^+ immobilization rates in organic horizon soil compared to the controls (Table 1), may be indicative of the imminent onset of N saturation symptoms. In fact, gross nitrification rates in organic horizon soils from the low-N plots were slightly greater than the control, and the difference was marginally significant ($P = 0.11$, Fig. 2b).

4.3. NO consumption as a source of NO_3^-

Our data suggest that a significant proportion of the NO_3^- produced in the hardwood plots may in fact have resulted from NO transformation. This mechanism of NO_3^- production could be represented in Fig. 1 as originating with NH_4^+ and then following the sequence of pathways b → f → g → NO_3^-. Approximately 5–20% of the N undergoing nitrification in incubating soils from the N-amended plots passed through the NO pool (Fig. 4a). The high subsurface NO consumption rates that were estimated from the field data and model simulations represent potential NO_3^- production rates corresponding to 20–25% of estimated gross nitrification rates in the hardwood high-N plot (Fig. 6a). Nitrate is the predominant end product of NO transformation in aqueous, soil, and gaseous systems (Schwartz and White, 1983; Atkinson et al., 1997; Conrad, 1995). Other potential products of NO transformation include nitrogen dioxide (NO_2) and nitrite (NO_2^-), both of which tend to be rapidly oxidized to NO_3^- via both biotic and abiotic reactions (Venterea and Rolston, 2002). The connection between NO transformation, gaseous diffusion, and NO_3^- production has been previously indicated in fertilized soils (Venterea and Rolston, 2002). It is also possible that a proportion of the consumed NO may have undergone incorporation into soil organic matter after conversion to NO_2^- (Fitzhugh et al., 2003; Davidson et al., 2003; Smith and Chalk, 1980).

4.4. The role of soil acidity in regulating N losses from temperate forests

It was previously shown that reduced soil pH at Harvard Forest may be promoting NO production via the HNO_2 pathway (Venterea et al., 2003). The current data also indicate that gross nitrification rates actually were higher at lower soil pH in the hardwood stand ($P < 0.10$, $r^2 = 0.35–0.42$) and to a greater extent in the pine stand ($P < 0.02$, $r^2 = 0.61–0.87$). This is consistent with previous data which suggested that acidophilic and/or acid-tolerant autotrophic bacteria are prevalent in N saturated forest soils (De Boer et al., 1992). This result is most likely the consequence of nitrification-induced acidification (Tietema et al., 1992) accumulating over the duration of the experiment, but it does appear to contradict commonly held assumptions about the inhibitory effects of low soil pH on nitrification. We also found that NH_4^+ immobilization and NO_3^- consumption rates per unit of available NO_3^- were each positively correlated with soil pH in hardwood organic horizon soils ($r^2 = 0.49$ and 0.71, respectively). Thus, while not appearing to significantly inhibit nitrification, nitrification- and/or deposition-induced acidity may in fact have enhanced N losses in multiple ways, by (i) contributing to increased nitrification by inhibiting heterotrophic competition for NH_4^+, (ii) contributing to increased NO_3^- leaching by inhibiting heterotrophic NO_3^- assimilation, and (iii) promoting NO and perhaps NO_3^- losses due to NO formed via the HNO_2 pathway.

5. Conclusions and questions

Gross nitrification rates measured in laboratory incubations in the current study displayed the same pattern of response to N additions as field measurements of NO_3^- leaching and NO emissions at the Harvard Forest Chronic N Amendment Study (Magill et al., 2004; Venterea et al., 2003). This coherent pattern suggests that enhanced gross nitrification is the fundamental process driving the more salient symptoms of N saturation in the high-N plots in both stands and the low-N plot in the pine stand. Another level of

understanding is required to explain the differential forest response. The questions of what particular constraints on nitrification prevail in either forest, and exactly how these constraints are lessened upon chronic N addition, need further study. The current data do suggest that decreased heterotrophic demand for NH_4^+ in response to N addition may be an important factor in this respect in the hardwood stand, while other constraints may be more important in the pine stand. Further study is also needed to clarify the multiple potential roles of reduced soil pH in enhancing N losses, and the role of NO consumption as a source of NO_3^-, in both N-limited and N-saturated forest soils. The current data suggest that such studies would benefit from the simultaneous determination of multiple gross rates. The repeated measurement of fundamental N process rates in different forests systems during different stages of N saturation would appear to be particularly illuminating.

Acknowledgements

The authors acknowledge Sabrina LaFave, Lynn Christensen, Jessica Kriebel, Lisa Martel, and Abraham Parker for their assistance with the laboratory analysis, and David Harris for analyzing the ^{15}N samples. We also thank Jennifer King, Martin Burger, and two anonymous reviewers for their comments. This work was funded by EPA NCERQA (Grant R827674) and the National Science Foundation Long-Term Ecological Research (LTER) Program. Additional support was provided during manuscript preparation by the Agricultural Research Service of the US Department of Agriculture.

References

Aber, J., Nadelhoffer, K., Steudler, P., Melillo, J., 1988. Nitrogen saturation in northern forest ecosystems. BioScience 39, 378–386.

Aber, J., Magill, A., Boone, R., Melillo, J., Steudler, P., Bowden, R., 1993. Plant and soil responses to chronic nitrogen additions at the Harvard Forest, Massachusetts. Ecol. Appl. 3, 156–166.

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., Fernandez, I., 1998. Nitrogen saturation in temperate forest ecosystems: hypothesis revisited. BioScience 48, 921–934.

Atkinson, R., Baulch, D., Cox, R., Hampson, J.R.F., Kerr, J., Rossi, M., Troe, J., 1997. Evaluated Kinetic and Photochemical Data for Atmospheric Chemistry: Supplement VI. IUPAC Subcommittee on Gas Kinetic Data Evaluation for Atmospheric Chemistry. J. Phys. Chem. Ref. Data 26, 1329–1499.

Baldwin, I., Olson, R., Reiners, W., 1983. Protein binding phenolics and the inhibition of nitrification in subalpine balsam fir soils. Sol Biol. Biochem. 15, 419–423.

Berntson, G., Aber, J., 2000. Fast nitrate consumption in N saturated temperate forest soils. Soil Biol. Biochem. 32, 151–156.

Bird, R., Stewart, W., Lightfoot, E., 1960. Transport Phenomena. Wiley, New York.

Brooks, P., Stark, J., McInteer, B., Preston, T., 1989. Diffusion method to prepare extracts for automated nitrogen-15 analysis. Soil Sci. Soc. Am. J. 53, 1707–1711.

Conrad, R., 1995. Metabolism of nitric oxide in soil and soil microorganisms and regulation of flux into the atmosphere. In: Murrell, J., Kelly, D. (Eds.), Microbiology of Atmospheric Trace Gases: Sources, Sinks and Global Processes. Springer, New York, pp. 167–201.

Crutzen, P., 1979. The role of NO and NO_2 in the chemistry of the troposphere and stratosphere. Ann. Rev. Earth Planet. Sci. 7, 443–472.

Dail, D., Davidson, E., Chorover, J., 2001. Rapid abiotic transformation of nitrate in an acid forest soil. Biogeochemistry 54, 131–146.

Davidson, E., Hart, S., Shanks, C., Firestone, M., 1991. Measuring gross nitrogen mineralization, immobilization, and nitrification by ^{15}N isotope pool dilution and intact soil cores. J. Soil Sci. 42, 335–349.

Davidson, E., Chorover, J., Dail, D., 2003. A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis. Global Change Biol. 9, 228–236.

De Boer, W., Tietema, A., Klein Gunnewiek, P.J.A., Laanbroek, H.J., 1992. The chemolithotrophic ammonium-oxidizing community in a nitrogen saturated acid forest soil in relation to pH-dependent nitrifying activity. Soil Biol. Biochem. 24, 229–234.

Firestone, M., Davidson, E., 1989. Microbiological basis of NO and N_2O production and consumption in soil. In: Andreae, M., Schimel, D. (Eds.), Exchange of Trace Gases Between Terrestrial Ecosystems and the Atmosphere. Wiley, Chichester, pp. 7–21.

Fitzhugh, R., Lovett, G., Venterea, R., 2003. Biotic and abiotic immobilization of nitrogen in soils under different tree species of the Catskill Mountains, New York. Global Change Biol. 9, 1591–1601.

Galloway, J., Schlesinger, W., Levy, H., Michaels, A., Schnoor, J., 1995. Nitrogen fixation: anthropogenic enhancement—environmental response. Global Biogeochem. Cycles 9, 235–252.

Hart, S., Stark, J., Davidson, E., Firestone, M., 1994. Nitrogen mineralization, consumption and nitrification. In: Weaver, W. (Ed.), Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties. Soil Science Society of America, Madison, pp. 985–1018.

Hutchinson, G.L., Mosier, A.R., 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Sci. Soc. Am. J. 45, 311–316.

Klemedtsson, L., Klemedtsson, A., Moldan, F., Weslien, P., 1997. Nitrous oxide emission from Swedish forest soils in relation to liming and simulated increased N-deposition. Biol. Fertil. Soils 25, 290–295.

Klemedtsson, L., Svenssoon, B.H., Rossall, T., 1988. A method of selective inhibition to distinguish between nitrification and denitrification as sources of oxide in soil. Biol. Fertil. Soils 6, 112–119.

Lovett, G., Weathers, K., Arthur, M., Schultz, J., 2004. Nitrogen cycling in a northern hardwood forest: do species matter? Biogeochemistry 67, 289–308.

Magill, A., Aber, J., Currie, W., Nadelhoffer, K., Martin, M., Melillo, J., Steudler, P., 2004. Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA. For. Ecol. Manage. 196, 7–28.

Magill, A., Aber, J., Berntson, G., McDowell, W., Nadelhoffer, K., Melillo, J., Steudler, P., 2000. Long-term nitrogen additions and nitrogen saturation in two temperate forests. Ecosystems 3, 238–253.

Melillo, J., Aber, J., Muratore, J., 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63, 621–626.

Moldrup, P., Olesen, T., Yamaguchi, T., Schjonning, P., Rolston, D., 1999. Modeling diffusion and reaction in soils. IX. The Buckingham–Burdine–Campbell equation for gas diffusivity in undisturbed soil. Soil Sci. 164, 542–551.

Nadelhoffer, K., Downs, M., Fry, B., 1999. Sinks for ^{15}N-enriched additions to an oak forest and a red pine plantation. Ecol. Appl. 9, 72–86.

Pauling, L., 1970. General Chemistry. Dover, New York.

Rainey, S., Nadelhoffer, K., Silver, W., Downs, M., 1999. Effects of chronic nitrogen additions on understory species in a red pine plantation. Ecol. Appl. 9, 949–957.

Reich, P., Lassoie, J., Amundson, R., 1983. Reduction in growth of hybrid poplar following field exposure to low levels of O_3 and (or) SO_2. Can. J. Bot. 62, 2835–2841.

Remde, A., Slemr, F., Conrad, R., 1989. Microbial production and uptake of nitric oxide in soil. FEMS Microbiol. Ecol. 62, 221–230.

Schwartz, S., White, W., 1983. Kinetics of reactive dissolution of nitrogen oxides into aqueous solution. In: Schwartz, S. (Ed.), Trace Atmospheric Constituents. Wiley, New York, pp. 1–116.

Skiba, U., Sheppard, L., Pitcairn, C., van Dijk, S., Rossall, M., 1999. The effect of N deposition on nitrous oxide and nitric oxide emissions from temperate forest soils. Water Air Soil Pollut. 116, 89–98.

Smith, C., Chalk, P., 1980. Fixation and loss of nitrogen during transformations of nitrite in soils. Soil Sci. Soc. Am. J. 44, 288–291.

Stark, J., Hart, S., 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Sci. Soc. Am. J. 60, 1846–1855.

Stark, J., Hart, S., 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385, 61–64.

Stohl, A., Williams, E., Wotawa, G., Kromp-Kolb, H., 1996. A European inventory of soil nitric oxide emissions and the effect of these emissions on the photochemical formation of ozone. Atmos. Environ. 30, 3741–3755.

Tietema, A., Riemer, L., Verstraten, J.M., van der Maas, M.P., van Wijk, A.J., van Voorthuyzen, I., 1992. Nitrogen cycling in acid forest soils subject to increased atmospheric nitrogen input. For. Ecol. Manage. 57, 29–44.

Tietema, A., 1998. Microbial carbon and nitrogen dynamics in coniferous forest floor material along a European nitrogen deposition gradient. For. Ecol. Manage. 101, 29–36.

Venterea, R., Rolston, D., 2000. Mechanisms and kinetics of nitric and nitrous oxide production during nitrification in agricultural soil. Global Change Biol. 6, 303–316.

Venterea, R., Rolston, D., 2002. Nitrogen oxide trace gas transport and transformation. II. Model simulations compared with data. Soil Sci. 167 (1), 49–61.

Venterea, R., Groffman, P., Verchot, L., Magill, A., Aber, J., Steudler, P., 2003. Nitrogen oxide gas emissions from temperate forest soils receiving long-term nitrogen inputs. Global Change Biol. 9, 346–357.

Verchot, L., Holmes, Z., Mulon, L., Groffman, P., Lovett, G., 2001. Gross vs. net rates of N mineralization and nitrification as indicators of functional differences between forest types. Soil Biol. Biochem. 33, 1889–1901.

Wright, R., Rasmussen, L., 1998. Introduction to the NITREX and EXMAN projects. For. Ecol. Manage. 101, 1–7.