



# 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 ( $\text{NO}_3^-$ ) consumption, ammonium ( $\text{NH}_4^+$ ) immobilization, nitrous oxide ( $\text{N}_2\text{O}$ ) 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  $\text{NO}_3^-$  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  $\text{g N m}^{-2}$  per year, and in soils from a hardwood stand receiving 15  $\text{g N m}^{-2}$  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  $\text{NH}_4^+$  immobilization, and rates of  $\text{NO}_3^-$  consumption per unit of available  $\text{NO}_3^-$ , 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  $\text{N}_2\text{O}$  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  $\text{NO}_3^-$  production.

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## 1. Introduction

Many forests in Europe and North America receive elevated levels of atmospheric nitrogen (N) deposition

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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

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 ( $\text{NO}_3^-$ ) 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  $\text{NO}_3^-$  export had higher gross nitrification rates compared to N-limited forests, and that gross  $\text{NO}_3^-$  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  $\text{NO}_3^-$  consumption, or a combination of both. It is also not clear if changes in gross N mineralization and/or ammonium ( $\text{NH}_4^+$ ) immobilization may be affecting the availability of  $\text{NH}_4^+$  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 ( $\text{O}_3$ ) (Crutzen, 1979). Soil NO emissions can significantly influence local  $\text{O}_3$  levels (Stohl et al., 1996), and therefore may contribute to the negative effects of  $\text{O}_3$  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  $\text{NO}_3^-$  production and distribution in the soil profile (Venterea and Rolston, 2002). Emissions of nitrous oxide ( $\text{N}_2\text{O}$ ), an important greenhouse gas and regulator of stratospheric  $\text{O}_3$ , have also shown some response to experimental N additions at HF and other forests (Venterea et al., 2003; Klemetsson 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  $\text{NO}_3^-$  production.

## 2. Methods

### 2.1. Site description

Experiments were conducted at the Chronic N Amendment Study at HF in central Massachusetts, USA ( $42^\circ30'\text{N}$ ,  $72^\circ10'\text{W}$ ), 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 ( $\text{NH}_4\text{NO}_3$ ) to simulate atmospheric N deposition in excess of background wet plus dry deposition of  $\sim 0.8 \text{ g N m}^{-2}$  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 ( $+5 \text{ g N m}^{-2}$  per year), and (iii) high-N plots ( $+15 \text{ g N m}^{-2}$  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 ( $\text{O}_i$  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  $\times$  200 mm long) was inserted into the soil to a depth of 150 mm. Each core sample was separated by organic ( $\text{O}_e + \text{O}_a$ ) 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<sup>-1</sup>) enriched with 99% <sup>15</sup>N (Isotec,<sup>1</sup> Miamisburg, OH) for determination of gross rates of N mineralization and NH<sub>4</sub><sup>+</sup> consumption, and the other sub-sample was amended with 2 ml of potassium nitrate solution (45 µg N ml<sup>-1</sup>) enriched with 99% <sup>15</sup>N (Isotec) for determination of gross rates of nitrification and NO<sub>3</sub><sup>-</sup> consumption using <sup>15</sup>N dilution techniques (Hart et al., 1994). After spreading each sample in a thin layer (~5–10 mm deep) in a plastic dish, <sup>15</sup>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 <sup>15</sup>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<sub>4</sub><sup>+</sup>-N and nitrite plus nitrate (NO<sub>2</sub><sup>-</sup>-N + NO<sub>3</sub><sup>-</sup>-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 <sup>15</sup>N content of the initial and final NH<sub>4</sub><sup>+</sup> pools (for gross mineralization and NH<sub>4</sub><sup>+</sup> consumption) and NO<sub>3</sub><sup>-</sup>

pools (for gross nitrification and NO<sub>3</sub><sup>-</sup> consumption). The at.% <sup>15</sup>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 <sup>15</sup>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<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> during the course of incubation. Previous experiments using sub-samples of the same samples used in the current experiments indicated that NO<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> production in these soils. Therefore, we calculated gross rates of non-autotrophic NH<sub>4</sub><sup>+</sup> consumption as the difference between the total gross NH<sub>4</sub><sup>+</sup> consumption and gross nitrification rates. Following Davidson et al. (1991), we refer to this derived process rate as the gross NH<sub>4</sub><sup>+</sup> *immobilization* rate to distinguish this quantity from the total gross NH<sub>4</sub><sup>+</sup> consumption rate.

The <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>3</sub><sup>-</sup> additions to these soils increased the soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations by approximately 2–3 µg N g<sup>-1</sup>, 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 <sup>15</sup>NO<sub>3</sub><sup>-</sup> or due to varying levels of ambient NO<sub>3</sub><sup>-</sup>, we also calculated NO<sub>3</sub><sup>-</sup> consumption rates per unit of extractable NO<sub>3</sub><sup>-</sup>. Assuming that soil inorganic N levels were at steady state, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> 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 <sup>15</sup>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.% <sup>15</sup>N in the soil NH<sub>4</sub><sup>+</sup> pool in our samples prior to spiking with <sup>15</sup>N solutions. The at.% <sup>15</sup>N values in soils from the control and low-N

<sup>1</sup> 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<sub>2</sub>O 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<sub>2</sub>O using gas chromatography with electron capture detection. Rates of N<sub>2</sub>O production on a dry soil basis ( $\mu\text{g N g}^{-1} \text{h}^{-1}$ ) were calculated from the change in headspace N<sub>2</sub>O concentration compared to the mean N<sub>2</sub>O 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<sub>2</sub>O 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<sub>x</sub>-free air through the jar prior to entering a chemiluminescent NO<sub>x</sub> analyzer (LMA-3D, Unisearch, Ontario, Canada) (Venterea and Rolston, 2000). Rates of net NO production ( $P_{\text{net}}$ ) on a dry soil mass basis ( $\mu\text{g N g}^{-1} \text{h}^{-1}$ ) were calculated from the difference between NO concentration in the air upstream and downstream of the soil, the air flow rate (approximately  $0.06 \text{ m}^3 \text{ h}^{-1}$ ), and the dry soil mass. Net NO production rates were measured at varying NO concentrations ([NO]) over the range of <600 to  $1.1 \times 10^6 \mu\text{g N m}^{-3}$  (<1–2000 parts per billion volume [ppbv]) by blending NO gas standards (Scott-Marrin, Riverside, CA) with humidified, NO<sub>x</sub>-free air and supplying the mixture as influent. The data obtained allowed for calculation of the gross NO

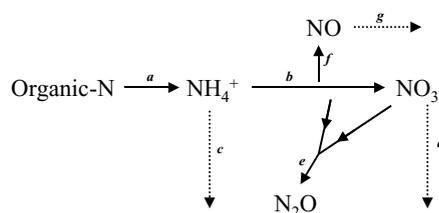


Fig. 1. Nitrogen transformation rates measured in incubating soils from Harvard Forest Chronic N Amendment Study plots: (a) N mineralization, (b) nitrification, (c) ammonium (NH<sub>4</sub><sup>+</sup>) immobilization, (d) nitrate (NO<sub>3</sub><sup>-</sup>) consumption, (e) sum of nitrification- and denitrification-derived nitrous oxide (N<sub>2</sub>O) 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.

production rate ( $P_{\text{NO}}$ ,  $\mu\text{g N g}^{-1} \text{h}^{-1}$ ) and consumption rate coefficient ( $k_c$ ) according to

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

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

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 (Klemmedtsson et al., 1988). Possible sources of N<sub>2</sub>O production include nitrification and denitrification (Firestone and Davidson, 1989). The contributions of each of these processes to the total N<sub>2</sub>O 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 ( $\theta$ , g  $H_2O$  g<sup>-1</sup> soil) was determined per above, which allowed for calculation of dry soil bulk density ( $\rho$ , g m<sup>-3</sup>). At each location, the NO production rate ( $P_{A,i}$ ) contributed by each horizon ( $i$ ) expressed on an areal basis ( $\mu\text{g N m}^{-2} \text{h}^{-1}$ ) 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

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

where  $[\text{NO}]$  is the gas-phase NO concentration ( $\mu\text{g N m}^{-3}$  gas),  $S_g$  a gas-phase sink term ( $\mu\text{g N m}^{-3}$

soil h<sup>-1</sup>) 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 ( $\text{m}^3 \text{gas m}^{-1} \text{soil h}^{-1}$ ). 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 ( $\epsilon$ ), total porosity ( $\phi$ ), 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 ( $P_{NO}$ ,  $k_c$ ,  $\theta$ ,  $\rho$ ) 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  $\phi$  was varied over the range of 0.45–0.75 in order to examine the sensitivity of simulation results to these parameters. Values of  $\epsilon$  were calculated from assumed values of  $\phi$  and measured values of  $\theta$  and  $\rho$ . 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  $\phi$  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

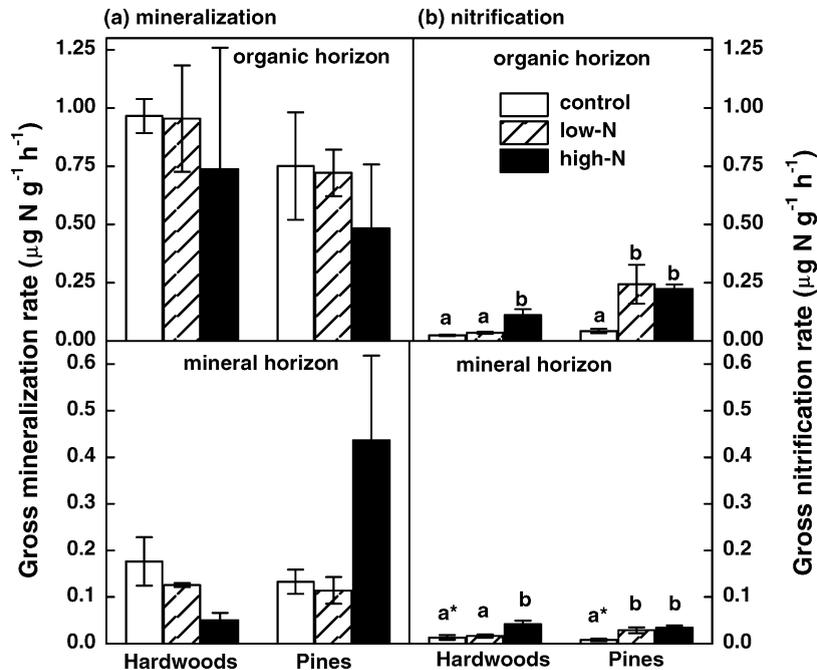


Fig. 2. Rates of (a) gross N mineralization, and (b) gross nitrification in incubating organic horizon soils (upper plates) and mineral horizon soils (lower plates) from Chronic N Amendments Study plots sampled May–June 2001 (mean  $\pm$  S.E.). Within each forest and soil type, bars with different letter 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  $\text{NO}_3^-$  consumption with level of N-addition (Table 1). Rates of  $\text{NO}_3^-$  consumption were positively correlated with substrate (KCl-extractable  $\text{NO}_3^-$ ) concentrations in the pine ( $r^2 = 0.66$ – $0.85$ ) and hardwood stands ( $r^2 = 0.53$ – $0.88$ ). As previously reported, KCl-extractable  $\text{NO}_3^-$  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  $\text{NO}_3^-$  consumption rates were normalized by dividing by the mean of the initial and post-

incubation KCl-extractable  $\text{NO}_3^-$  concentrations. In contrast to absolute rates,  $\text{NO}_3^-$  consumption rates per unit of available  $\text{NO}_3^-$  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  $\text{NO}_3^-$  consumption rate per unit of available  $\text{NO}_3^-$  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,  $\text{NO}_3^-$  consumption per unit of available  $\text{NO}_3^-$  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  $\text{NH}_4^+$  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  $\text{NH}_4^+$  immobilization were not significantly correlated with soil  $\text{NH}_4^+$  concentrations or gross N mineralization rates in either stand. In the hardwood stand, the mean total  $\text{NH}_4^+$  consumption rate (i.e.,

Table 1  
Rates ( $\mu\text{g N g}^{-1} \text{h}^{-1}$ ) of  $\text{NH}_4^+$  immobilization and  $\text{NO}_3^-$  consumption in soil samples collected in June 2001<sup>a,\*\*</sup>

	Gross $\text{NH}_4^+$ immobilization <sup>b,**</sup>		Gross $\text{NO}_3^-$ consumption	
	Mineral horizon	Organic horizon	Mineral horizon	Organic horizon
<b>Hardwoods</b>				
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**
<b>Pines</b>				
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*

<sup>a</sup> 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.

<sup>b</sup> Calculated as the difference between gross  $\text{NH}_4^+$  consumption and gross nitrification rate.

\*\*  $P < 0.05$ .

\*  $P < 0.10$ .

gross nitrification plus gross  $\text{NH}_4^+$  immobilization), was significantly higher ( $P = 0.003$ ) in soils from the control plot ( $0.93 \mu\text{g g}^{-1} \text{h}^{-1}$ ) compared to both the low-N ( $0.45 \mu\text{g g}^{-1} \text{h}^{-1}$ ) and high-N plots ( $0.19 \mu\text{g g}^{-1} \text{h}^{-1}$ ). In contrast, total  $\text{NH}_4^+$  consumption did not vary significantly across treatments in the pine stand, but tended to be higher ( $P = 0.094$ ) in the

high-N plots ( $1.5 \mu\text{g g}^{-1} \text{h}^{-1}$ ) compared to the control and low-N plots ( $0.72$ – $0.74 \mu\text{g g}^{-1} \text{h}^{-1}$ ). In the hardwood stand, as a percentage of total  $\text{NH}_4^+$  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.

Our recoveries of added  $^{15}\text{NO}_3^-$  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  $\text{NH}_4^+$  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  $\text{NO}$  production rate ( $P_{\text{NO}}$ ) was significantly elevated above the respective controls (Fig. 4a). As a percentage of gross nitrification,  $P_{\text{NO}}$  values ranged from 1.2 to 19%. Gross  $\text{NO}$  production rates were positively correlated with gross nitrification rate ( $r^2 = 0.68$ ) and  $\text{NO}_3^-$  turnover time ( $r^2 = 0.72$ ) in organic horizon soils from the hardwood

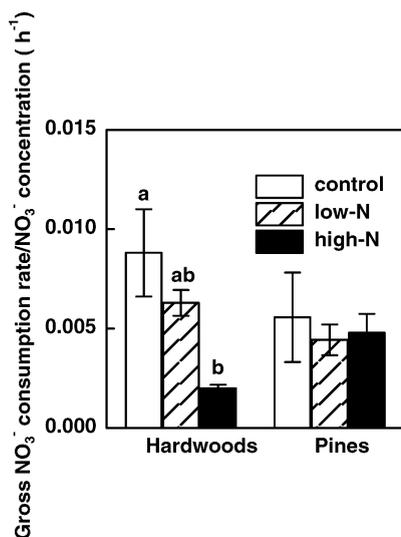


Fig. 3. Rates of  $\text{NO}_3^-$  consumption per unit concentration of  $\text{NO}_3^-$  in incubating organic horizon soils from Chronic N Amendment Study plots sampled May–June 2001 (mean  $\pm$  S.E.). Within each forest, bars with different letter designation are significantly different based on least significant differences ANOVA ( $P < 0.10$ ).

Table 2  
Inorganic N pool turnover times (days) in soil samples collected in June 2001<sup>a,\*\*</sup>

	NH <sub>4</sub> <sup>+</sup>		NO <sub>3</sub> <sup>-</sup>	
	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**

<sup>a</sup> 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.

<sup>\*\*</sup>  $P < 0.05$ .

\*  $P < 0.10$ .

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

Mean rates of N<sub>2</sub>O production in soils from the N-treated plots were generally  $\leq 1\%$  of gross nitrification rates and  $\leq 15\%$  of  $P_{\text{NO}}$  values (Fig. 4b). Production of N<sub>2</sub>O tended to increase with level of N addition, although not consistently. Rates of N<sub>2</sub>O production were positively correlated with KCl-extractable NO<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> consumption rate in pine organic ( $r^2 = 0.90$ ) and hardwood mineral horizon soil ( $r^2 = 0.78$ ), and with NO<sub>3</sub><sup>-</sup> 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  $\rho$  values measured in the hardwood organic horizon ( $2 \times 10^5$  to  $6 \times 10^5$  g m<sup>-3</sup>) and  $\varepsilon$  estimates of 0.2–0.8 m<sup>3</sup> gas m<sup>-3</sup> 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  $\mu\text{g N m}^{-2} \text{h}^{-1}$  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).

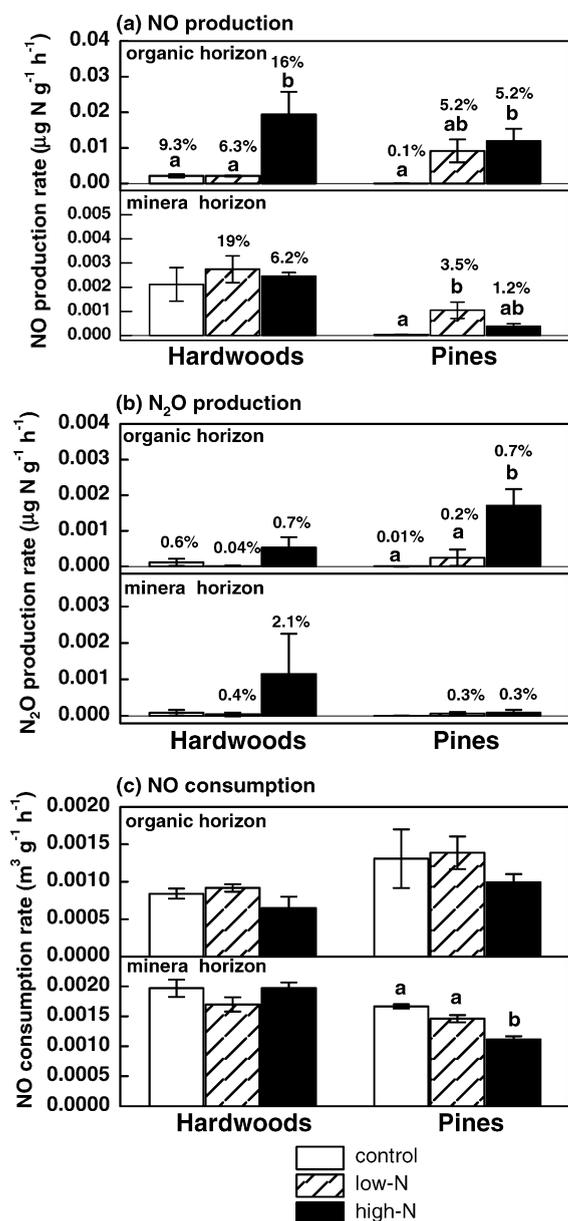


Fig. 4. Mean rates of (a) NO production, (b) N<sub>2</sub>O production, and (c) NO consumption in organic horizon soils (upper plates) and mineral horizon soils (lower plates) from Chronic N Amendment Study plots sampled May–June 2001 ( $\pm$ S.E.,  $n = 3$ ). Within each forest and soil type, bars with 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<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> leaching displayed elevated gross nitrification compared to soils from two other forests which had no measurable NO<sub>3</sub><sup>-</sup> losses. Gross nitrification rates measured by Tietema (1998) were in the range of 0.16–0.28  $\mu\text{g N g}^{-1} \text{h}^{-1}$ , 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<sub>3</sub><sup>-</sup> consumption is contributing to increased NO<sub>3</sub><sup>-</sup> leaching, i.e., NO<sub>3</sub><sup>-</sup> consumption rates actually tended to increase with increasing level of N input (Table 1). However, when NO<sub>3</sub><sup>-</sup> consumption rates were normalized with respect to the available NO<sub>3</sub><sup>-</sup> (Fig. 3), the data indicate that, in soils from the N-amended hardwood plots, NO<sub>3</sub><sup>-</sup> consumption rates have not increased sufficiently to keep pace with the increasing supply of NO<sub>3</sub><sup>-</sup>. 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<sub>3</sub><sup>-</sup>) 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<sub>3</sub><sup>-</sup> consumption kinetics have responded to N additions and may be important in controlling NO<sub>3</sub><sup>-</sup> leaching. In contrast, there was no evidence that either the absolute (Table 1) or normalized (Fig. 3) rates of NO<sub>3</sub><sup>-</sup> consumption have decreased as a result of chronic N inputs to the pine stand.

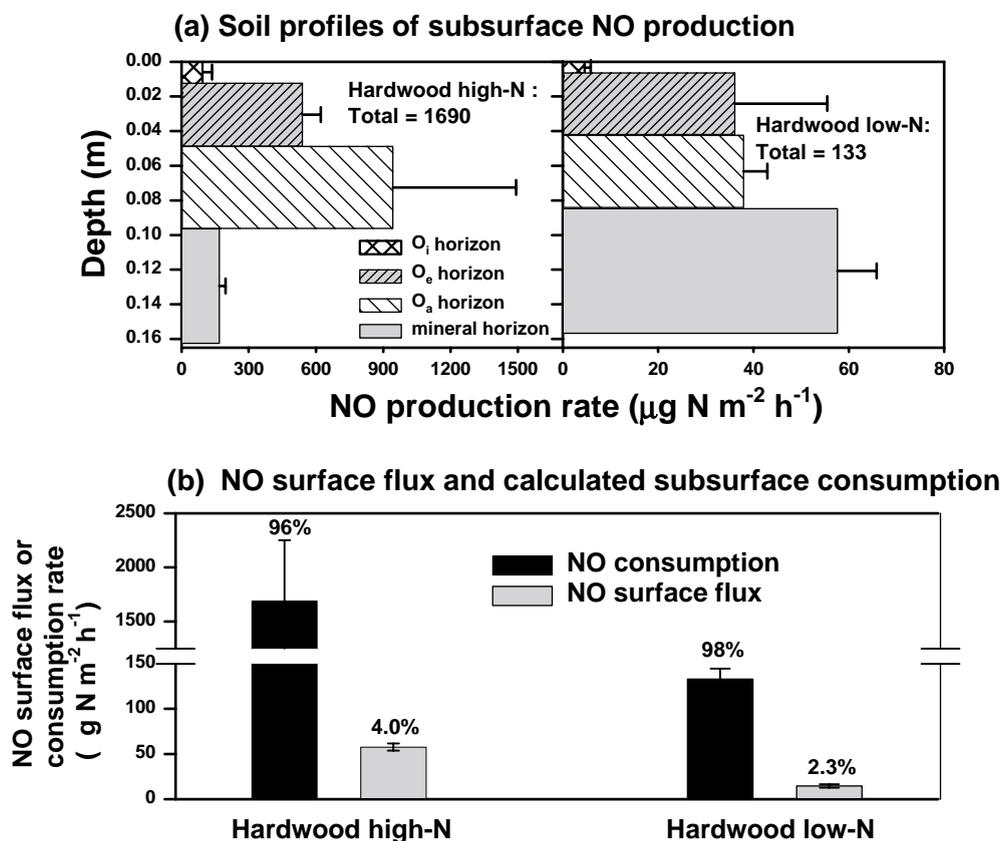


Fig. 5. (a) Mean subsurface NO production rates contributed by individual soil horizons from the hardwood high-N, and low-N plots obtained in the field immediately after measuring NO surface flux at the same location, and (b) subsurface NO consumption (left vertical axis) and surface NO flux (right vertical 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  $\pm$  S.E.,  $n = 3$ .

The current findings are consistent with [Berntson and Aber \(2000\)](#), who found lower absolute rates of  $\text{NO}_3^-$  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  $\text{NO}_3^-$  concentrations were not reported by [Berntson and Aber \(2000\)](#), direct comparisons with respect to the normalized  $\text{NO}_3^-$  consumption rates cannot be made.

[Berntson and Aber \(2000\)](#) achieved recoveries of  $\leq 50\%$  of the  $^{15}\text{NO}_3^-$  applied to soils from the Chronic N Amendment plots that were extracted 15 min after  $^{15}\text{NO}_3^-$  application. These data, together with the

findings of [Davidson et al. \(2003\)](#) and [Dail et al. \(2001\)](#), provided evidence that much of the applied  $^{15}\text{NO}_3^-$  was rapidly immobilized and further suggested that  $\text{NO}_3^-$  produced via nitrification may also be rapidly immobilized via abiotic pathways. Our recoveries of applied  $^{15}\text{NO}_3^-$  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  $^{15}\text{NO}_3^-$  occurring during the first 30 min after addition, our results are in support of a rapid process mediating at least part of the  $\text{NO}_3^-$  consumption in these soils. We found no significant differences in  $^{15}\text{NO}_3^-$  recoveries between treatments which might suggest that N inputs are affecting rates of this rapid immobilization process. The absolute

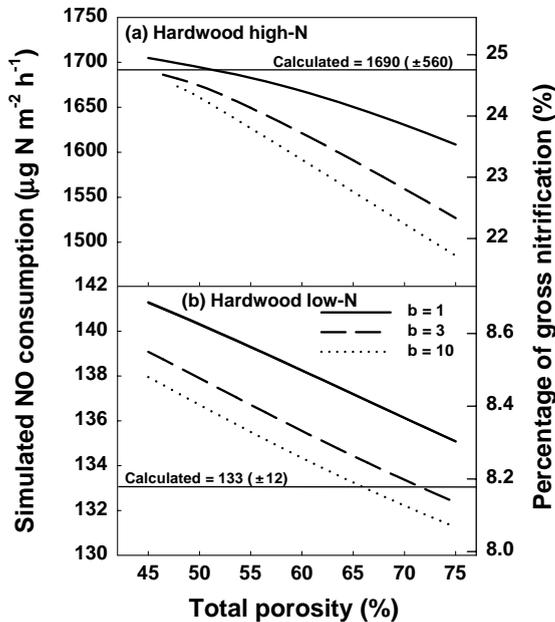


Fig. 6. Simulated rates of NO consumption on an areal basis (left-hand axis) and as a percentage of areal gross nitrification rates (right-hand axis) in hardwood (a) high-N and (b) low-N plots predicted from diffusion-reaction model (Eq. (2)) as a function of soil total porosity and the Campbell soil-water 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  $\text{NO}_3^-$  consumption found here in mineral horizon soils ( $0.003\text{--}0.04 \mu\text{g N g}^{-1} \text{h}^{-1}$ ) 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  $\text{NO}_3^-$  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)  $\text{NO}_3^-$  consumption per unit of KCl-extractable  $\text{NO}_3^-$  decreased with N addition in the hardwood soils, but did not vary in the pine soils, and (2)  $\text{NH}_4^+$  immobilization (i.e., non-autotrophic  $\text{NH}_4^+$  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  $\text{NO}_3^-$  leaching in the pine stand was *not* a result of a suppression in  $\text{NO}_3^-$  consumption arising from the N additions, or lower rates of  $\text{NO}_3^-$  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  $\text{NH}_4^+$ -assimilation capacity of the heterotrophic microbial community, which in turn favored the nitrifying microbial community in their competition for available  $\text{NH}_4^+$ . The lack of such a decrease in heterotrophic  $\text{NH}_4^+$  assimilation capacity in the pine soils as a prerequisite for enhanced nitrification suggests that the competition between autotrophs and heterotrophs for available  $\text{NH}_4^+$  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  $\text{NO}_3^-$  leaching or NO emissions, the significant ( $P < 0.05$ ) decrease in  $\text{NH}_4^+$  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 $\text{NO}_3^-$

Our data suggest that a significant proportion of the  $\text{NO}_3^-$  produced in the hardwood plots may in fact have resulted from NO transformation. This mechanism of  $\text{NO}_3^-$  production could be represented in Fig. 1 as originating with  $\text{NH}_4^+$  and then following the sequence of pathways  $b \rightarrow f \rightarrow g \rightarrow \text{NO}_3^-$ . 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  $\text{NO}_3^-$  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 ( $\text{NO}_2$ ) and nitrite ( $\text{NO}_2^-$ ), both of which tend to be rapidly oxidized to  $\text{NO}_3^-$  via both biotic and abiotic reactions (Venterea and Rolston, 2002). The connection between NO transformation, gaseous diffusion, and  $\text{NO}_3^-$  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  $\text{NO}_2^-$  (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  $\text{HNO}_2$  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  $\text{NH}_4^+$  immobilization and  $\text{NO}_3^-$  consumption rates per unit of available  $\text{NO}_3^-$  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  $\text{NH}_4^+$ , (ii) contributing to increased  $\text{NO}_3^-$  leaching by inhibiting heterotrophic  $\text{NO}_3^-$  assimilation, and (iii) promoting NO and perhaps  $\text{NO}_3^-$  losses due to NO formed via the  $\text{HNO}_2$  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  $\text{NO}_3^-$  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  $\text{NH}_4^+$  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  $\text{NO}_3^-$ , 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.

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