



Soil emissions of nitric oxide in two forest watersheds subjected to elevated N inputs

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Received 19 November 2003; received in revised form 4 February 2004; accepted 21 March 2004

Abstract

The production of nitric oxide (NO) in forest soils can indicate that the ecosystem is progressing toward a state of nitrogen (N) saturation. Soil NO emissions may also have impacts on local tropospheric ozone (O₃) levels. During 2000–2001, we made first-time measurements of NO emissions in two paired watershed studies. In each study, one watershed had been amended with aerial applications of 2.5–3.5 g N m⁻² per year above background atmospheric deposition rates since 1989, and an adjacent watershed served as a reference. In plots at the Fernow Experimental Forest (FEF) in West Virginia and the Bear Brook Watershed in Maine (BBWM), NO emissions in N-amended watersheds (0.61–6.8 μg NO-N m⁻² h⁻¹) were higher than in the reference watersheds (0.21–1.4 μg NO-N m⁻² h⁻¹). In the N-amended watershed at BBWM, NO fluxes in plots dominated by hardwood species were higher than in plots dominated by softwood species, in contrast to previous studies in other forests. Field NO fluxes were correlated with mineral soil nitrate (NO₃⁻) concentrations ($r^2 = 0.65$, $P = 0.016$) across all plots, suggesting that NO emissions may be a reliable indicator of NO₃⁻ leaching potential. Laboratory experiments indicated that nitrification was the dominant source of NO at both sites. At BBWM, increased NO emissions in N-amended soil appeared to result from more rapid nitrification. In contrast, reduced soil pH in N-amended soil at FEF may have caused increased protonation of nitrification-derived nitrite, and the subsequent abiotic formation of NO, even though nitrification rates were not significantly higher than in unamended soil. The results suggest that enhanced soil NO emissions are a characteristic response in forests subjected to elevated N inputs. One possible consequence of higher NO emissions is an increase in O₃-related phytotoxicity. This effect may mitigate the ability of forests to accumulate carbon in response to N inputs or increasing atmospheric CO₂.

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Keywords: N deposition; Nitrification; Ozone; Soil acidity

1. Introduction

Rates of atmospheric N deposition to forests in the northeastern US have remained essentially constant over the past two decades, ranging from >4 g N m⁻² per year in high elevation sites downwind of industrial

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or agricultural areas to $<0.3 \text{ g N m}^{-2}$ per year in remote forests (Lavery et al., 2002; NADP, 2002; Lovett et al., 1982). Persistent N inputs that exceed a forest ecosystem's biological demand may lead to "N saturation", the later stages of which are characterized by nutrient imbalances, soil acidification, NO_3^- leaching, and, ultimately, forest decline (Fenn et al., 1998; Aber et al., 1998).

Several N addition experiments have been initiated in North America and Europe over the past two decades (Magill et al., 2000; Gundersen et al., 1998; Wright and Rasmussen, 1998). These studies have largely focused on the impact of N deposition on NO_3^- leaching and concomitant effects on water quality, soil fertility, and forest productivity. Increased soil N oxide gas emissions have also been proposed as an important response to chronic N inputs, but there have been few measurements to test this hypothesis (Butterbach-Bahl et al., 1997; Skiba et al., 1999). Recent measurements at the Harvard Forest chronic-N-amendment study showed that elevated NO emissions occurred consistently in plots exhibiting increased nitrification and NO_3^- leaching, compared to plots not displaying these symptoms of N saturation (Venterea et al., 2003a).

Soil emissions of NO represent a pathway of ecosystem N export that has potential impacts on proximal forest vegetation and downwind ecosystem quality. Once emitted to the lower atmosphere, NO is rapidly oxidized to nitrogen dioxide (NO_2), and NO and NO_2 together (NO_x) play a central role in regulating tropospheric O_3 production (Crutzen, 1979). Because O_3 formation in rural areas tends to be limited by atmospheric NO_x concentrations, soil NO emissions may affect local ambient O_3 levels (National Research Council, 1992). Also, because NO and NO_2 are eventually converted to nitric acid (HNO_3), NO emissions represent sources of N and acid deposition to downwind ecosystems (Crutzen, 1979).

Two of the longest running examinations of forest responses to simulated N deposition have been conducted at the Fernow Experiment Forest in West Virginia (FEF) and the Bear Brook Watershed in Maine (BBWM). At each of these locations, paired watershed N addition studies were initiated in 1989. In both studies, N has been added via aerial application to one watershed with an adjacent watershed serving as a reference. While the N addition rates are similar at

FEF and BBWM (3.55 and 2.52 g N m^{-2} per year, respectively), the two sites receive substantially different rates of background N deposition (1.90 and 0.84 g N m^{-2} per year, respectively). Several studies have characterized changes in soil N cycling, vegetation, and soil and water quality in response to N inputs at both sites (e.g., Gilliam et al., 1996, 2001; Peterjohn et al., 1996; Adams et al., 1997; Edwards et al., 2002; Kahl et al., 1993; Fernandez et al., 1999, 2003; Jefts et al., 2004). A comparative description of the two studies through 1996 is presented by Fernandez and Adams (2000). The main objectives of the present study were to test the hypotheses that (i) persistent N inputs to temperate forest soils results in elevated soil NO emissions, and (ii) the response will vary among forest types or landscape components. A secondary objective was to investigate processes regulating NO production in soils at BBWM and FEF.

2. Methods

2.1. Study locations and experimental design

The FEF is located in central West Virginia ($39^\circ 03' \text{N}$ latitude, $79^\circ 49' \text{W}$ longitude) and occupies approximately 1900 ha. Since January 1989, one watershed within the forest (WS 3, 34.3 ha) has received experimental N inputs of 3.55 g N m^{-2} per year in the form of $(\text{NH}_4)_2\text{SO}_4$ in addition to background atmospheric N deposition (wet plus dry) of approximately 1.90 g N m^{-2} per year. An adjacent watershed (WS 7, 24.2 ha) has received no experimental N inputs or any other experimental treatments. Aerial applications to WS 3 are made via helicopter as granular $(\text{NH}_4)_2\text{SO}_4$ as follows: 0.71 g N m^{-2} in March and November, and 2.13 g N m^{-2} in July. Watersheds 3 and 7 range in elevation from 730 to 825 m, and are comprised of approximately 30 year old stands of mixed hardwood tree species dominated by black cherry (*Prunus serotina* Ehrh.) and sugar maple (*Acer saccharum* Marsh.). Other important species include black birch (*Betula lenta* L.), American beech (*Fagus grandifolia* Ehrh.), and northern red oak (*Quercus rubra* L.). Soils are mostly sandy and silt loams (Typic Dystrochrepts) formed from sandstone and shale. Annual precipitation is 1450 mm, and mean monthly air temperatures range from -2°C in January

to about 9 °C in July (Gilliam et al., 1996; Peterjohn et al., 1996; Adams et al., 1997; Gilliam et al., 2001).

The BBWM is comprised of two contiguous forested watersheds located in eastern Maine (44°52'N latitude, 68°6'W longitude). Since November 1989, one of the watersheds (West WS, 10.3 ha), has received experimental N inputs of 2.52 g N m⁻² per year in addition to background atmospheric N deposition (wet plus dry) of approximately 0.84 g N m⁻² per year. The adjacent watershed (East WS, 11.0 ha) received no experimental N inputs or any other experimental treatments. Applications to BBWM West WS are made as granular (NH₄)₂SO₄ via helicopter in six equal amounts at bimonthly intervals. Both watersheds encompass the upper 265–475 m of Lead Mountain. In the upper reaches of both watersheds, vegetation is dominated by red spruce (*Picea rubens* Sarg.) with smaller amounts of balsam fir (*Abies balsamea* (L.) Mill.). At lower elevation, dominant species include mixed northern hardwoods dominated by American beech, sugar maple, and red maple (*Acer rubrum* L.), with a mixture of these softwoods and hardwoods mid-slope. Soils are mostly fine sandy loams (Typic and Lithic Haplorthods) formed from dense basal till derived from siltstone and granite. Annual precipitation is 1300 mm, and mean air temperature is 4.9 °C (Kahl et al., 1993; Fernandez et al., 1999, 2003; Pellerin et al., 2002; Elvir et al., 2003).

In summer 2000, plots were established within each of the watersheds described above as locations for the current set of measurements. At BBWM, two 10 m × 10 m plots were established within each of the softwood- and hardwood-dominated zones within each of the two watersheds (total of eight plots). At FEF, two 15 m diameter plots were established within “high” and “low” elevation zones within each of the two watersheds (total of eight plots). The high elevation plots were located at elevations within 5 m of the crest of each watershed, and the low elevation plots were located at elevations within 5 m of the stream gauging stations at the base of each watershed.

2.2. Field nitric oxide fluxes

Within each of the above plots, three permanent gas flux chamber base rings were installed. Chamber base rings constructed of 287 mm diameter (ID) sections of

polyvinyl chloride (PVC) pipe were inserted to a depth of 20–40 mm for the duration of the study. A PVC cylinder (287 mm ID × 40 mm) fitted with stainless steel inlet and outlet fittings were placed on top of the base rings during each measurement. Upon chamber placement, a continuous gas stream (0.03–0.09 m³ h⁻¹) was withdrawn from the chamber and delivered to a chemiluminescent NO analyzer (Unisearch,¹ Ont., Canada). Concentrations of NO in gas recirculating through the analyzer and chamber headspace were recorded at 10–30 s intervals for 4–5 min after placement of the chamber top. Fluxes of NO were calculated from the rate of increase in chamber NO concentrations, the chamber volume, and cross-sectional area. The gas flux chamber design and measurement methods were identical to those used to measure NO fluxes at Harvard Forest, and further method details are presented by Venterea et al. (2003a).

At FEF, NO fluxes were measured at approximately monthly intervals from August to November 2000 and April to November 2001. At BBWM, NO fluxes were measured at approximately monthly intervals during August to November 2000 and in May, July and October of 2001. During each NO measurement, temperatures at 10 and 50 mm below the surface were measured using portable thermistor probes (Fisher, Chicago) inserted into the soil immediately adjacent to each chamber base ring.

2.3. Soil sampling and analysis

On the same day of each gas flux sampling, soil samples were taken from 2 to 4 locations within each plot and mixed together to generate composite samples, which were subsequently analyzed gravimetrically for water content. At BBWM, two composite samples were collected from each plot, one from each of the organic and mineral horizons. A section of PVC plastic pipe (50 mm ID × 200 mm long) was inserted into the soil to a depth of 150 mm. Each core was separated into organic and mineral soil material based on visual and tactile observation. The segregated organic and mineral material was then combined with

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

respective material from other cores taken from within the plot in order to generate the two composite samples. Due to the absence of a well-defined organic soil horizon at FEF, a single composite sample was collected over the top 100 mm using either a trowel or a PVC pipe as described above. Composite samples were dried in the laboratory at 105 °C for mineral soils or 65 °C for organic soil from BBWM for 24–48 h, and moisture content ($\text{g H}_2\text{O g}^{-1}$ dry soil) was determined by difference in mass before and after drying.

In 2001, additional soil sampling was carried out at FEF in May and August, and at BBWM in July and September. Composite samples were collected using methods described above from all eight plots at each site. In the laboratory, samples were first passed through a 6 mm-mesh sieve and homogenized manually by mixing in plastic bags. Separate samples (60–100 g) of each composite were amended with 2.0 mL of potassium nitrate solution ($45 \mu\text{g N mL}^{-1}$) or ammonium nitrate solution ($45 \mu\text{g N mL}^{-1}$) enriched with 99% ^{15}N (Sigma–Aldrich, St. Louis) for determination of gross nitrification rate or gross N mineralization rate, respectively, using ^{15}N dilution techniques (Hart et al., 1994; Venterea et al., 2004). Each of the two amended samples were divided into four sub-samples (15–25 g each), two of which were extracted with 2 N KCl at a 5:1 soil:solution mass ratio, 10–30 min after the addition of the ^{15}N solution. The KCl extracts were subsequently analyzed for $\text{NH}_4^+\text{-N}$ and total $\text{NO}_2^-\text{-N} + \text{NO}_3^-\text{-N}$ using an automated colorimetric analyzer (Perstorp Analytical, Silver Spring, MD). The other two sub-samples were transferred to 250 mL glass jars with screw-on lids for incubation at 20 °C. After 2 days, approximately 5 g of soil was removed from the jars and extracted with 2 M KCl for subsequent analysis of total $\text{NO}_2^-\text{-N} + \text{NO}_3^-\text{-N}$ (for gross nitrification rate) or $\text{NH}_4^+\text{-N}$ (for gross N mineralization rate). Soils remaining in the jars were incubated for an additional 12 days, and then extracted with 2 M KCl for subsequent analysis of total $\text{NO}_2^-\text{-N} + \text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$. Net nitrification rates were calculated from the pre-incubation and 14 days post-incubation total $\text{NO}_2^-\text{-N} + \text{NO}_3^-\text{-N}$ concentrations, and net N mineralization rates were calculated from the pre-incubation and 14 days post-incubation total inorganic N concentrations. Diffusion techniques (Brooks et al., 1989;

Stark and Hart, 1996) were used to prepare samples for determination of the at.% ^{15}N of the pre- and 2 days post-incubation $\text{NH}_4^+\text{-N}$ or $\text{NO}_2^-\text{-N} + \text{NO}_3^-\text{-N}$ pools using isotope ratio mass spectrometry (MS). All ^{15}N analyses were performed at the Stable Isotope Facility, University of California, Davis. Rates of gross nitrification, N mineralization, NH_4^+ consumption, and NO_3^- consumption were calculated using published equations (Hart et al., 1994). For unknown reasons, efficiencies of the ^{15}N diffusion techniques applied to the $^{15}\text{NO}_3^-$ -amended samples from BBWM were so low that the accuracy of the MS analysis was below acceptable limits. Therefore, gross nitrification rate data were not obtained for BBWM soils.

Samples collected from FEF in August 2001 and from BBWM in July 2001 were also analyzed for NO production rate in the laboratory. Immediately prior to the 2 days sub-sampling for gross nitrification (above), NO production rate was determined in the incubating soils by sealing the jars 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 analyzer (Venterea and Rolston, 2000; Venterea et al., 2003a). Rates of NO production were calculated from the difference between NO concentration in air upstream and downstream of the soil, the airflow rate, and the dry soil mass. Rates of NO production were also determined in soils exposed to 30–40 Pa of acetylene (C_2H_2) gas, in parallel soil incubation jars. This amount of C_2H_2 inhibits autotrophic nitrification without significantly affecting denitrification (Klemetsson et al., 1988).

Samples collected from FEF in August 2001 and from BBWM in July 2001 were analyzed for pH by mixing with 1 N KCl at a soil:solution mass ratio of 2:1 (mineral soil) or 5:1 (organic soil). After settling for 60 min, solution was poured off for pH determination.

2.4. Data analysis

For each set of field NO flux measurements, values obtained at the three chamber locations within each plot were averaged to obtain a plot mean for each elevation zone at FEF or species type at BBWM within each of the treated and reference watersheds. Preliminary data analysis indicated that NO fluxes in the N-amended watersheds over all sampling dates at both

sites were positively correlated with fluxes in the reference watersheds when paired by sampling date and elevation or species ($r^2 = 0.49$, $P < 0.0001$). Based on this finding and the paired nature of the experimental design (two levels for all main effects at two sites), we used paired t -tests to evaluate (a) the effect of experimental N inputs on NO fluxes, (b) the effect of elevation at FEF (high or low) or species at BBWM (hardwoods or softwoods), and (c) to compare fluxes at the two sites (Zar, 1996). The treatment (N) effect was examined overall, and within each elevation or species category. Elevation, species, and site effects were examined separately within the N-amended and reference watersheds. For each set of laboratory measurements, two values obtained from duplicate analysis of two soil composite samples were averaged to obtain plot means. Due to the lower sampling frequency for soil variables, the main effect of N addition on soil variables was evaluated using one-way analysis of variance (ANOVA), similar to the analysis used in other long-term N addition studies (e.g., Magill et al., 2000; Venterea et al., 2003a). Soil pH data were transformed to H^+ concentrations for statistical analysis. Paired-sample analysis, ANOVA, and linear regression were performed using Statgraphics Plus 5.0 (Manugistics, Rockville, MD). Non-linear regression analysis was performed using Sigma Plot 8.0 (SPSS, UK). P -values for all regression analyses are <0.05 unless indicated.

3. Results

On nearly every occasion that field measurements were made during 2000–2001 at FEF and BBWM, mean NO emission rates were higher in plots within the N-amended watersheds compared to corresponding plots within the respective reference watersheds ($P < 0.022$, Fig. 1). Differences due to species at BBWM were evident in the N-amended watershed (West WS), where the mean NO flux in the hardwood plots ($4.4 \pm 1.2 \mu\text{g N m}^{-2} \text{h}^{-1}$) was greater than in the softwood plots ($0.61 \pm 0.16 \mu\text{g N m}^{-2} \text{h}^{-1}$) ($P = 0.021$). In the N-amended watershed at FEF (WS 3), the overall mean NO flux in the low elevation plots ($6.8 \pm 2.5 \mu\text{g N m}^{-2} \text{h}^{-1}$) was not significantly different ($P = 0.12$) from the mean NO flux in the high elevation plots ($2.8 \pm 1.1 \mu\text{g N m}^{-2} \text{h}^{-1}$).

Within the reference watersheds, mean NO fluxes were very similar in the low and high elevation plots at FEF ($P = 0.56$) and in the hardwood and softwood plots at BBWM ($P = 0.47$). Fluxes in the N-amended and reference watersheds at FEF (high and low elevation plots combined) tended to be higher when compared to respective fluxes in the N-amended and reference watersheds at BBWM (hardwood and softwood plots combined), but the differences were not significant ($P = 0.23$ for N-amended watersheds, $P = 0.12$ for reference watersheds).

Mean NO fluxes across all plots at both sites were positively correlated with mean mineral soil NO_3^- concentrations ($r^2 = 0.65$, $P = 0.016$). Soil NO emissions within each site, watershed, species type, or elevation were not strongly correlated with soil water content ($r^2 < 0.33$) or soil temperatures at the 1 and 5 cm depths ($r^2 < 0.18$). Consistent with the patterns of field NO flux measurements, mean rates of NO production measured in the laboratory were consistently higher in soils from the N-amended WSs compared to soils from the reference WSs (Fig. 2), although the differences were not significant ($0.10 < P < 0.15$ in all cases). The application of 30–40 Pa of C_2H_2 consistently inhibited NO production in the incubating N-amended soils, indicating that autotrophic nitrification was responsible for the majority of NO produced.

Nitrification rates measured in laboratory incubations tended to be higher in the N-amended plots, although the differences did not occur consistently (Fig. 3). In August 2001, mean net nitrification rates (NRs) in soils from the high and low elevation plots in the N-amended watershed at FEF, and the mean gross nitrification rates (GRs) in soils from the low elevation N-amended plots, were more than two times higher than in soils from corresponding plots in the reference watershed (Fig. 3a). Net nitrification rates were significantly higher in the N-amended plots at BBWM in three out of eight of the data sets (Fig. 3b).

Soil NO_3^- concentrations tended to be higher in the N-amended watersheds compared to the reference watersheds (Tables 1 and 2). There was no consistent pattern and there were few significant differences with respect to the effect of N addition on soil NH_4^+ concentrations or N mineralization rates (Tables 1 and 2). Soil pH was lower in soils from the N-amended

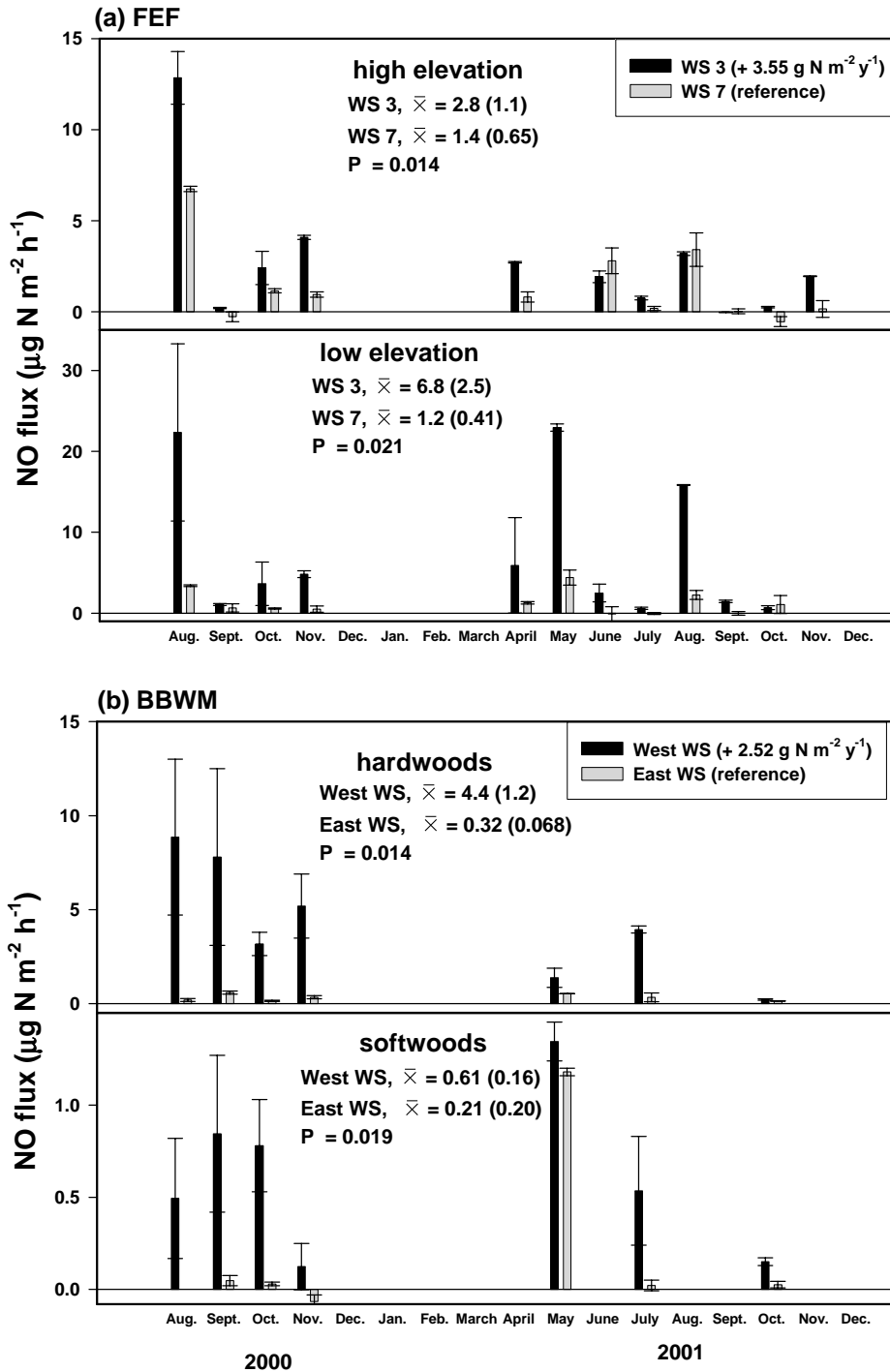


Fig. 1. Nitric oxide (NO) fluxes in reference and N-amended plots at (a) Fernow Experimental Forest, West Virginia (FEF), and (b) Bear Brook Watershed, Maine (BBWM) (mean \pm standard error, $n = 2$). P values are based on two-tailed paired t -tests of N treatment effect within each elevation or species category. Overall means (\bar{x}) and standard errors are shown.

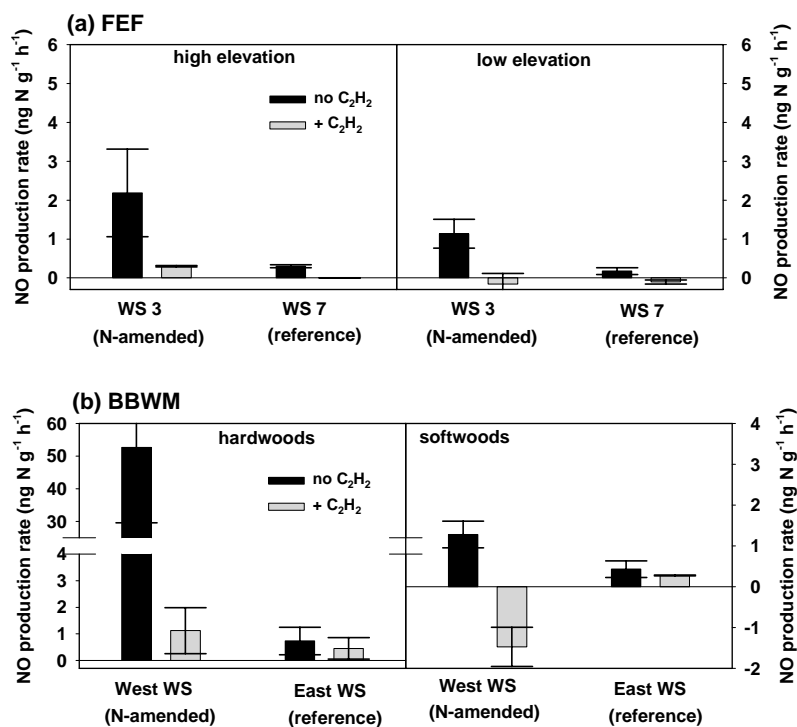


Fig. 2. Laboratory rates of nitric oxide (NO) production with and without the application of 30–40 Pa of acetylene (C₂H₂) in soils from reference and N-amended plots at (a) Fernow Experimental Forest, West Virginia (FEF) collected in August 2001, and (b) Bear Brook Watershed, Maine (BBWM) (organic soils collected in July 2001) (mean ± standard error, $n = 2$).

plots at FEF compared to reference plot soils but did not differ at BBWM (Table 3). There were no significant differences or consistent trends in gross rates of NH₄⁺ or NO₃⁻ consumption. Gross NH₄⁺ consumption varied from 20 to 55 μg N g⁻¹ per day in organic soils at BBWM, and from 1.9 to 5.4 μg N g⁻¹ per day in mineral soils from BBWM and FEF. Gross NO₃⁻ consumption varied from 0.43 to 2.6 μg N g⁻¹ per day in soils from FEF.

4. Discussion and conclusions

4.1. Forest type effects

The elevated NO fluxes observed at FEF and the BBWM hardwood plots are similar in magnitude to fluxes of 6–13 μg N m⁻² h⁻¹ in forest plots in Germany that receive >30 kg N ha⁻¹ per year of atmospheric deposition (Butterbach-Bahl et al., 1997). In the same study, spruce-dominated plots displayed

higher NO fluxes than beech-dominated plots. In the HF study, a similar pattern was observed in that the coniferous stand displayed elevated NO fluxes at a lower level of N addition as compared to the mixed hardwood stand. The current BBWM data are not consistent with these previous studies, in that the softwood (primarily spruce-fir) plots displayed lower fluxes than the hardwood (primarily beech-maple) plots in the N-amended watershed. The contrasting effects of forest types in these studies could be due in part to differences in species composition among the different forests. There is evidence that temperate forest soils that are more influenced by sugar maple and less influenced by red oak trees have lower C:N ratios and support higher rates of nitrification and NO₃⁻ export to headwater streams (Lovett et al., 2002; Venterea et al., 2003b). Thus, the greater proportion of sugar maple and lesser importance of oak species at BBWM as compared to HF may be supporting higher rates of nitrification and NO production in the BBWM hardwood stand.

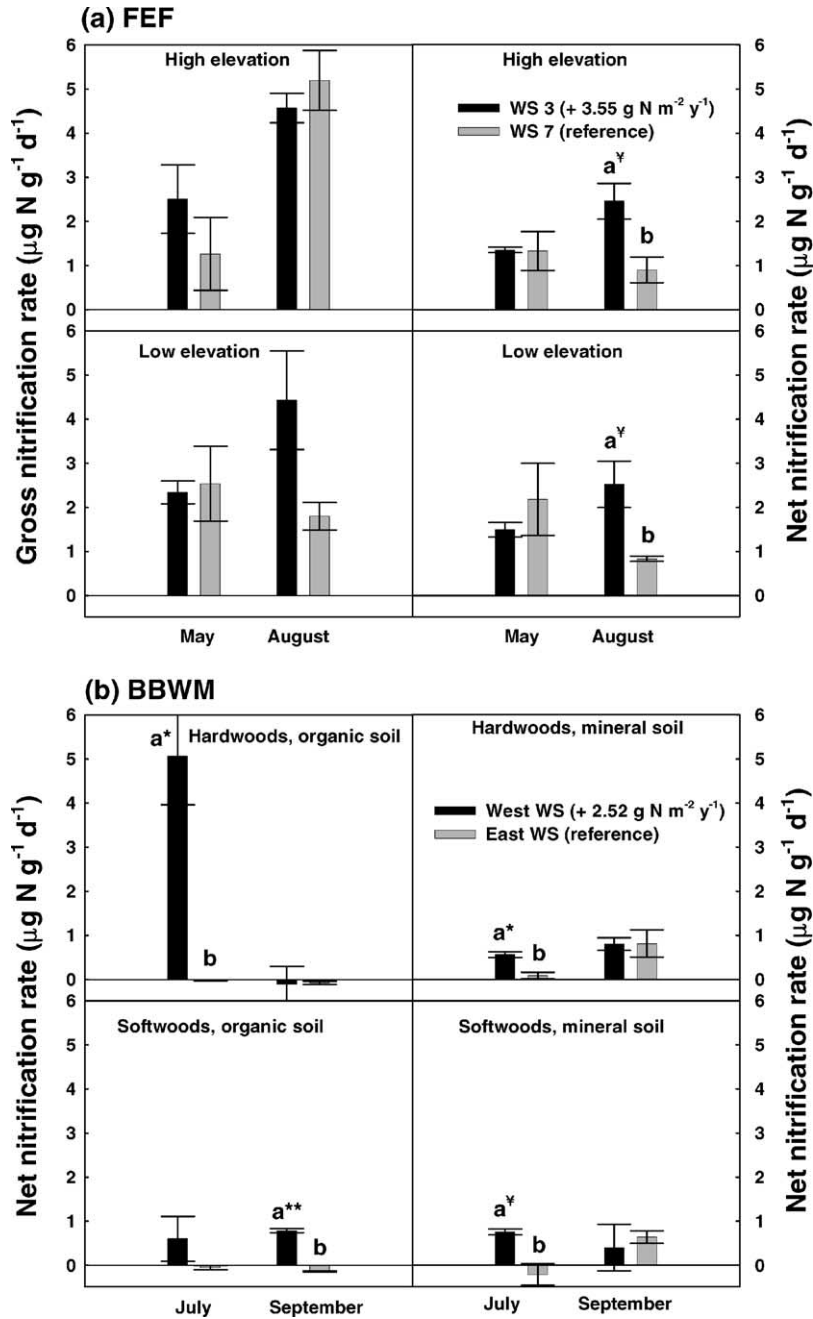


Fig. 3. Rates of (a) gross nitrification (left axis) and net nitrification (right axis) in soils from Fernow Experimental Forest, West Virginia (FEF), and (b) net nitrification in organic soil (left axis) and mineral soil (right axis) from Bear Brook Watershed, Maine (BBWM) (mean \pm standard error, $n = 2$). Bars with different letter designations are significantly different within each site and elevation or species: * $P < 0.05$; ** $P < 0.01$; $\gamma 0.05 < P < 0.10$.

Table 1
Inorganic N levels and N mineralization rates in soil samples from Fernow Experimental Forest, West Virginia^a

	NO ₃ ⁻ -N (μg N g ⁻¹)	NH ₄ ⁺ -N (μg N g ⁻¹)	Gross N mineralization (μg N g ⁻¹ per day)	Net N mineralization (μg N g ⁻¹ per day)
High elevation, May 2001				
N-amended	20 (6.9)	2.2 (0.04)**	4.6 (1.9)	-0.036 (0.078)
Reference	6.0 (1.1)	6.3 (0.27)	3.3 (0.37)	0.22 (0.21)
Low elevation, May 2001				
N-amended	15 (1.9)	4.7 (0.95)	3.2 (0.46)	0.0094 (0.32)
Reference	21 (6.3)	5.1 (0.42)	5.5 (2.3)	0.058 (0.24)
High elevation, August 2001				
N-amended	36 (3.6) a*	2.2 (1.0)	2.8 (0.46)	-0.038 (0.087)
Reference	9.2 (4.2) b	1.0 (0.51)	2.5 (0.31)	0.034 (0.0018)
Low elevation, August 2001				
N-amended	33 (10)	1.2 (0.15)	2.8 (0.078)	-0.0013 (0.040)
Reference	7.6 (0.031)	2.4 (0.71)	3.0 (0.31)	0.026 (0.024)

^a Mean values, $n = 2$ (standard error of mean in parentheses). Values followed by different letter designations are significantly different based on ANOVA comparisons of N-amended vs. unamended soils within each site and elevation or species with confidence level indicated as follows: * $P < 0.05$; ** $P < 0.01$.

4.2. Nitrification rates and site differences

While the sampling frequencies employed here for nitrification rates were low (two times per site), the

data suggest that enhancement of nitrification in response to experimental N inputs at BBWM appears to be more important than at FEF. At FEF, gross and net nitrification in soils from the N-amended

Table 2
Inorganic N levels and N mineralization rates in soil samples from Bear Brook Watershed, Maine^a

	NO ₃ ⁻ -N (μg N g ⁻¹)		NH ₄ ⁺ -N (μg N g ⁻¹)		Gross N mineralization (μg N g ⁻¹ per day)		Net N mineralization (μg N g ⁻¹ per day)	
	Organic	Mineral	Organic	Mineral	Organic	Mineral	Organic	Mineral
Hardwoods, July 2001								
N-amended	26 (2.1) a**	5.9 (0.33) a*	62 (47)	2.2 (0.55)	16 (1.7)	2.9 (0.48)	5.0 (2.6)	0.58 (0.072) a
Reference	0.32 (0.06) b	1.1 (0.57) b	30 (2.0)	7.0 (2.5)	17 (0.24)	3.8 (0.024)	4.6 (0.24)	0.096 (0.072) b
Softwoods, July 2001								
N-amended	9.6 (3.1)	11 (2.9)	50 (10)	7.2 (2.7)	11 (0.72) a*	3.4 (0.14)	0.60 (0.50)	0.39 (0.26)
Reference	1.1 (0.60)	2.7 (1.6)	59 (21)	32 (21)	15 (0.48) b	5.9 (1.5)	-0.048 (0.048)	0.08 (1.6)
Hardwoods, September 2001								
N-amended	180 (9.1)	4.1 (0.27) a*	180 (9.1) a*	11 (11)	15 (7.0)	2.2 (0.32)	2.0 (5.5)	0.57 (0.47)
Reference	110 (11)	1.1 (0.62) b	120 (11) b	0.31 (0.01)	31 (- ^b)	2.5 (0.87)	2.9 (1.5)	-0.55 (0.82)
Softwoods, September 2001								
N-amended	6.4 (6.2)	10 (2.5)	110 (41)	5.7 (5.6)	- (- ^b)	2.4 (1.5)	12 (0.95) a*	-0.18 (0.39) a[‡]
Reference	1.2 (0.39)	3.7 (1.2)	190 (13)	0.21 (0.10)	21 (4.1)	2.6 (0.48)	-1.9 (2.0) b	1.6 (0.19) b

^a Mean values, $n = 2$ (standard error of mean in parentheses). Values followed by different letter designations are significantly different based on ANOVA comparisons of N-amended vs. unamended soils within each site and elevation or species with confidence level indicated as follows: * $P < 0.05$; ** $P < 0.01$; [‡] $0.05 < P < 0.10$.

^b No replication or no data available due to elimination of data points where calculated gross N mineralization rate is less than zero.

Table 3

Soil pH in samples from Fernow Experimental Forest, West Virginia (FEF) collected in August 2001 and Bear Brook Watershed, Maine (BBWM) collected in July 2001

		Soil pH (1:1 M KCl) ^a	
		Organic soil	Mineral soil
FEF			
High elevation	N-amended	–	3.5 a [‡]
	Reference	–	4.0 b
Low elevation	N-amended	–	3.6 a*
	Reference	–	4.7 b
BBWM			
Hardwoods	N-amended	3.0	3.9
	Reference	2.9	3.5
Softwoods	N-amended	2.9	3.5
	Reference	2.6	3.1

^a Mean values shown, $n = 2$ (calculated after transformation to H^+ concentrations). Values with different letter designations are significantly different based on LSD comparisons of N-amended vs. unamended soils within each site and elevation or species with confidence level indicated as follows: * $P < 0.05$; [‡] $0.05 < P < 0.10$.

watersheds did not vary significantly from soils in the reference watershed (Fig. 3a). The higher rates of background atmospheric N deposition at FEF (1.90 g N m^{-2}) may be influencing soil N cycling to a greater extent than at BBWM where background deposition rates are less (0.84 g N m^{-2} per year), resulting in higher nitrification rates in the reference watershed at FEF. Over all the sampling dates, mean net nitrification rates in soils from the reference watershed at FEF ($1.3 \pm 0.27 \mu\text{g N g}^{-1}$ per day) were nearly 10 times higher than in soils from the reference watershed at BBWM ($0.14 \pm 0.10 \mu\text{g N g}^{-1}$ per day) ($P < 0.0001$). Mean NO emissions in the reference watersheds, while not significantly different ($P = 0.12$), also were greater at FEF ($1.36 \pm 0.36 \mu\text{g N m}^{-2} \text{ h}^{-1}$) than at BBWM ($0.27 \pm 0.10 \mu\text{g N m}^{-2} \text{ h}^{-1}$). Thus, these findings together with the results discussed below in Sections 4.3 and 4.4, suggest that (i) enhanced nitrification is primarily responsible for increased NO emissions at BBWM, while (ii) reduced soil pH may be critical in enhancing nitrification-derived NO production in the N-amended soils at FEF.

The range of gross nitrification rates in the N-amended and reference soils from FEF (1.7–5.5 and

1.4–5.8 $\mu\text{g N g}^{-1}$ per day, respectively) are similar to those observed in other N saturated forests. Tietema (1998) found rates in the range of 3.8–6.7 $\mu\text{g N g}^{-1}$ per day in soils from European coniferous forests exhibiting significant NO_3^- leaching. Soils from N-amended plots at HF displayed rates in the range of 1.2–4.8 $\mu\text{g N g}^{-1}$ per day, while rates in the control plots were $< 1 \mu\text{g N g}^{-1}$ per day (Venterea et al., 2004).

4.3. Mechanisms and kinetics of NO production

Previous studies in acidic forest and agricultural soils have suggested that abiotic reactions following the generation of NO_2^- via nitrification may be important sources of NO production (Venterea and Rolston, 2000; Venterea et al., 2003a). Once formed biologically, NO_2^- is protonated to form nitrous acid (HNO_2) to an extent that depends on the pH ($\text{pK}_a = 3.3$). This can be followed by rapid chemical disproportionation of HNO_2 and other reactions of HNO_2 with soil organic and/or mineral constituents, all of which may result in NO production (Nelson, 1982; Stevenson, 1994). Previous studies have found relationships between soil NO production rates and HNO_2 concentrations, which were determined from measured soil NO_2^- and pH levels (Venterea and Rolston, 2000; Venterea et al., 2003a). In the current study, we were unable to obtain reliable NO_2^- data due to high turbidity of soil KCl extracts. The analysis of low levels of NO_2^- in solutions containing organic matter can be problematic (Vandenabeele et al., 1990). In the absence of data, we assumed that NO_2^- concentrations were proportional to the gross nitrification rate (GR). This assumption is supported by a high degree of correlation between soil NO_2^- and net nitrification rates ($r^2 = 0.72$) observed in mineral soils from the red pine plots at HF (Venterea et al., 2003a), and between soil NO_2^- and gross nitrification rates ($r^2 = 0.91$) observed in agricultural soils during rapid nitrification (Venterea and Rolston, 2000). Under this assumption, a proxy estimate of HNO_2 concentrations ($[\text{HNO}_2^*]$) can be calculated from the acid–base equilibrium equation per Venterea and Rolston (2000) given by

$$[\text{HNO}_2^*] = \frac{GR \times 10^{-\text{pH}}}{10^{-\text{pH}} + 10^{-\text{pK}_a}} \quad (1)$$

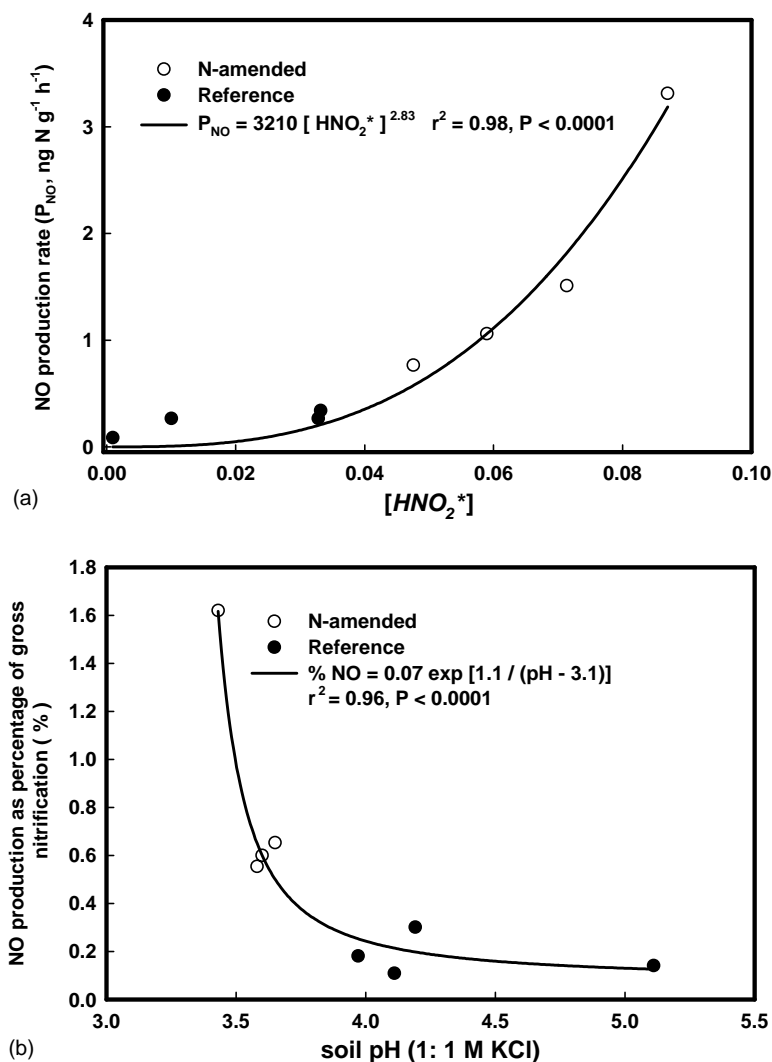


Fig. 4. (a) Nitric oxide (NO) production rate (P_{NO}) vs. a proxy estimate of nitrous acid concentration ($[\text{HNO}_2^*]$, using Eq. (1)), and (b) NO production rate as a percentage of gross nitrification rate vs. soil pH in soils sampled in August 2001 from reference and N-amended plots at Fernow Experimental Forest in West Virginia.

Production of NO was positively correlated with $[\text{HNO}_2^*]$ and increased according to a kinetic model with an apparent reaction order (b) of 2.83 and rate coefficient (a) of 3210 (Fig. 4a).

Comparison of process rates in soils from FEF indicated that the NO production represented approximately 0.15–1.6% of gross nitrification, and that the percentage of gross nitrification represented by NO production decreased exponentially as a

function of soil pH (Fig. 4b) (gross nitrification rates were not obtained from BBWM soils). Both relationships shown in Fig. 4 are supportive of a mechanism of NO production involving HNO_2 . However, these relationships do not preclude other potential sources, including the direct microbial reduction of NO_2^- to NO by autotrophic or heterotrophic bacteria (Conrad, 1995; Beaumont et al., 2002).

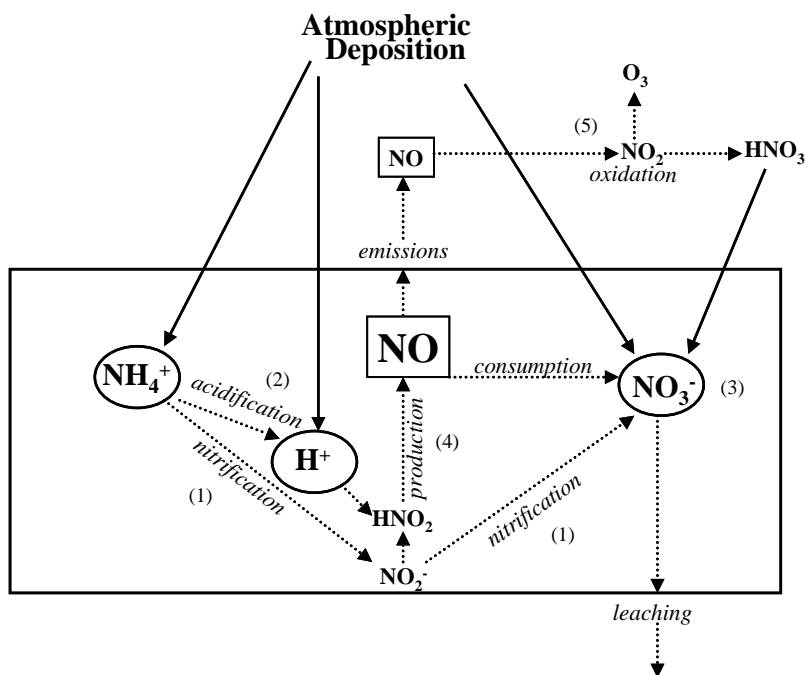


Fig. 5. Illustration of the multiple influences of persistent atmospheric deposition on pathways of N loss in forests soils, including increased: (1) nitrification, (2) acidity, (3) soil NO_3^- concentrations, and (4) NO production via HNO_2 decomposition. Other microbial sources of NO may also be important but are not illustrated. Also shown is (5) the role of NO emissions in contributing to local O_3 formation and downwind NO_3^- deposition.

The current data are therefore consistent with data from HF in indicating that atmospheric deposition may promote the production of NO in forest soils due to (i) the stimulation of nitrification, and/or (ii) the increase in soil acidity deriving directly from acidic deposition and/or as a by-product of nitrification. Each of these effects acting separately would promote the biotic–abiotic reaction pathway of NO production mediated by HNO_2 , and both acting together could have synergistic effects (Fig. 5). Also indicated in Fig. 5 are the possible fates of NO once produced, both within the soil and in the atmosphere. The rapid transformation of NO within the soil matrix, while mitigating NO emissions, serves as a potential source of soil NO_3^- (Venterea and Rolston, 2002; Venterea et al., 2004).

4.4. Soil pH

The lack of a decline in soil pH with N addition at BBWM suggests that the enhanced production of NO

observed in soils from the N-amended watershed at BBWM may have resulted from increased nitrification alone. The already low pH of soils from the reference WS at BBWM (2.6–3.5) compared to the reference watershed at FEF (4.0–4.4) and as compared to soils from the control plots at Harvard Forest (pH \sim 3.6 in mineral soil, pH \sim 3.0 in organic soil), indicates that BBWM soils would be more resistant to pH changes resulting from nitrification-induced H^+ production. For example, a pH of 2.6 at BBWM would represent \sim 63 times the active acidity as a pH of 4.4 at FEF, and thus a given H^+ input would evoke less of a pH change in the BBWM soil. The lower rates of experimental and background N addition at BBWM compared to FEF and HF may also be partly responsible for the lack of a pH decline at BBWM. In contrast, the lack of significant differences in gross or net nitrification rates in the N-amended versus reference soils at FEF (discussed above) suggests that reduced soil pH was primarily responsible for the enhanced NO production in the N-amended soils at FEF (Figs. 4–5, Table 3).

4.5. NO emissions as an indicator of ecosystem N status

The consistent co-occurrence of elevated NO emissions and other symptoms of N saturation (e.g., increased soil NO_3^-) observed at HF, FEF, and BBWM, including the correlation observed here between mean monthly NO flux and mineral soil NO_3^- concentrations ($r^2 = 0.65$), suggests that NO emissions may be a useful tool for assessing ecosystem change with respect to N saturation. Chamber-based NO flux methods, as previously indicated by Skiba et al. (1999), provide real-time, direct, and non-destructive determination of a dynamic property (flux). The method is also highly sensitive, i.e., fluxes of $<0.2 \mu\text{g N m}^{-2} \text{h}^{-1}$ ($<0.018 \text{ kg N ha}^{-1}$ per year) can be easily detected. This high sensitivity may facilitate NO measurements as an early indicator of impending changes, compared to other indicators such as NO_3^- leaching below the root zone or accumulation in stream water (Adams, 2003).

4.6. NO emissions and O_3 phytotoxicity

The current data indicate that NO flux is not a major component of the ecosystem N budget at FEF and BBWM. Even assuming that fluxes of 10, 5 and $1 \mu\text{g N m}^{-2} \text{h}^{-1}$ persisted for the entire year at FEF, BBWM (hardwoods), and BBWM (softwoods), respectively, which is almost certainly an overestimation (Fig. 1), NO emissions would represent $<1.6\%$ of total N inputs to each of these experimentally manipulated ecosystems. However, the impact of summertime NO emissions on tropospheric O_3 and its effects on proximal vegetation may be more important than the contribution to N budgets. The greater importance of O_3 -related phytotoxicity to important tree species in rural compared to urban areas has recently been documented (Gregg et al., 2003). It has also been estimated that 30–50% of all O_3 -monitoring sites in rural areas in the US would be non-compliant with new air quality standards proposed by the US Environmental Protection Agency (Saylor et al., 1998). Ambient O_3 levels in rural systems are expected to be more sensitive to soil NO emissions, since NO_x concentrations tend to limit O_3 production in these areas (National Research Council, 1992). Recent investigations have found that forest ecosystem O_3

exposure in the northeastern US is correlated with atmospheric N deposition rates (Ollinger et al., 2002). Ollinger et al. (2002) also point out that O_3 -related phytotoxicity may act to mitigate any increased productivity that might otherwise occur in response to increased N inputs and/or atmospheric CO_2 . Ozone effects have also recently been found to reduce the accretion of soil organic C that might otherwise occur under elevated CO_2 (Loya et al., 2003). Thus, persistent atmospheric deposition of N and acidity that results in elevated NO emissions may in effect mitigate the capacity of forest ecosystems to accumulate C and counteract CO_2 -induced climate change.

In many cases, the soil NO emission rates found here in the N-amended watersheds (Fig. 1) are significantly higher than the $2 \mu\text{g N m}^{-2} \text{h}^{-1}$ assumed for temperate forests in current models of biogenic gas emissions (e.g., US EPA, 2003). While modeling efforts have concluded that soil NO emissions may have measurable impacts on O_3 levels at the regional scale (Stohl et al., 1996), the impact of soil NO emissions on more local photochemistry has yet to be documented. Estimation of this effect is made more difficult by high uncertainties associated with the magnitude of forest canopy absorption of NO_x (primarily in the form of NO_2) (Jacob and Bakwin, 1991; Guenther et al., 2000). Quantifying the contribution of soil NO emissions to O_3 photochemistry deserves timely consideration given the continued deposition of N to forests in many parts of the world, and given current uncertainties regarding the responsiveness of forest productivity to persistent N inputs and increasing atmospheric CO_2 .

Acknowledgements

The authors gratefully acknowledge J. Demers, L. Godwin, J. Kriebel, S. LaFave, L. Martel, A. Parker, S. Jefts, and A. Wellman for their untiring assistance with the field and laboratory measurements, and M. Burger for reviewing an earlier draft of the manuscript. This work was funded by EPA NCERQA (Grant R827674). We are grateful to International Paper for use of the BBWM site, and the Maine Agricultural and Forest Experiment Station for logistical support. Additional support was provided by USDA-ARS during manuscript preparation.

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