Nitrogen oxide gas emissions from temperate forest soils receiving long-term nitrogen inputs

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Abstract

From spring 2000 through fall 2001, we measured nitric oxide (NO) and nitrous oxide (N_2O) fluxes in two temperate forest sites in Massachusetts, USA that have been treated since 1988 with different levels of nitrogen (N) to simulate elevated rates of atmospheric N deposition. Plots within a pine stand that were treated with either 50 or $150 \, kg \, N \, ha^{-1} \, yr^{-1}$ above background displayed consistently elevated NO fluxes ($100–200 \, \mu g \, N \, m^{-2} \, h^{-1}$) compared to control plots, while only the higher N treatment plot within a mixed hardwood stand displayed similarly elevated NO fluxes. Annual NO emissions estimated from monthly sampling accounted for 3.0–3.7% of N inputs to the high-N plots and 8.3% of inputs to the Pine low-N plot. Nitrous oxide fluxes in the N-treated plots were generally < 10% of NO fluxes. Net nitrification rates (NRs) and NO production rates measured in the laboratory displayed patterns that were consistent with field NO fluxes. Total N oxide gas flux was positively correlated with contemporaneous measurements of NR and NO_3^- concentration. Acetylene inhibited both nitrification and NO production, indicating that autotrophic nitrification was responsible for the elevated NO production. Soil pH was negatively correlated with N deposition rate. Low levels ($3–11 \, \mu g \, N \, kg^{-1}$) of nitrite ($NO_2^-$) were detected in mineral soils from both sites. Kinetic models describing NO production as a function of the protonated form of NO_2^- (nitrous acid [HNO_2]) adequately described the mineral soil data. The results indicate that atmospheric deposition may generate losses of gaseous NO from forest soils by promoting nitrification, and that the response may vary significantly between forest types under similar climatic regimes. The lowering of pH resulting from nitrification and/or directly from deposition may also play a role by promoting reactions involving HNO_2.

Keywords: chemodentrification, N deposition, nitric oxide, nitrification, nitrous oxide

Introduction

Many forests in Europe and North America continue to receive elevated levels of atmospheric N deposition, deriving mainly from fossil fuel combustion and fertilizer production and use (Fenn *et al.*, 1998; Gundersen *et al.*, 1998). Total N deposition rates can range from above $50 \, kg \, N \, ha^{-1} \, yr^{-1}$ in high elevation sites downwind of

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industrial or agricultural areas to below $3 \, kg \, N \, ha^{-1} \, yr^{-1}$ in remote forests (NADP, 2002; Lovett *et al.*, 1982; Tietema, 1993). Significant increases in N deposition rates worldwide have been predicted based on projected increases in energy and fertilizer consumption (Galloway *et al.*, 1994; Hall & Matson, 1999). There is increasing concern that anthropogenic N inputs may not only exceed plant uptake capacity but may also have deleterious impacts on entire ecosystems. Potential effects include soil acidification (van Breemen *et al.*, 1982), nutrient imbalances and losses (Johnson *et al.*, 1991), nitrate (NO_3^-) leaching to groundwater and streams (Kahl *et al.*, 1993), soil emissions of N oxide gases

(Skiba *et al.*, 1999) and, ultimately, forest decline (Aber *et al.*, 1998). More information is needed regarding the partitioning of N inputs between different soil and plant pools, the variation in responses among forest types, and the effect on soil N cycling processes such as nitrification, denitrification, and N trace gas production.

While soil-to-atmosphere emissions of N oxide gases from forest soils have been proposed as a likely response to persistent N additions (Aber *et al.*, 1998; Fenn *et al.*, 1998), there have been only a few measurements of N_2O flux (Brumme & Beese, 1992; Klemedtsson *et al.*, 1997; Peterjohn *et al.*, 1998), and fewer measurements of both N_2O and NO (Butterbach-Bahl *et al.*, 1997; Skiba *et al.*, 1999), in response to N deposition in temperate forests. Quantification of NO and N_2O emissions may help to improve total ecosystem N budget estimates for N-impacted forests (Magill *et al.*, 2000). Soil emissions of N oxide gases themselves may have important impacts, including possible effects on regional tropospheric ozone levels, contribution to downwind N deposition, enhanced destruction of stratospheric ozone, and increases in total greenhouse gases (Crutzen, 1979, 1981).

The chronic-nitrogen-addition experiment at the Harvard Forest (HF) in central Massachusetts, USA is one of the longest running and most intensive studies of N deposition in temperate forest ecosystems. Since 1988, a variety of plant, soil, and whole ecosystem responses to experimental N additions have been monitored in two adjacent sites comprised of mixed hardwood and red pine, respectively (Bowden *et al.*, 1991; Aber *et al.*, 1993; Magill *et al.*, 1997, 2000; Nadelhoffer *et al.*, 1999). While small increases in N_2O emissions have been observed in the pine forest, N_2O emissions have been estimated to account for $< 0.4\%$ of the total ecosystem N budget at HF (Magill *et al.*, 1997, 2000). Increases in soil nitrification rates in response to N inputs have also been observed at HF, with the pine forest responding sooner than the hardwood forest (Magill *et al.*, 2000). These results suggest that nitrification-driven emissions of NO (and possibly N_2O) may also be increasing, since previous studies in forest soils have found strong correlations between nitrification potential and N oxide fluxes (Verchot *et al.*, 1999; Davidson *et al.*, 2000). However, emissions of NO had not been measured at HF prior to the present study.

The objectives of the present study were (i) to make updated field measurements of N_2O flux and first-time measurements of NO flux from long-term N deposition study plots at HF, (ii) to compare fluxes between the mixed hardwood and red pine plots, (iii) to examine the influence of climatic factors on emissions rates, and (iv) to investigate the microbial and chemical processes responsible for the elevated NO emissions which were observed.

Methods and materials

Site description and long-term experimental design

Experiments were conducted at the chronic-N addition plots 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 received additions of NH_4NO_3 to simulate atmospheric N deposition in excess of background wet plus dry deposition of $8\,kg\,N\,ha^{-1}\,yr^{-1}$ (Magill *et al.*, 2000). One of the sites (hereafter referred to as 'Hardwoods') is comprised of a mixture of deciduous tree species that regenerated following clear cutting ca. 1950. The forest is dominated by black oak (*Quercus velutina* Lam.) and red oak (*Q. rubra* L.). The other forest (hereafter referred to as 'Pines') is comprised primarily of even-aged red pine (*Pinus resinosa* Ait.) planted in 1926. Experimental areas within each forest are divided into four $30\,m \times 30\,m$ (0.09 ha) plots, and each plot is further subdivided into $5\,m \times 5\,m$ (0.0025 ha) subplots. Annual treatments are as follows: (i) control plot (no additions), (ii) low-N addition plot ($+50\,kg\,N\,ha^{-1}\,yr^{-1}$), and (iii) high-N addition plot ($+150\,kg\,N\,ha^{-1}\,yr^{-1}$). Applications of NH_4NO_3 solution are made in six equal treatments beginning the first week of May and then at 4-week intervals ending in mid-September. Further experimental details are described by Magill *et al.* (1997). The low-N treatment level was selected to represent the upper range of actual N deposition rates (Lovett *et al.*, 1982; Tietema, 1993; Gundersen *et al.*, 1998). The high-N treatment level was employed in order to explore hypotheses related to the concept of N saturation (Aber *et al.*, 1989, 1998; Fenn *et al.*, 1998). The objective was to accelerate ecosystem responses that otherwise might occur over several decades, so they might become evident within a more practical experimental period (10–20 years).

Field NO and N_2O fluxes

Within each plot, three interior subplots were randomly selected for closed chamber NO and N_2O flux measurements. Plot locations were fixed for the entire study. Field gas fluxes were measured at approximately monthly intervals during snow-free periods beginning 11 June 2000 and concluding 24 November 2001. During each NO measurement, soil temperatures at 10 and 50 mm below the soil surface were measured using soil temperature probes (Fisher Scientific). Monthly flux measurements were made during mid-morning to early afternoon (10:00–14:00 local time), and were timed to occur when soil temperatures in the upper 50 mm were approximately equal to the mean of minimum and

maximum daily values. All reported monthly gas flux data were collected at least 7 days after N application, except the August 2000 and September 2000 data, which were collected 2 days after N application.

The gas flux chamber design was identical to that used in previous studies of N_2O fluxes at HF (Bowden *et al.*, 1990, 1991). Chambers consisted of 287-mm diameter (ID) by 40 mm high polyvinyl chloride (PVC) cylinders, which were placed on permanently installed PVC base rings immediately prior to measurement. At sampling intervals of approximately 15, 30 and 60 min following placement of the chamber, 9-mL gas samples were collected using polypropylene syringes from gas sampling ports in the centre of the chamber top. Replicate samples of ambient air taken during measurement periods were used as time 0 samples. Samples were transferred to evacuated glass vials, which were stored at room temperature prior to N_2O analysis by gas chromatography (GC) with electron capture detection. Flux of N_2O was calculated from the linear rate of change in N_2O concentration, the chamber internal volume and soil surface area.

For NO flux measurement, a chamber of the same dimensions as above fitted with inlet and outlet fittings were used. Upon chamber placement, a continuous gas stream ($0.03–0.09 \, m^3 \, h^{-1}$) was withdrawn from the chamber and delivered to a chemiluminescent NO_x ($NO + NO_2$) analyzer (Unisearch Models LMA-3 and LMA-3D) using a vacuum pump within the analyzer. Prior to entering the analyzer, chamber gas was passed through granular CrO_3, which converts NO to NO_2. Outlet air from the analyzer was passed through $KMnO_4$-impregnated alumina granules, which act as a NO_x scrubber (Purafil, Inc.) and anhydrous $CaSO_4$ (desiccant) prior to return to the chamber. In this configuration, the analyzer detects total NO_x. Measurements were periodically made with bypass of the CrO_3 converter to confirm that emissions of NO_x consisted mainly ($> 99\%$) of NO and not NO_2. Therefore, we hereafter use the term 'NO flux' to denote flux measured as total NO_x. Concentrations of NO_x in the recirculating gas were recorded at 10–30 s intervals for 4–5 min after placement of the chamber top. The analyzer was calibrated within 1–24 h prior to measurements using gas streams containing NO in the range of 1–100 ppbv. Fluxes of NO (F_{NO}, $\mu g \, N \, m^{-2} \, h^{-1}$) were calculated from

$$F_{NO} = \frac{dC}{dt} \frac{V}{A} + C_A \frac{Q}{A} \qquad (1)$$

where dC/dt is the rate of change of NO concentration ($\mu g \, N \, m^{-3}$) over time (h) determined by linear regression, V is the internal chamber volume (m^3), A is the soil surface area ($0.065 \, m^2$), C_A is the average NO concentration during the time interval of regression, and Q is the recirculation air flow rate ($m^3 \, h^{-1}$). Deriving from mass balance considerations, the final term in Eqn 1 accounts for the removal of NO from the chamber headspace during recirculation.

Additional experiments were done to examine responses in gas fluxes to experimental N addition or rain events, and to record daytime fluctuations in gas fluxes on an hourly time-scale. A previous study at this site found no short-term response in N_2O emission to NH_4NO_3 applications over a period of 1–24 days (Magill *et al.*, 1997). In the present study, field NO fluxes were measured 48 h and 1 h prior to, and 24 h, 48 h and 72 h following the NH_4NO_3 application of 29 May 2001. The response of NO fluxes to wetting was examined in August and October 2001. After measuring baseline gas fluxes, a volume of deionized water equivalent to a 25-mm rainfall event was applied to the area inside of and surrounding each chamber base ring to a distance of 0.25 m with a manual sprinkler. In August 2001, gas fluxes were measured 6 and 30 h after water was applied. In October 2001, fluxes were measured 24 h after water was applied and gas fluxes were measured only in plots which had previously displayed elevated NO fluxes. No actual rainfall was recorded during the 48 h preceding each wetting experiment. Daytime fluctuations in NO flux and soil temperatures were examined on two days in 2001 (3 July and 27 October). On these dates, measurements were made in the morning (7:00–8:30 LT), mid-day (12:00–13:30 LT) and afternoon (16:00–18:00 LT). The experiments examining daytime fluctuations and responses to N addition and wetting focused on NO emissions. Previous studies at HF have closely examined the dynamics of N_2O flux (Bowden *et al.*, 1990, 1991; Magill *et al.*, 1997).

Soil sampling and analysis

On the same day of each monthly gas flux sampling, soil samples (5–10 g each) were taken by hand trowel from 3 to 4 interior subplots and mixed together to generate two composite samples, one each from the organic ($O_e + O_a$) and mineral horizons. These samples were dried in the laboratory at $105 \, °C$ (mineral soil) or $65 \, °C$ (organic soil) for 24–48 h in order to determine gravimetric soil water content. Additional soil samples were collected in summer 2000 (24 August) and spring 2001 (1 June) for determination of inorganic N concentrations, soil pH and rates of net nitrification, N mineralization and NO production. These samples were taken at least 72 h following the August 2000 and May 2001 N applications from interior subplots other than those used for gas flux sampling. Prior to sampling, the upper layer (approximately 50–200 mm thick) of litter (O_i horizon) was removed from the sampling area. A section of split PVC pipe (50-mm ID × 200-mm long) was then pounded into the soil to a

depth of 150 mm. Each core sample was separated into organic ($O_e + O_a$ horizon) and mineral material, and delivered to the laboratory for processing within 1–3 days of collection.

Samples were first passed through a 6-mm-mesh sieve. Two subsamples ($\sim$5 g each) of mineral and organic sample were extracted for 1–2 h in 20 mL of 2 M KCl. Extracts were filtered through Whatman No. 42 paper and stored at 4 °C prior to determination of ammonium-N ($NH_4^+ - N$) and nitrate plus nitrite-N ($NO_2^- - N + NO_3^- - N$) using an automated colorimetric analyzer (Perstorp Analytical, 3000 series). On the same day, two additional subsamples (10–30 g) were placed into separate 250-mL glass jars, sealed loosely, and incubated in a humid atmosphere at 20 °C. After 14 days, a subsample ($\sim$5 g) was extracted with 2 M KCl as above. Potential net nitrification rates were calculated from the net increase in $NO_2^- - N + NO_3^- - N$ concentrations, and potential net N mineralization rates were calculated from the net increase in total inorganic N occurring during the incubation period. Rates were expressed on a dry soil mass basis (μg N kg^{-1} h^{-1}). Soil pH was measured in separate subsamples of soils collected in August 2000 by manually mixing soil with 1 M KCl at a soil-to-liquid mass ratio of 2:1 (mineral soil) or 5:1 (organic soil). After settling for 30–90 min, solution was poured off for determination of pH using a combination electrode.

Instantaneous rates of NO production in the incubating soils were determined after 3–4 days of incubation by sealing each jar with a specially fitted lid attached to a dynamic flow-through system, which allowed for the continuous delivery of a humidified air stream through the jar prior to entering the NO_x analyzer. Rates of NO production on a dry soil mass basis (μg N kg^{-1} h^{-1}) were calculated from the difference between NO concentration in air upstream and downstream of the soil, the air flow rate, and the dry soil mass, as previously described (Venterea & Rolston, 2000a).

The role of autotrophic nitrification in regulating NO_3^- production and NO production was examined using acetylene (C_2H_2) inhibition. In parallel with the 14-day incubation experiments, separate subsamples (10–30 g) of each sample collected in June 2001 were placed in 250-mL glass jars and treated with gas-phase C_2H_2 on the first day of incubation. Headspace concentrations of 30–40 Pa C_2H_2 were achieved by injecting 5 mL of 1000 Pa C_2H_2 through a butyl rubber septum into each jar. Following C_2H_2 addition, jars were kept sealed for 24–48 h, opened for 5–10 min to allow equilibration with ambient air, and then were sealed loosely. Soils were re-treated with C_2H_2 a second time during the incubation (after 5–10 days). Headspace concentrations of C_2H_2 in the range of 10–100 Pa (0.01–0.1%) have been shown to inhibit autotrophic nitrifying bacteria (e.g. *Nitrosomonas* sp.), which

mediate the oxidation of NH_4^+ to NO_2^- (Klemedtsson et al., 1988). Rates of net nitrification and NO production were determined in the C_2H_2-treated soils as for non-treated soils. Prior to measuring NO production, jars were flushed with C_2H_2-free air for approximately 5 min at 0.06 m^3 h^{-1} in order to assure that residual C_2H_2 levels in the jars were well below concentrations of 100 Pa shown in previous studies to interfere with NO production rate measurements (Bollmann & Conrad, 1997).

The role of chemodenitrification reactions mediated by nitrous acid (HNO_2) in regulating NO production was examined in samples collected in August 2000. Separate replicate subsamples (20–30 g) from each plot were extracted for 10 min with 40 mL of 1 M KCl immediately after measurement of NO production rate. Extracts were centrifuged at 3000 rpm for 20 min and adjusted to pH 7.5–8.5 using dilute sodium hydroxide to minimize complexation of NO_2^- with soil organic matter. Extracts were analyzed for NO_2^- with a Shimadzu UV-1601 spectrophotometer within 4 h of extraction (Vandenabeele et al., 1990). Soil pH was measured on a separate portion of the subsample used for the NO production rate and NO_2^- measurements. Calculated HNO_2 concentrations were determined using the acid dissociation constant (pKa = 3.3), soil pH, and $NO_2^- - N$ concentrations as previously described (Venterea & Rolston, 2000a).

Within each forest type, the effect of N addition was evaluated using one-way analysis of variance (ANOVA) with the level of N addition as the main factor and with subplot measurements considered as treatment replicates, consistent with previous data analysis at the HF chronic-N study (Aber et al., 1993; Rainey et al., 1999; Magill et al., 2000). All statistical analysis was performed using Statgraphics (Manugistics, Rockville, MD).

Results

Field NO and N$_2$O fluxes

Nitric oxide fluxes in the N-treated forest plots were consistently elevated above control plots during most of the sampling period (Fig. 1a). In the Pine site, NO fluxes in both the low-N and high-N plots were consistently elevated above the control plot. In contrast, NO fluxes in the Hardwood low-N plot were not different from the control plot, while fluxes in the high-N plot were elevated and similar in magnitude to fluxes in the Pine high-N plot (50–200 μg N m^{-2} h^{-1}). Using mean monthly plot values over the entire sampling period, NO flux was positively correlated ($P < 0.05$) with soil temperature at the 10-mm depth ($r^2 = 0.34$) and soil temperature at the 50-mm depth ($r^2 = 0.30$–0.33) within each of the N-treated Pine plots. In contrast, no significant correlation with soil temperature was evident in the N-treated Hardwood

plots, or within either of the control plots. There were no significant correlations between soil water content and NO fluxes in either forest.

Fluxes of N_2O were generally $< 10 \, \mu g \, N \, m^{-2} \, h^{-1}$ (Fig. 1b). Although there were significant differences in N_2O fluxes between treatment and control plots at certain times, particularly in the Pine forest, these differences were not as dramatic or consistent as for NO flux. In general, less seasonal variation in N_2O fluxes was evident as compared to the NO flux data. In plots displaying elevated NO fluxes (Fig. 1a), the ratio of N_2O to NO flux (N_2O:NO ratio) was generally < 0.10. The mean N_2O:NO ratio ranged from 0.04 in the Hardwood high-N plot to 0.06 and 0.08 in the Pine low-N and high-N plots, respectively, and was not correlated with organic or mineral soil water content in any of the plots or overall. In the control plots, N_2O fluxes were generally less than zero. No significant correlations between N_2O flux and soil temperature or mineral soil water content were found. Organic soil water content and N_2O flux tended to

be negatively correlated. The negative relationship was significant ($P < 0.05$) in the Hardwood control plot ($r^2 = 0.56$) and the Pine low-N plot ($r^2 = 0.41$).

Nitric oxide fluxes measured 24 h after the NH_4NO_3 application of 29 May 2001 were approximately five and seven times higher than pre-application fluxes in the Pine low-N and high-N plots, respectively (Fig. 2a). An increase of approximately 25% above pre-application levels was observed in the Hardwood high-N forest after 24 h, although this change was within the range of day-to-day variation observed prior to application and 24–48 h after application. Fluxes returned to pre-application levels at both sites 48 h following N addition.

Soil NO fluxes measured 6 and 30 h following a simulated 25-mm rainfall event in mid-August 2001, and 24 h following a similar event in late-October 2001 displayed no detectable differences from pre-wetting fluxes (Fig. 2b). Soil temperatures (T_s) within each plot varied by 2–6 °C at the 10-mm depth, and by only 1–2 °C at the 50-mm depth, during daylight hours on 3 July and 27 October 2001 (Fig. 3). In the July experiment, NO fluxes within each plot were significantly ($P < 0.05$) and positively correlated with T_s at both the 10-mm depth
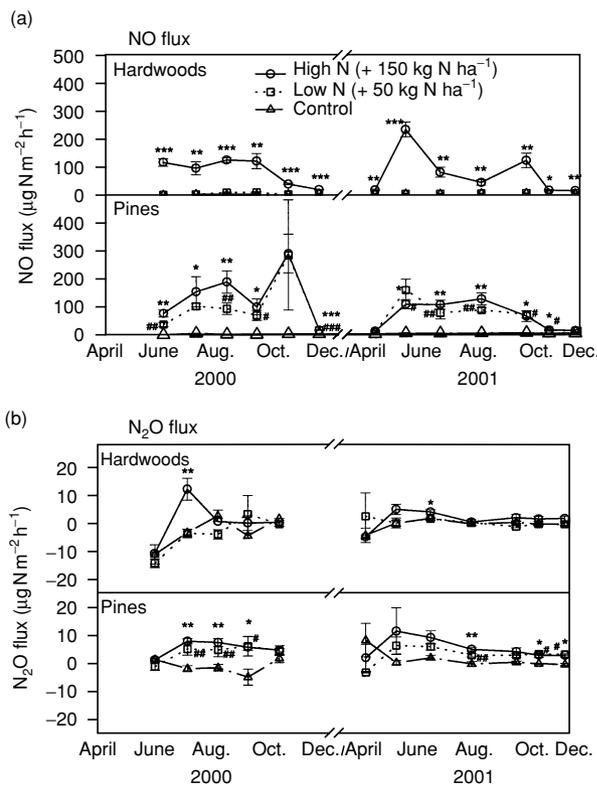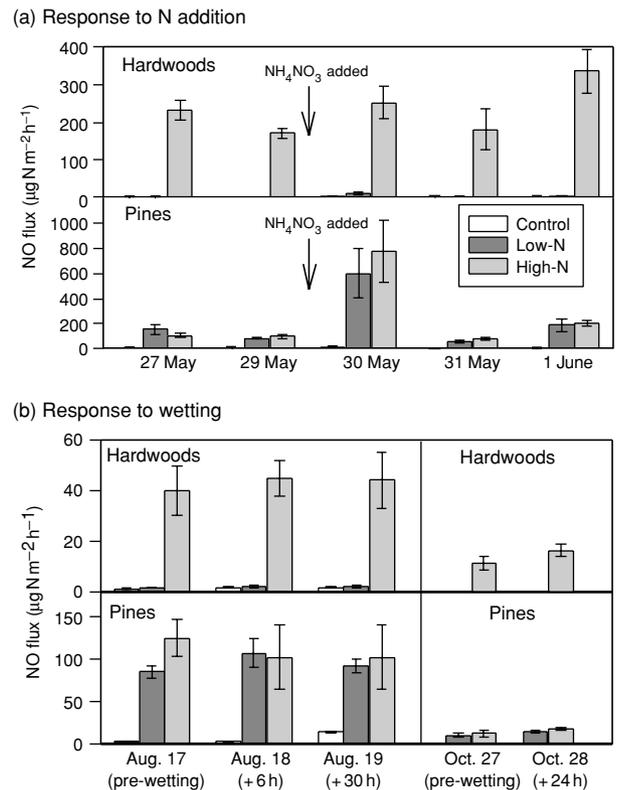

Fig. 1 Fluxes of (a) NO and (b) N_2O during June 2000 through November 2001. Values are the means of three measurements in the control, low-N, and high-N treatment plots at approximately monthly intervals. Symbols indicate if the low-N (#) or high-N (*) plot values are significantly different from the control at any time based on ANOVA with least significant differences multiple range test. # or *$P < 0.05$; ## or **$P < 0.01$; ### or ***$P < 0.001$.

Fig. 2 Response of NO flux to (a) NH_4NO_3 applied on 29 May 2001 and (b) simulated rainfall events on 18 August and 27 October 2001.
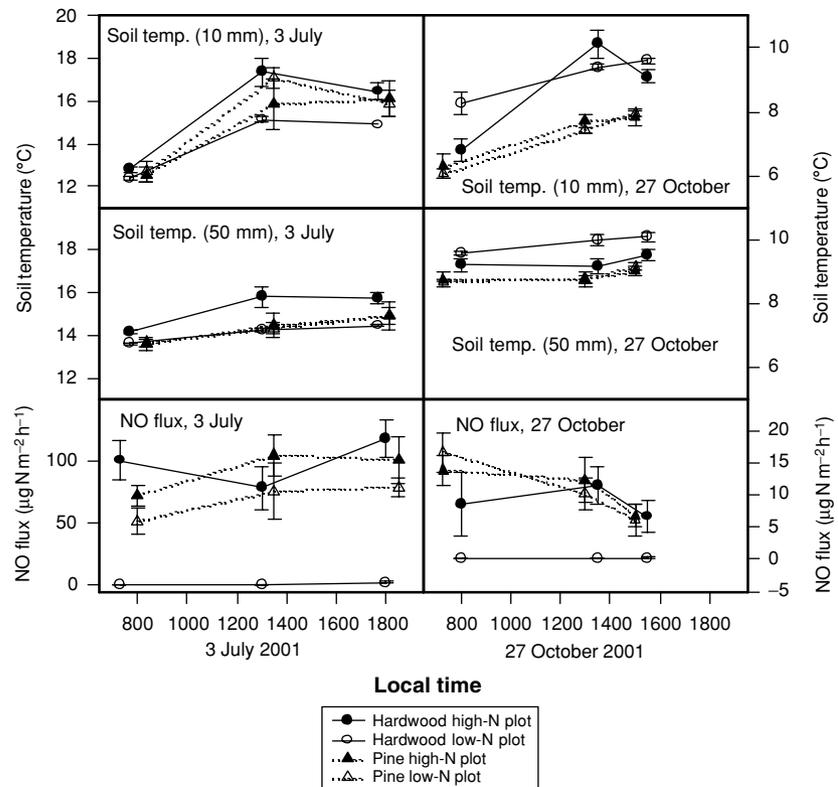
Fig. 3 Soil temperatures at 10 and 50-mm depths and NO fluxes measured during course of day on 3 July 2001 and 27 October 2001.

($r^2 = 0.35$–0.60) and 50-mm depth ($r^2 = 0.46$–0.91) except in the Hardwood high-N plot ($r^2 < 0.1$, $P > 0.5$). In the October experiment, only the Hardwood low-N plot displayed a positive correlation with T_s ($r^2 = 0.69$–0.73, $P < 0.05$), while the Pine low-N plot displayed a significant negative correlation with T_s at the 10-mm depth ($r^2 = 0.54$, $P < 0.05$). In most cases, NO fluxes measured toward the middle of the day were intermediate in magnitude between early morning and afternoon fluxes (Fig. 3).

Soil analysis

Net nitrification rates (NRs) determined in 14-day laboratory incubations of soils collected in August 2000 and June 2001 displayed the same pattern demonstrated by field NO fluxes over the course of the study: i.e., NRs in the Hardwood and Pine high-N plots, and Pine low-N plots, were elevated above control plots (Fig. 4a). Nitric oxide production rates in organic soils displayed the same pattern as field NO fluxes and NRs, and a similar though not entirely consistent pattern was evident in NO production rates in mineral soils (Fig. 4b). The application of acetylene in parallel soil incubations resulted in near-complete inhibition of both nitrification and NO production in mineral and organic soil (Fig. 4a, b). Soil

NO_3^- concentrations displayed the same pattern as laboratory-measured NRs, while soil NH_4^+ concentrations and N mineralization rates displayed no consistent pattern (Table 1).

Nitrite (NO_2^-) levels could not be detected ($< 2 \, \mu g \, N \, kg^{-1}$) in any of the organic soils, but were detectable (3–$11 \, \mu g \, N \, kg^{-1}$) in mineral soils collected August 2000. Nitric oxide production rate (P_{NO}) in Pine mineral soils was positively correlated ($r^2 = 0.66$, $P < 0.001$) with NO_2^- concentration ($r^2 < 0.05$ in Hardwood soils). A kinetic model following that developed by Venterea & Rolston (2000a, b) and represented by

$$P_{NO} = a[HNO_2]^b \qquad (2)$$

was fitted to the data by non-linear regression, where a is the NO production rate coefficient for HNO_2-mediated NO production, and b is the empirical reaction order. The obtained regression models were statistically significant ($P < 0.001$) with reaction order values of 3.6 and 2.4 for Hardwoods ($r^2 = 0.84$) and Pines ($r^2 = 0.90$), respectively (Fig. 5). Soil pH in mineral and organic soils was lower in the N-treated plots compared to the control plots, and were positively correlated with the annual rate of N deposition (Table 2).
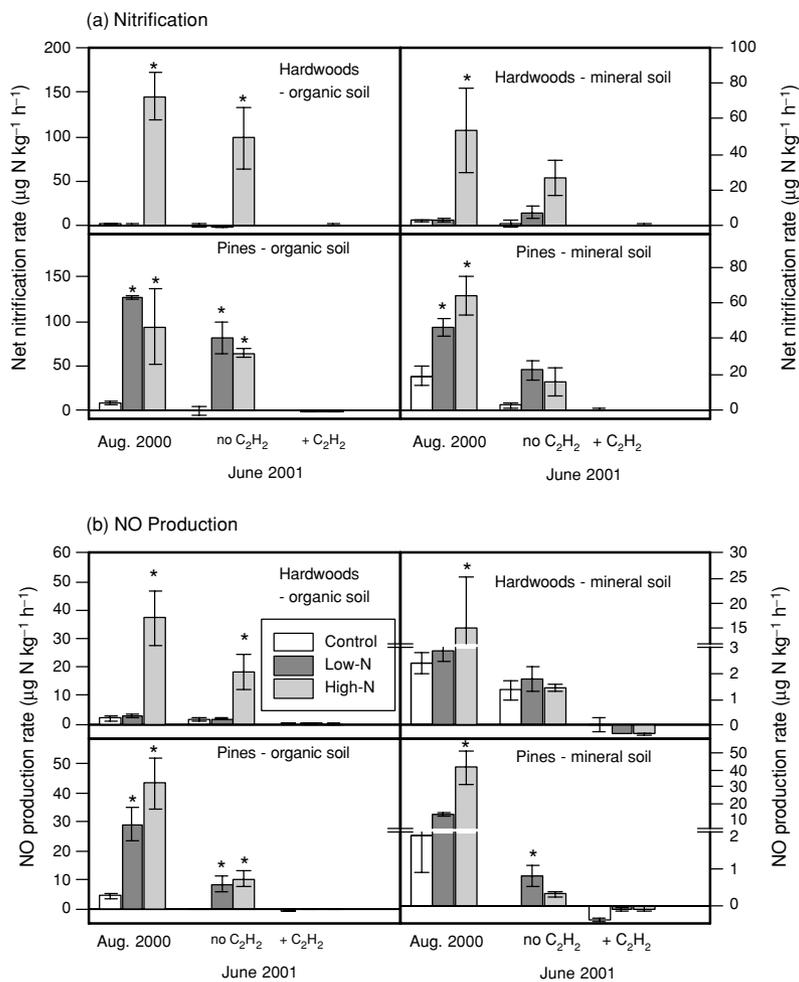
Fig. 4 Rates of (a) net nitrification and (b) NO production in organic and mineral soils sampled August 2000 (incubated without C_2H_2) and June 2001 (incubated with and without C_2H_2). Values are the means of three measurements in the control, low-N and high-N treatment plots. Symbols indicate if the low-N or high-N plot values are significantly different from the control at any time using least significant differences multiple range test. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Note breaks in vertical axis scale for mineral soil NO production rate.

Discussion

Patterns in N oxide gas emissions

Our findings extend previous results from the Harvard Forest chronic-N addition study to include elevated NO flux along with enhanced NO_3^- production and mobility as a response to persistent N inputs (Bowden *et al.*, 1991; Magill *et al.*, 2000). Soil NO fluxes were of similar magnitude to the other major N loss mechanism at HF, i.e., NO_3^- leaching below the root zone. Typical summertime NO fluxes ($100–200 \, \mu g \, N \, m^{-2} \, h^{-1}$, Fig. 1a) were equivalent to 15–45% of annual NO_3^- leaching rates in the high-N plots and $> 100\%$ of NO_3^- losses in the Pine low-N plots estimated for 1996 (Magill *et al.*, 2000). Estimates of NO_3^- losses for the period 1996–2001, which are currently being prepared for publication (A. Magill, personal communication), will allow for contemporaneous comparisons of NO_3^- and NO losses.

The differential response in the two forest types observed here is consistent with trends previously observed at HF. As of 1996, significant increases in nitrification rates were observed in the Pine low-N plot and in both the Pine and Hardwood high-N plots, while NO_3^- leaching was observed only in the high-N plots (Magill *et al.*, 2000). The current findings provide new indication that the Pine low-N plot has advanced beyond initial stages of N saturation, as it is now exhibiting responses similar to both high-N plots with respect to enhanced nitrification and NO emissions (Aber *et al.*, 1998). The Hardwood low-N plot continues to resist changes in N cycling rates.

The elevated NO fluxes observed at HF are similar to fluxes of $20–130 \, \mu g \, N \, m^{-2} \, h^{-1}$ in spruce-dominated plots in Germany which receive $> 30 \, kg \, N \, ha^{-1} \, yr^{-1}$ of deposition (Butterbach-Bahl *et al.*, 1997). Emissions of NO from beech-dominated plots in this forest were lower ($6–47 \, \mu g \, N \, m^{-2} \, h^{-1}$). Thus, the pattern of higher NO

Table 1 Concentrations of nitrate-N and ammonium-N and net N mineralization rates in soils from Harvard Forest chronic-N plots*

| | Nitrate-N concentration ($mg\,N\,kg^{-1}$) | | | | Ammonium-N concentration ($mg\,N\,kg^{-1}$) | | | | Net N mineralization rate ($\mu g\,N\,kg^{-1}\,h^{-1}$) | | | |
| | August 2000 | | June 2001 | | August 2000 | | June 2001 | | August 2000 | | June 2001 | |
	Mineral	Organic	Mineral	Organic	Mineral	Organic	Mineral	Organic	Mineral	Organic	Mineral	Organic
Hardwoods												
Control	0.22 (0.04) a	0.24 (0.08) a	0.69 (0.18) a	0.11 (0.01) a	0.72 (0.09)	7.3 (1.2)	110 (19)	94 (21)	30 (3.8)	300 (51)	4600 (780)	3900 (874)
Low-N	0.48 (0.14) a	1.13 (0.59) a	0.88 (0.16) a	0.95 (0.29) a	1.3 (0.33)	6.9 (2.4)	84 (21)	130 (7.1)	54 (14)	290 (99)	3500 (880)	5400 (290)
High-N	23 (6.1) b	68 (14) b	13 (1.3) b	26 (5.8) b	1.0 (1.4)	9.5 (3.6)	47 (7.8)	47 (46)	42 (56)	400 (150)	1960 (320)	2000 (1900)
Pines												
Control	3.6 (0.68) a	2.5 (1.4) a	0.91 (0.27) a	1.15 (0.50) a	3.4 (1.2) a	34 (12)	33 (27)	66 (25)	33 (2.2) a	100 (38)	−1900 (3700)	**8500 (2600) a**
Low-N	17 (2.1) b	64 (4.5) b	6.5 (1.5) b	27 (6.8) b	4.5 (0.20) a	65 (44)	6.2 (2.8)	43 (9.5)	58 (7.9) b	270 (83)	1700 (390)	**4800 (660) a**
High-N	31 (4.2) c	79 (7.8) b	15 (0.89) c	38 (6.0) b	14 (1.1) b	140 (31)	1.2 (0.21)	69 (19)	76 (9.6) b	100 (17)	670 (280)	**−3400 (3100) b**

*Mean values for each plot are shown, $n = 3$ (standard error of mean in parentheses). Values with same lower case letter designation are not significantly different ($P > 0.05$) based on least significant differences comparisons within each forest type, soil type and sampling date. Significant differences indicated in bold type.
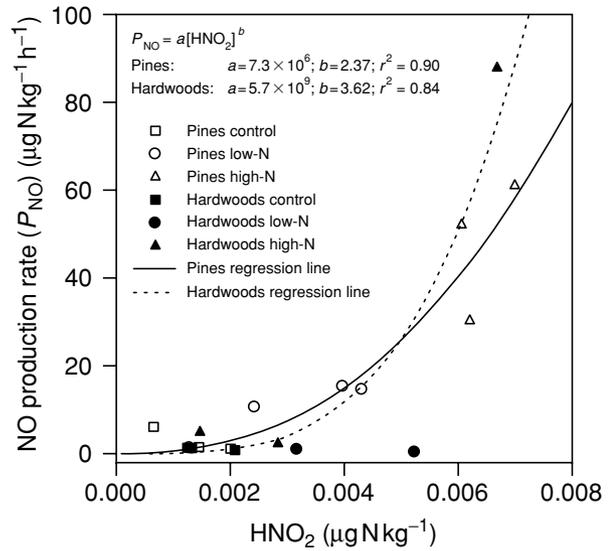

Fig. 5 Laboratory-measured rates of NO production in mineral soils sampled August 2000 vs. calculated nitrous acid (HNO_2) concentrations. Lines are results of non-linear regression analysis using kinetic models in the form of Eqn 2, $P < 0.001$.

fluxes in the N-impacted coniferous forest as compared to the deciduous forest in Germany is similar to the pattern observed in the low-N plots at HF, and is consistent with the idea that deciduous forests in general may have a greater capacity to retain N inputs than coniferous forests with similar land-use histories (Aber *et al.*, 1998).

Questions remain as to the factors controlling differences in response between the two forests. Data through 1996 show that vegetation in the Hardwood plots had assimilated only slightly more of the N added since 1988 than vegetation in the Pine plots. Based on ecosystem N budget calculations through 1996, the major N sink is believed to be some component of soil organic matter, accounting for $\geq 70\%$ of inputs (Magill *et al.*, 2000). Consideration of our first-time estimates of annual NO emissions would reduce the proportion of inputs attributed to soil retention by modest amounts (~ 3–8%, see below). Our data do not suggest that net N mineralization rates (MRs) are limiting nitrification rates in the Hardwood low-N plot by substrate limitation, as there were no consistent trends or differences in soil NH_4^+ or MRs (Table 1). This is consistent with previous findings, which have shown no response, or even a negative response, in MRs to N additions (Aber *et al.*, 1998). Thus, possible explanations for the delayed response in nitrification in the Hardwood forest compared to the Pine forest include those which have been previously discussed in detail by Aber *et al.* (1998), i.e., higher rates of N assimilation by heterotrophic microbes, abiotic processes, and/or mycorrhizae, all of which may compete

Table 2 Soil pH in mineral and organic soils from Harvard Forest chronic-N plots[†]

	Hardwoods				Pines			
	Control	Low-N	High-N	$r^{2\ddagger}$	Control	Low-N	High-N	$r^{2\ddagger}$
Mineral soil	**3.75 (0.06) a**	**3.55 (0.14) ab**	**3.21 (0.10) b**	**0.998****	**3.64 (0.17) a**	**3.31(0.07) ab**	**2.94 (0.04) b**	**0.976****
Organic soil	3.12 (0.19)	2.94 (0.06)	2.66 (0.10)	0.996**	2.94 (0.07) a	2.63 (0.04) b	2.55 (0.07) b	0.755

[†]Mean values for each plot are shown, $n = 3$ (standard error of mean in parentheses). Values with same lower case letter designation are not significantly different ($P > 0.05$) based on least significant differences comparisons within each forest type and soil type. Significant differences indicated in bold type.

[‡]From linear regression of annual N deposition rate vs. soil pH in each forest. Significance level indicated: *$P < 0.05$, **$P < 0.10$; ***$P < 0.01$.

with nitrifying microbes for N, and/or differences in land-use history. Elucidation of these mechanisms remains a critical goal of ongoing research (e.g. Berntson & Aber, 2000).

It is certainly not clear to what extent the responses observed after 12–13 years of artificially high rates of N addition in the high-N plots mimic the cumulative effects which might be observed over longer periods with deposition rates more representative of impacted areas. However, data from the high-N plots at HF have been valuable in suggesting that the two forest types have finite, and differential, capacities to retain added N. In the absence of the high-N treatments, there would be no basis for estimating the limits of N retention in the Hardwood forest (Aber et al., 1998). The results also support general hypotheses about the process of N saturation, whereby N oxide emissions and NO_3^- leaching are proposed as ecosystem responses which are likely to be non-linear with respect to cumulative N inputs (Aber et al., 1998; Fenn et al., 1998), as demonstrated here.

Monthly flux measurements may not be sufficient to very accurately estimate contributions to the overall ecosystem N mass balance because of generally high temporal variability of N oxide fluxes (e.g. Williams et al., 1992). Nonetheless, our short-term data (Figs 2,3) do not indicate that variability at time-scales of days or hours was so extreme as to completely preclude such estimation in the absence of additional data. We therefore applied a set of assumptions used in a previous study to estimate annual N_2O emissions at HF (Bowden et al., 1991), i.e., each sampling date was treated as the mid-point of a sampling period during which gas fluxes were assumed to equal the mean plot flux measured on that date. The estimated total NO emissions were similar in the Hardwood and Pine high-N plots, representing 3.0% (equivalent to 4.7 kg N ha^{-1} yr^{-1}) and 3.7% (5.9 kg N ha^{-1} yr^{-1}), respectively, of total N inputs (background plus experimental) during June 2000–November 2001. The integrated NO mass flux from the Pine low-N plot represented the greatest proportion (8.3% or

4.8 kg N ha^{-1} yr^{-1}) of total N inputs. Emissions from the Hardwood and Pine control plots were 1.3 and 2.3%, respectively, of total inputs, while NO emitted from the Hardwood low-N plot represented the smallest proportion (0.3% or 0.16 kg N ha^{-1} yr^{-1}). These amounts compare to NO_3^- leaching losses representing approximately 2% of inputs to the low-N plots in both forests, and 15 and 4% of inputs to the high-N plots in the Pine and Hardwood forests, respectively, during 1988–1996 (Magill et al., 2000). Total N_2O emissions were < 5% of total NO emissions and < 0.3% of total N inputs in all plots.

Significant positive correlations ($P < 0.01$) were found between net nitrification rates and total N oxide gas fluxes (i.e. $NO + N_2O$) measured at approximately the same time as soil sample collection ($r^2 = 0.49$ in mineral and 0.65 in organic soil). Similarly, total N oxide gas flux was correlated ($P < 0.01$) with soil NO_3^- concentrations in mineral ($r^2 = 0.58$) and organic soil ($r^2 = 0.52$). These relationships are consistent with the hole-in-the-pipe (HIP) model which describes N oxide gas flux primarily as a function of N cycling rates and other indices of N availability, with partitioning between NO and N_2O based on water-filled pore space (Davidson et al., 2000). Soil NH_4^+ concentrations and net N mineralization rates were not correlated ($P > 0.42$) with total N oxide flux. These findings are also consistent with the predominance of nitrification, and not N mineralization, as the main process regulating N oxide emissions at HF.

The short-term responses to N addition observed in the Pine stand following N application (Fig. 2a) represent experimental artefacts of the N treatments, since actual N deposition would occur in more dilute inputs distributed over time. These results point out potential limitations in N deposition simulation studies, some of which have used N application methods similar to those at HF (Kahl et al., 1993; Peterjohn et al., 1998), while others have used more frequent, dilute applications (Klemedtsson et al., 1997). No other studies, to our knowledge, have examined such short-term responses in NO flux. We

hypothesize that the pulse of NO flux in the Pine plots was due to transient increases in the production of NO_2^- by NH_4^+-oxidizing nitrifiers accompanied by a lag in the response of NO_2^--oxidizing nitrifiers (Venterea & Rolston, 2000c).

Sources of NO production

Simultaneous inhibition of nitrification and NO production by acetylene (Fig. 4) indicated that NO production was due to reactions accompanying the autotrophic oxidation of NH_4^- to NO_2^- (Davidson et al., 1986; Klemedtsson et al., 1988). Similar conclusions regarding nitrification as the source of NO production in tropical forest soils have been obtained using similar techniques (Davidson, 1992a,b). The correlation between NO production and HNO_2 (Fig. 5) further suggests abiotic decomposition of HNO_2 (i.e. 'chemodenitrification') during nitrification as an important NO-production mechanism. Nitrous acid-mediated reactions have been indicated as an important mechanism of NO production in agricultural soils (Blackmer & Cerrato, 1986; Venterea & Rolston, 2000a,b) as well as forest and grassland soils (Davidson, 1992a,b; Yamulki et al., 1997). Since chemodenitrification requires the protonation of nitrification-derived NO_2^-, these results together with the pH data (Table 2) suggest that acidity may be an important factor in promoting N losses in forests impacted by atmospheric deposition. Thus, management practices such as amending soils with lime, which has been shown to reduce emissions of N_2O from forest soils (Brumme & Beese, 1992; Klemedtsson et al., 1997), may also be effective in reducing NO emissions. Liming of spruce plots in Southern Germany did in fact result in a 25–30% reduction in NO fluxes (Butterbach-Bahl et al., 1997).

Previous studies have also considered the role of soil organic matter (SOM) in enhancing HNO_2-mediated NO production (Stevenson et al., 1970). Venterea & Rolston (2000a) found a positive correlation between the NO production rate coefficient (a in Eqn [2]), and organic C in three agricultural soils containing 0.3–1.4% organic C. Organic C concentrations in mineral soils at HF are 5–8% (Aber et al., 1993), and the rate coefficients observed in HF mineral soils are approximately 6–8 times greater than the corresponding a values in these agricultural soils (Venterea & Rolston, 2000a). Thus, the current data are consistent with a mechanism of NO production involving reactions between HNO_2 and SOM.

There are methodological challenges in further elucidating the mechanisms of NO production in forest soils. Nitrite levels were not detectable in the organic soils, although organic soils generally displayed higher rates of NO production than mineral soils (Fig. 4b). Organic matter, in particular compounds containing phenolic groups, can interfere with NO_2^- analysis in soil extracts under acidic conditions (Vandenabeele et al., 1990). While we attempted to minimize these interferences, it is not known to what extent these effects influenced our determination of NO_2^- levels. Thus, the importance of chemodenitrification in the organic soils cannot be eliminated. Conversely, the relationships in Fig. 5 do not preclude the importance of other sources of NO production coupled to nitrification, such as biological NO_2^- reduction (Conrad, 1995). The statistical relationship between HNO_2 concentrations and NO production in the Hardwood mineral soils was largely influenced by a single data point exhibiting the highest rate of NO production and HNO_2 concentration (Fig. 5). Thus, the Hardwood kinetic data are not as convincing as the Pine data with respect to the role of HNO_2 in mediating nitrification-derived NO production. This could possibly have been related to variation in soil organic matter quantity or quality within mineral soil samples from the Hardwood plots, reactions between NO_2^- and SOM which may act to fix some fraction of the NO_2^-/HNO_2 (Smith & Chalk, 1980), and/or interferences in the measurement of low NO_2^- levels in organic-rich soils, as discussed above.

Climatic factors

Soil temperature accounted for 30–34% of the overall variance in monthly NO flux within each Pine N-treated plot, but no significant correlations were observed in the Hardwood monthly data. While some previous studies have shown a positive correlation between NO flux and soil temperature (e.g. Williams et al., 1992), recent experimental and modeling studies have suggested that NO consumption, as well as production, exert significant control over net NO emissions, and also that the sensitivity of NO consumption rates to soil temperature may significantly impact overall temperature effects on net NO emissions (Stark et al., 2002; Venterea & Rolston, 2002). Thus, our data may indicate a greater importance of NO consumption in the Hardwood plots with respect to temperature controls. Increased NO consumption with temperature may also explain the decreases in NO flux observed during the afternoon of 27 October 2001 when soil temperatures at the 10-mm depth continued to increase (Fig. 3).

There were no significant correlations between soil water content and NO fluxes. This is consistent with the fact that soil water content may have both negative and positive effects on NO emissions, i.e., increased water availability may enhance nitrification (Schmidt, 1982) while at the same time impeding gaseous diffusion and thereby promoting NO transformation within the soil

matrix prior to release at the soil surface (Davidson *et al.*, 2000; Venterea & Rolston, 2000c). While rain-induced pulses in NO emissions persisting for several days have been observed in seasonally dry ecosystems, including tropical forests and savannas, and chaparral and grasslands in Mediterranean climates (e.g. Davidson 1992a; Otter *et al.*, 1999), similar effects in temperate forests have yet to be demonstrated, and were not supported by our data (Fig. 2b). The amount of water added in the present experiments (25 mm) was selected based on the frequency and quantity of rainfall events occurring at HF. During 2000–2001, nine rainfall events exceeded 25 mm in a 24-h period, but all were < 37 mm, while one event produced 53 mm in a 24-h period. Thus, our results suggest that rain-induced pulses of gas emissions were not important in controlling overall NO emissions during the study period.

Acknowledgements

References

Aber J, Magill A, Boone R *et al.* (1993) Plant and soil responses to chronic nitrogen additions at the Harvard forest, Massachusetts. *Ecological Applications*, **3**, 156–166.

Aber J, McDowell W, Nadelhoffer K *et al.* (1998) Nitrogen saturation in temperate forest ecosystems: hypothesis revisited. *Bioscience*, **48**, 921–934.

Aber J, Nadelhoffer K, Steudler P *et al.* (1989) Nitrogen saturation in northern forest ecosystems. *Bioscience*, **39**, 378–386.

Berntson G, Aber J (2000) Fast nitrate immobilization in N saturated temperate forest soils. *Soil Biology and Biochemistry*, **32**, 151–156.

Blackmer A, Cerrato M (1986) Soil properties affecting formation of nitric oxide by chemical reactions of nitrite. *Soil Science Society of America Journal*, **50**, 1215–1218.

Bollmann A, Conrad R (1997) Enhancement by acetylene of the decomposition of nitric oxide in soil. *Soil Biology and Biochemistry*, **29**, 1057–1066.

Bowden R, Melillo J, Steudler P *et al.* (1991) Effects of nitrogen additions on annual nitrous oxide fluxes from temperate forest soils in the north-eastern United States. *Journal of Geophysical Research-Atmospheres*, **96** (D5), 9321–9328.

Bowden R, Steudler P, Melillo J *et al.* (1990) Annual nitrous oxide fluxes from temperate forest soils in the north-eastern United States. *Journal of Geophysical Research-Atmospheres*, **95** (D9), 13997–14005.

van Breemen N, Burrough P, Velthorst E *et al.* (1982) Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall. *Nature*, **299**, 548–550.

Brumme R, Beese F (1992) Effects of liming and nitrogen fertilization on emissions of CO_2 and N_2O from a temperate forest. *Journal of Geophysical Research-Atmospheres*, **97** (D12), 12851–12858.

Butterbach-Bahl K, Gasche R, Breuer L *et al.* (1997) Fluxes of NO and N_2O from temperate forest soils: impact of forest type, N deposition and of liming on the NO and N_2O emissions. *Nutrient Cycling in Agroecosystems*, **48**, 79–90.

Conrad R (1995) Soil microbial processes involved in production and consumption of atmospheric trace gases. In: *Advances in Microbial Ecology* (ed. Jones J), pp. 207–250. Plenum Press, New York.

Crutzen P (1979) The role of NO and NO_2 in the chemistry of the troposphere and stratosphere. *Annual Review of Earth and Planetary Sciences*, **7**, 443–472.

Crutzen P (1981) Atmospheric chemical processes of the oxides of nitrogen, including nitrous oxide. In: *Denitrification, Nitrification and Atmospheric N_2O* (ed. Delwiche C), pp. 17–44. John Wiley, Chichester.

Davidson E (1992a) Pulses of nitric oxide and nitrous oxide flux following wetting of dry soil: an assessment of probable sources and importance relative to annual fluxes. *Ecological Bulletins*, **42**, 149–155.

Davidson E (1992b) Sources of nitric oxide and nitrous oxide following wetting of dry soil. *Soil Science Society of America Journal*, **56**, 95–102.

Davidson E, Swank W, Perry T (1986) Distinguishing between nitrification and denitrification as sources of gaseous nitrogen production in soil. *Applied and Environmental Microbiology*, **52**, 1280–1286.

Davidson E, Keller M, Erickson H *et al.* (2000) Testing a conceptual model of nitrous and nitric oxide. *Bioscience*, **50**, 667–680.

Fenn M, Poth M, Aber J *et al.* (1998) Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. *Ecological Applications*, **8**, 706–733.

Galloway J, Levy II H, Kasibhatla P (1994) Year 2020: consequences of population growth and development on deposition of oxidized nitrogen. *Ambio*, **23**, 120–123.

Gundersen P, Emmett B, Kjonaas O *et al.* (1998) Impacts of nitrogen deposition on nitrogen cycling: a synthesis. *Forest Ecology and Management*, **101**, 37–55.

Hall S, Matson P (1999) Nitrogen oxide emissions after nitrogen additions in tropical forests. *Nature*, **400**, 152–155.

Johnson D, van Miegroet W, Lindberg S *et al.* (1991) Nutrient cycling in red spruce forests of the Great Smoky Mountains. *Canadian Journal of Forest Research*, **21**, 769–787.

Kahl J, Norton S, Fernandez I *et al.* (1993) Experimental inducement of nitrogen saturation at the watershed scale. *Environmental Science and Technology*, **27**, 565–568.

Klemedtsson L, Klemedtsson A, Moldan F *et al.* (1997) Nitrous oxide emission from Swedish forest soils in relation to liming and simulated increased N-deposition. *Biology and Fertility of Soils*, **25**, 290–295.

Klemedtsson L, Svenssoon B, Rossall T (1988) A method of selective inhibition to distinguish between nitrification and denitrification as sources of oxide in soil. *Biology and Fertility of Soils*, **6**, 112–119.

Lovett G, Reiners W, Olson R (1982) Cloud droplet deposition in sub-alpine balsam fir forests: hydrological and chemical inputs. *Science*, **218**, 1303–1304.

Magill A, Aber J, Berntson G *et al.* (2000) Long-term nitrogen additions and nitrogen saturation in two temperate forests. *Ecosystems*, **3**, 238–253.

Magill A, Aber J, Hendricks J *et al.* (1997) Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. *Ecological Applications*, **7**, 402–415.

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

NADP (2002) *National Atmospheric Deposition Program 2001 Summary.* NADP Report, 2002-01, Illinois State Water Survey, Champaign, IL [http://nadp.sws.uiuc.edu/lib/data/2001as.pdf].

Otter L, Yang W, Scholes M *et al.* (1999) Nitric oxide emissions from a southern African savanna. *Journal of Geophysical Research-Atmospheres*, **104** (D15), 18471–18485.

Peterjohn W, McGervey R, Sexstone A *et al.* (1998) Nitrous oxide production in two forested watersheds exhibiting symptoms of nitrogen saturation. *Canadian Journal of Forest Research*, **28**, 1723–1732.

Rainey S, Nadelhoffer K, Silver W *et al.* (1999) Effects of chronic nitrogen additions on understory species in a red pine plantation. *Ecological Applications*, **9**, 949–957.

Schmidt E (1982) Nitrification in soil. In: *Nitrogen in Agricultural Soils* (ed. Stevenson F), pp. 253–288. American Society of Agronomy, Madison.

Skiba U, Sheppard L, Pitcairn C *et al.* (1999) The effect of N deposition on nitrous oxide and nitric oxide emissions from temperate forest soils. *Water, Air and Soil Pollution*, **116**, 89–98.

Smith C, Chalk P (1980) Fixation and loss of nitrogen during transformations of nitrite in soils. *Soil Science Society of America Journal*, **44**, 288–291.

Stark J, Smart D, Hart S *et al.* (2002) Regulation of nitric oxide emissions from forest and rangeland soils of western North America. *Ecology*, **83**, 2278–2292.

Stevenson F, Harrison R, Wetselaar R *et al.* (1970) Nitrosation of soil organic matter. III. Nature of gases produced by reaction of nitrite with lignins, humic substances and phenolic constituents under neutral and slightly acidic conditions. *Soil Science Society of America Proceedings*, **34**, 430–435.

Tietema A (1993) Mass loss and nitrogen dynamics in decomposing acid forest litter in the Netherlands at increased nitrogen deposition. *Biogeochemistry*, **20**, 45–62.

Vandenabeele J, Verhaegen K, Sudradjat Y *et al.* (1990) Interferences in simple N-determination methods. II. Nitrite in the presence of organic compounds. *Environmental Technology*, **11**, 1129–1135.

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

Venterea R, Rolston D (2000b) Nitric and nitrous oxide emissions following fertilizer application to agricultural soil: biotic and abiotic mechanisms and kinetics. *Journal of Geophysical Research-Atmospheres*, **105** (D12), 15117–15129.

Venterea R, Rolston D (2000c) Mechanistic modeling of nitrite accumulation and nitrogen oxide gas emissions during nitrification. *Journal of Environmental Quality*, **29**, 1741–1751.

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

Verchot L, Davidson E, Cattanio J *et al.* (1999) Land use change and biogeochemical controls of nitrogen oxide emissions from soils in eastern Amazonia. *Global Biogeochemical Cycles*, **13**, 31–46.

Williams E, Geunther A, Fehsenfeld F (1992) An inventory of nitric oxide emissions from soils in the United States. *Journal of Geophysical Research-Atmospheres*, **97** (D7), 7511–7519.

Yamulki S, Harrison R, Goulding K *et al.* (1997) N$_2$O, NO and NO$_2$ fluxes from a grassland: effect of soil pH. *Soil Biology and Biochemistry*, **29**, 1199–1208.