Nitrous oxide production in two forested watersheds exhibiting symptoms of nitrogen saturation

William T. Peterjohn, Richard J. McGervey, Alan J. Sexstone, Martin J. Christ, Cassie J. Foster, and Mary B. Adams

Abstract: A major concern about N saturation is that it may increase the production of a strong greenhouse gas, nitrous oxide (N_2O). We measured N_2O production in two forested watersheds, a young, fertilized forest (WS 3) and an older, unfertilized forest (WS 4), to (*i*) assess the importance of N_2O production in forests showing symptoms of N saturation; (*ii*) estimate the contribution of chemoautrophic nitrification to total N_2O production; and (*iii*) examine the relative importance of factors that may control N_2O production. During the study period, mean monthly rates of N_2O production (3.41–11.42 $\mu g\ N \cdot m^{-2} \cdot h^{-1}$) were consistent with measurements from other well-drained forest soils but were much lower than measurements from N-rich sites with poorly drained soils. Chemoautotrophic nitrification was important in both watersheds, accounting for 60% (WS 3) and 40% (WS 4) of total N_2O production. In WS 3, N_2O production was enhanced by additions of $CaCO_3$ and may be constrained by low soil pH. In WS 4, N_2O production on south-facing slopes was exceptionally low, constrained by low NO_3^- availability, and associated with a distinct assemblage of woody vegetation. From this observation, we hypothesize that differences in vegetation can influence N cycling rates and susceptibility to N saturation.

Résumé : Une préoccupation majeure au sujet de la saturation en azote vient du fait qu'elle pourrait augmenter la production d'un gaz à effet de serre très actif, l'ozyde d'azote (N_2O). Nous avons mesuré la production de N_2O dans deux bassins versants boisés : une jeune forêt fertilisée (WS 3) et une forêt plus vieille non fertilisée (WS 4), dans le but de (*i*) évaluer l'importance de la production de N_2O dans des forêts montrant des signes de saturation en N; (*ii*) estimer la contribution de la nitrification chimio-autotrophe à la production totale de N_2O et (*iii*) examiner l'importance relative des facteurs qui pourraient contrôler la production de N_2O. Pendant la durée de l'étude, les taux mensuels moyens de production de N_2O (3,41–11,42 $\mu g\ N \cdot m^{-2} \cdot h^{-1}$) étaient cohérents avec les mesures pour d'autres sols forestiers bien drainés mais étaient beaucoup plus faibles que les mesures pour des sites riches en N avec des sols mal drainés. La nitrification chimio-autotrophe était importante dans les deux bassins versants et comptait pour 60% (WS 3) et 40% (WS 4) de la production totale de N_2O. Dans le bassin WS 3, la production de N_2O a été favorisée par des additions de $CaCO_3$ et pourrait être inhibée par un faible pH du sol. Dans le bassin WS 4, la production de N_2O sur les pentes exposées au sud était exceptionnellement faible, limitée par la faible disponibilité de NO_3^- et associée à un assemblage distinct de la végétation ligneuse. À partir de cette observation, nous formulons l'hypothèse que des différences dans la végétation peuvent influencer les taux de recyclage de N et la sensibilité à la saturation en N.

[Traduit par la rédaction]

Introduction

Human activities have approximately doubled the rate of N input to the terrestrial biosphere and this change is substantially altering terrestrial ecosystems (Vitousek et al. 1997). Some of the negative consequences of enhanced N deposition for forest ecosystems were recognized as early as 1985 when Nihlgard (1985) proposed his "ammonium hypothesis." Based on observations of forest damage in Europe and Scandinavia, Nihlgard suggested that high levels of N input have "over-saturated" these forests with N in relation to the availability of water and other essential nutrients. During the last decade, continued research into the effects of chronic N deposition on forest ecosystems has led to the current view of N saturation as a continuum of changes that supplies N in excess of the ability of an ecosystem to retain it (Ågren and Bosatta 1988; Aber et al. 1989; Aber 1992).

Concern over nitrogen saturation arises because the increased availability of inorganic N may (*i*) degrade water quality by increasing surface- and ground-water concentrations of NO_3^- (Stoddard 1994); (*ii*) enhance the loss of nutrient cations such as Ca^{2+} and Mg^{2+} from forest soils (Likens et al. 1996; Adams et al. 1997); (*iii*) contribute to forest decline by creating nutrient imbalances, reducing frost hardiness and increasing the sensitivity of the vegetation to

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attacks by insects and pathogens (Schulze 1989); and (*iv*) increase emissions of nitrous oxide (N_2O) to the atmosphere by denitrification and (or) nitrification (Tietema and Verstraten 1991). Enhanced emission of N_2O is of particular interest because N_2O is a strong greenhouse gas (Houghton et al. 1995) and contributes to stratospheric ozone depletion (Cliff and Thiemens 1997; Yung and Miller 1997).

Symptoms of N saturation have been documented for several forest ecosystems in Europe, Japan, and the United States (Hauhs et al. 1989; Gundersen 1991; Johnson and Lindberg 1992; Stoddard 1994; Ohrui and Mitchell 1997). Among the sites in the United States, one of the better documented examples of N saturation comes from over 20 years of research at the Fernow Experimental Forest in West Virginia. Seven symptoms of N saturation were recently identified when two watersheds at this location where compared (Peterjohn et al. 1996). These symptoms include long-term increases in stream-water concentrations of NO_3^- and base cations, a low retention of inorganic N inputs, little seasonal variability in stream-water NO_3^- concentrations, and a rapid increase in NO_3^- loss following fertilization of a young aggrading forest with NH_4SO_4 (Fig. 1). However, no previous measurements of net N_2O production have been made at the Fernow Experimental Forest, despite its potential importance in N-saturated ecosystems. Therefore, the purpose of this study was to examine net N_2O production at two sites in the Fernow Experimental Forest to (*i*) determine the importance of net N_2O production in soils from contrasting forests that exhibit symptoms of N saturation; (*ii*) estimate the relative contribution of nitrification to net N_2O production; and (*iii*) examine the relative importance of factors that may control net N_2O production on these watersheds.

Methods

Study sites

This study was conducted on two adjacent watersheds (WS 3 and WS 4) located in the Fernow Experimental Forest near Parsons, WV (39°3′15″N, 79°41′15″W). Both watersheds are small (ca. 36 ha) and have similar soils, geology, hydrology, climate, and atmospheric N-deposition regimes. Both watersheds also exhibit several symptoms of N saturation (Peterjohn et al. 1996). The soils are primarily a Calvin channery silt loam (loamy-skeletal, mixed, mesic Typic Dystrochrept) that is derived from the acidic sandstone and shale of the Hampshire formation (Losche and Beverage 1967). Between 1951 and 1990 the annual precipitation and streamflow for WS 3 averaged 148 and 66.6 cm, respectively (Adams et al. 1994). During the same period, annual precipitation and streamflow for WS 4 averaged 146 and 64.0 cm. Precipitation is uniformly distributed throughout the year, whereas streamflow is strongly seasonal with peak flows occurring between November and April. Mean monthly air temperatures range from about –2°C in January to about 20°C in July (Adams et al. 1994). The mean annual temperature is about 9°C. Atmospheric N deposition at these sites is high. Between 1984 and 1993, N inputs (NO_3^--N + NH_4^+-N) in bulk precipitation were estimated to be 11.5 kg·ha^{-1} per year for WS 3 and 11.3 kg·ha^{-1} per year for WS 4 (Adams et al. 1997). For both watersheds, 55% of these inputs are in the form of NO_3^--N.

Despite their similarities, WS 3 and WS 4 differ in important ways that result from dissimilar land-use histories. Watershed 3 was heavily cut around 1905, 1958, 1963, and 1970 (Adams et al. 1994). After each cutting, the forest regenerated naturally and is currently a young-growth stand dominated by *Prunus serotina* Ehrh., *Acer rubrum* L., *Betula lenta* L., and *Fagus grandifolia* Ehrh.. As part of a forest fertilization experiment (Adams et al. 1993, 1997), aerial applications of NH_4SO_4 on WS 3 were begun in 1989. To mimic the seasonal pattern of ambient nutrient deposition, three applications of fertilizer are made each year: 7.1 kg N·ha^{-1} in March, 7.1 kg N·ha^{-1} in November, and 21.3 kg N·ha^{-1} in July. The total amount of N added each year (35.5 kg N·ha^{-1}) approximately triples the amount of N received from the atmosphere in bulk precipitation.

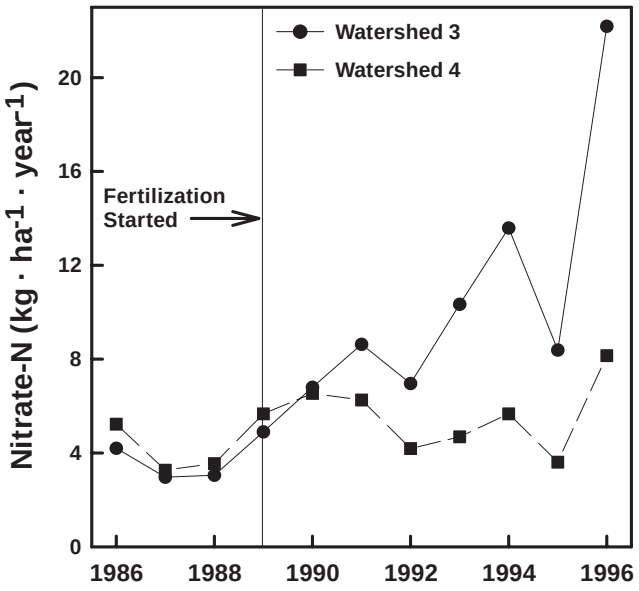


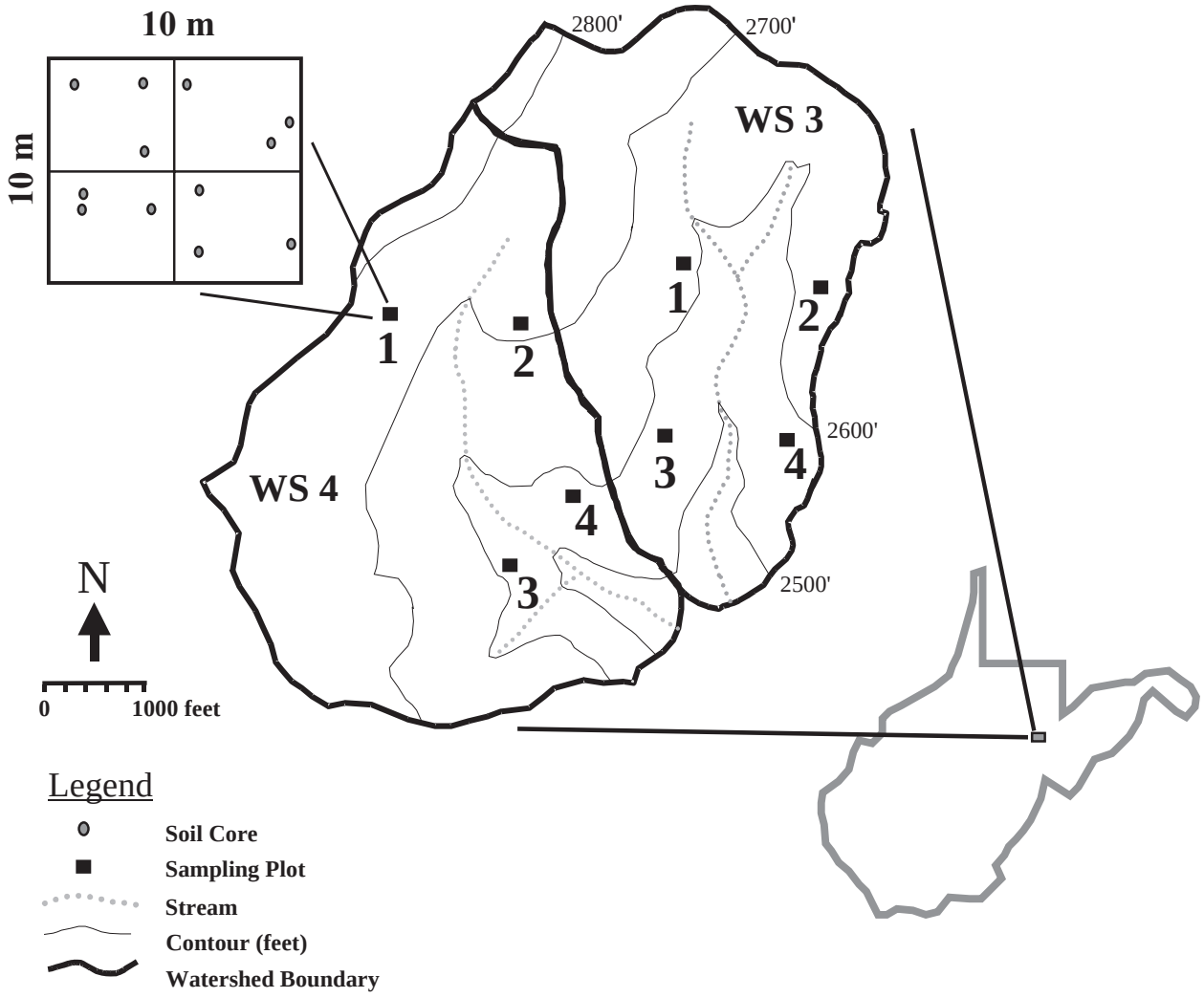
Fig. 1. Annual discharge of NO_3^--N from watersheds 3 and 4 at the Fernow Experimental Forest, West Virginia (M.B. Adams, unpublished data). Watershed 3 was last cut in 1972 and has received 35.5 kg N·ha^{-1} per year as NH_4SO_4 in three applications per year since 1989. Watershed 4 is adjacent to watershed 3 and was last cut in 1905. Watershed 4 has not been fertilized.

In contrast to WS 3, WS 4 has been relatively undisturbed since it was heavily cut around 1905. The only disturbance since that time has been the removal of dead American chestnut trees (*Castanea dentata* (Marsh.) Borkh.) in the 1940s (Adams et al. 1993, 1997). As a result, the forest on WS 4 is a mature stand of trees dominated by *Acer saccharum* Marsh., *Quercus rubra* L., *A. rubrum*, and *P. serotina* (M.J. Christ, unpublished data).

Field sampling

We collected intact soil cores each month from June through October 1994 and used them to measure net N_2O production and the relative contribution of nitrification as a source of N_2O. Additional cores were collected in May of 1995 to investigate factors that may control net N_2O production at these sites. All intact cores were obtained from four 10 × 10 m plots located at midslope positions in each watershed (Fig. 2). The plots were divided into 5 × 5 m quadrants and on every sampling occasion we collected three cores from each quadrant for a total of 96 cores per sampling occasion. Cores were collected by driving sharpened sections (5 × 8 cm) of schedule-40 polyvinyl chloride pipe into the soil with a small sledgehammer. After excavating each core, we sealed them in plastic bags and stored them on ice for transport to the laboratory. On each sampling occasion, we also measured the soil temperature (5-cm depth) and air temperature at each plot.

Fig. 2. Location of study sites and sampling plots. Watershed 3 (WS 3) is a young and aggrading forest that has received 35 kg N·ha^{-1} per year since 1989. Watershed 4 (WS 4) is a mature and unfertilized forest.



Rate measurements

Prior to measuring rates of net N_2O production, intact soil cores were typically stored for <10 days at 4°C. Sample storage at this temperature has previously been shown to have little effect on subsequent rate measurements (Parkin et al. 1984; Breitenbeck and Bremner 1987).

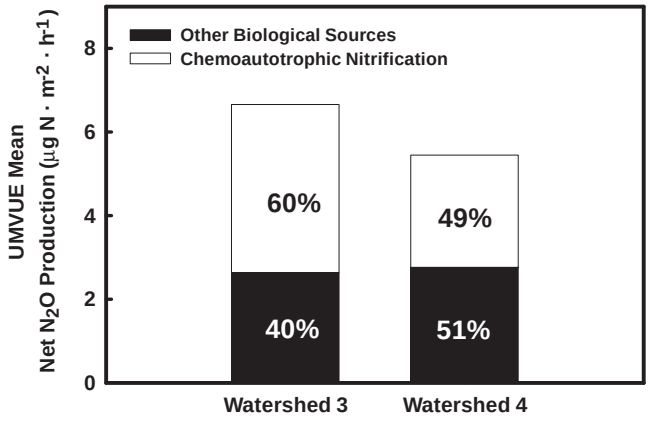
To measure rates of net N_2O production, we warmed the intact cores to room temperature (23°C) and sealed each in an airtight, one-pint (ca. 430 mL), canning jar. A rubber septum was fitted into the top of each jar and was pierced by a single syringe needle attached to an airtight stopcock. This arrangement allowed the air in each jar to be easily mixed and repeatedly sampled using a syringe. At the beginning of each measurement, 40 mL of air were injected into each jar and mixed by repeatedly pumping the syringe. An initial sample of 20 mL was then removed for analysis, leaving a 20-mL overpressure. After 24 h, we thoroughly mixed the air in each jar and removed a second, 20-mL sample. Following the final sample, the chambers were vented, sealed, and stored at 4°C for subsequent measurements of net N_2O production by sources other than nitrification.

To measure the rate of net N_2O production by sources other than nitrification, we used the procedure described above to measure net N_2O production in the presence of 10 Pa of CaC_2-generated acetylene. This amount of acetylene inhibits chemoautotrophic nitrification but typically does not affect N_2O production by other biological sources of N_2O, including denitrification (Davidson et al. 1986; Robertson and Tiedje 1987). Thus, the difference between measurements made with and without 10 Pa of acetylene represents the rate of N_2O produced solely by the process of chemoautotrophic nitrification.

Following the final measurements of N_2O production, we determined the air volume of each jar with the core in place (ca. 330 mL), the soil-moisture content, and the water-filled pore space (WFPS) of each sample. The volume of air in each jar was determined by injecting 20 mL of air and measuring the pressure increase with a pressure transducer (Parkin et al. 1984). Soil moisture was measured gravimetrically after drying for >24 h at 110°C. The WFPS was calculated using measurements of soil moisture in combination with porosity values. Porosity was measured using the gas pycnometer method of Danielson and Sutherland (1986).

Gas samples obtained from the jars and calibration standards were stored in syringes fitted with airtight stopcocks. All samples and standards were then analyzed for N_2O using a Shimadzu 14A gas chromatograph equipped with a ^{63}Ni electron-capture detector. The oven temperature was 40°C, the detector temperature was

Fig. 3. Uniformly minimum variance unbiased estimates of the mean N_2O production rates by chemoautrophic nitrification and other biological sources. Means are for measurements made during the study period (June–October 1994).



300°C, and the carrier gas was 5% CH_4 in Ar flowing at 11.6 mL·min^{-1} through a 10 ft (1 ft. = 0.305 m) stainless-steel column packed with Porapak Q (80:100 mesh).

Rates of N_2O production were calculated by multiplying the volume of air in each jar by the change in N_2O concentration and dividing the result by the incubation time. Concentrations were corrected for dissolved N_2O (Tiedje 1982), and production estimates were corrected for the soil temperatures measured at the time of sampling by assuming a Q_{10} of 2 (Tiedje et al. 1989).

Amendment experiment

To investigate the factors controlling net N_2O production, we conducted an amendment experiment in the laboratory. Three of the 12 soil cores collected from each plot were randomly assigned to one of four treatments: 28 mL of deionized water (equivalent to a 1.4-cm rainstorm); 28 mL of a 1 mM KNO_3 solution; 28 mL of a 1 mM dextrose solution; or 28 mL of a solution containing 50 g $CaCO_3$/L (equivalent to 7 Mg $CaCO_3$/ha). Each soil core was placed in a canning jar and the treatment solution was slowly applied to the top the core. After the treatments were added, the cores acclimated for 24 h at 4°C before the excess solution was drained, and the rate of net N_2O production was measured following the procedure described above. For this experiment we did not determine the relative importance of nitrification to the overall rate of net N_2O production. Following the measurement of net N_2O production, we determined the air volume, soil moisture, and WFPS using the methods described above. We also measured soil pH in 0.01 M $CaCl_2$ (1:2 soil/solution) extracts using an Orion 290A pH meter (McClean 1982).

Data analysis

Field measurements of denitrification and other soil parameters are often lognormally distributed (Parkin et al. 1988; Parkin 1993). Our measurements of net N_2O production closely approximated a lognormal distribution, so we estimated the population means using the uniformly minimum variance unbiased estimator (UMVUE; Gilbert 1987; Parkin and Robinson 1992). This estimator is preferred when the coefficient of variation (CV) of the untransformed data is >1.0 (Parkin et al. 1988). To calculate UMVUE values (which require log transformations), all measurements from an individual core were excluded from the final data set if negative values were obtained for the estimates of net N_2O production by nitrification or net N_2O production by other biological sources. Negative values for nitrification are not biologically possible. Negative values for other biological processes are theoretically possible but rarely reported (Robertson and Tiedje 1987). Use of these criteria removed ca. 20% of the samples for the entire study period but left sample sizes ranging from 29 to 58 for an individual sampling plot.

To evaluate the treatment effects in our amendment experiment, we used a two-way hierarchical ANOVA on the log-transformed data. This procedure tests for significant differences in the geometric mean and is appropriate for this experiment because the intact soil cores served as the experimental units (Parkin and Robinson 1992; Parkin 1993). All statistical calculations were performed using the computer program STATISTICA release 5 (StatSoft 1995). The statistical model we used treated the sampling plots as a random factor and included the following terms: watershed; plot nested within watershed; treatment type; and the appropriate interactions. Because of the high spatial variability inherent in measurements of net N_2O production, we tested for significance at the level of $\alpha = 0.10$.

Results

Rate measurements

During the study period (June–October), the rate for net N_2O production was 22% higher for WS 3 (6.6 ± 0.7 μg N·m^{-2}·h^{-1}; mean ± SE) than WS 4 (5.4 ± 1.4 μg N·m^{-2}·h^{-1}). Although this difference is not statistically significant, it resulted from a greater production of N_2O by chemoautotrophic nitrification in WS 3 (Fig. 3). In WS 3, chemoautotrophic nitrification accounted for 60% of net N_2O production. In contrast, it accounted for 49% of net N_2O production in WS 4.

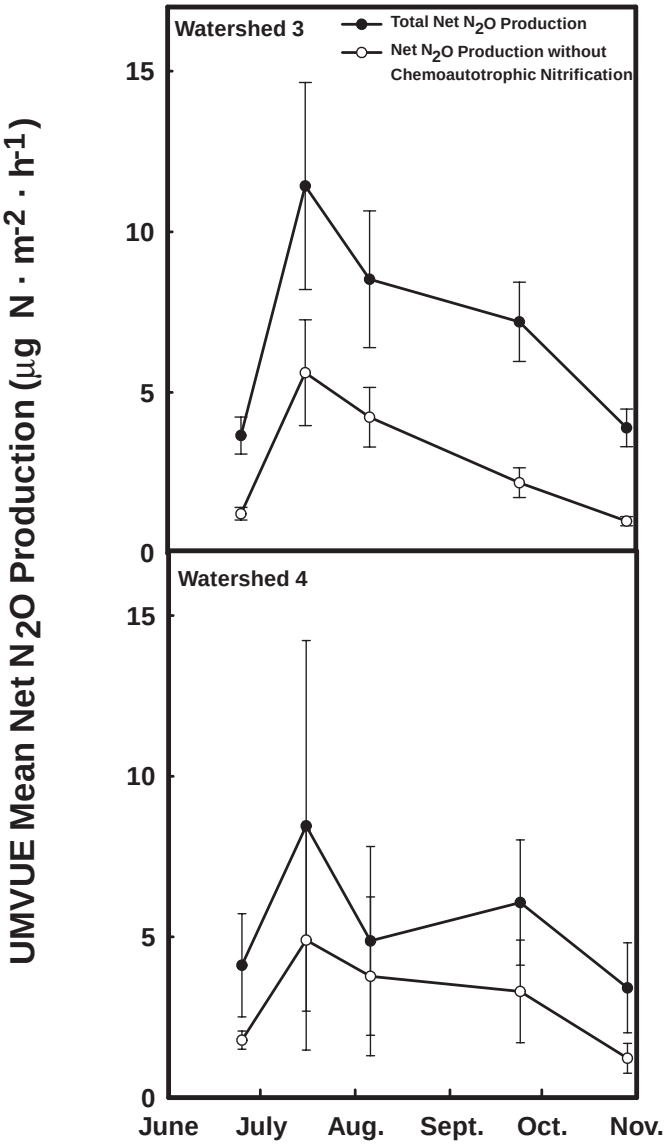
In both watersheds, the rates of net N_2O production showed a seasonal pattern with production peaking in July (Fig. 4). A different seasonal pattern was found for the relative contribution of chemoautotrophic nitrification to net N_2O production. Nitrification contributed the most to net N_2O production in June and October (ca. 70% for WS 3 and 60% for WS 4) and the least in August (ca. 50% for WS 3 and 23% for WS 4).

In addition to temporal differences in net N_2O production, spatial differences were also apparent within a given watershed (Fig. 5). In WS 3, similar rates of net N_2O production were measured in plots 1–3 but rates measured in plot 4 were ca. 60% lower. A more meaningful spatial pattern occurred in WS 4. In this watershed, net N_2O production was 93% lower in plots located on south-facing slopes (plots 2 and 4; Fig. 2) than in plots on more east-facing slopes (plots 1 and 3). These aspect-related differences within WS 4 were not associated with large differences in either average soil temperature (17.8 vs. 17.4°C for south- vs. east-facing slopes), air temperature (14.0 vs. 14.0°C), WFPS (37.6 vs. 39.1%), or soil pH (3.92 vs. 3.91). No large differences or apparent spatial patterns were observed for the relative contribution of chemoautotrophic nitrification to net N_2O production.

Amendment experiment

After adding 28 mL of solution and allowing it to drain for 24 h, the soils in this experiment had an average WFPS (ca. 80%) that was double the value measured in samples used to estimate field rates of N_2O production. It is likely that these high values for WFPS contributed to the relatively high rates of N_2O production. Net N_2O production in soil

Fig. 4. Uniformly minimum variance unbiased estimates of the mean monthly N_2O production rates in the presence and absence of an inhibitor of chemoautrophic nitrification. Error bars are SE.



cores receiving only deionized water had a mean production rate (ca. 1540 μg $N \cdot m^{-2} \cdot h^{-1}$) that was >200 times the average rate measured under field conditions (ca. 6 μg $N \cdot m^{-2} \cdot h^{-1}$).

When tested at the level of $\alpha = 0.10$, ANOVA revealed a significant plot effect ($p < 0.0001$), plot × treatment interaction ($p = 0.0007$), and watershed × treatment interaction ($p = 0.0829$). In light of the significant watershed × treatment interaction, we used separate ANOVA procedures to analyze the data from each watershed independently. The statistical model used in these ANOVAs included the following factors: plot, treatment, and the plot × treatment interaction.

When data from WS 3 were analyzed independently, ANOVA revealed a nearly significant treatment effect ($p = 0.1064$) and no significant plot ($p = 0.75$) or plot × treatment interaction ($p = 0.45$). In the absence of a significant interaction, we eliminated plot from the statistical model and found a significant difference ($p = 0.029$; Tukey HSD test) between net N_2O production in cores receiving KNO_3 and those receiving $CaCO_3$ (Fig. 6). Overall, it appears that additions of $CaCO_3$ stimulated net N_2O production and that supplemental KNO_3 tended to suppress net N_2O production relative to the deionized water and dextrose treatments.

When the data from WS 4 were analyzed independently, ANOVA revealed a highly significant sampling plot effect ($p < 0.0001$) and plot × treatment interaction ($p = 0.0001$). In light of the significant interaction, we used separate ANOVA procedures to analyze the data from each plot independently. For plots with a more easterly aspect (plots 1 and 3), there were no significant treatment effects (Fig. 7). In contrast, net N_2O production in plots with a more southerly aspect differed significantly in response to the treatment solutions. In plot 2, a significant treatment effect ($p = 0.037$) was created by the lower production rates in cores receiving dextrose and the higher production rates in cores receiving KNO_3. In plot 4, the addition of KNO_3 increased net N_2O production rates above all other treatments and created a significant overall treatment effect ($p = 0.00152$).

Discussion

Rate measurements

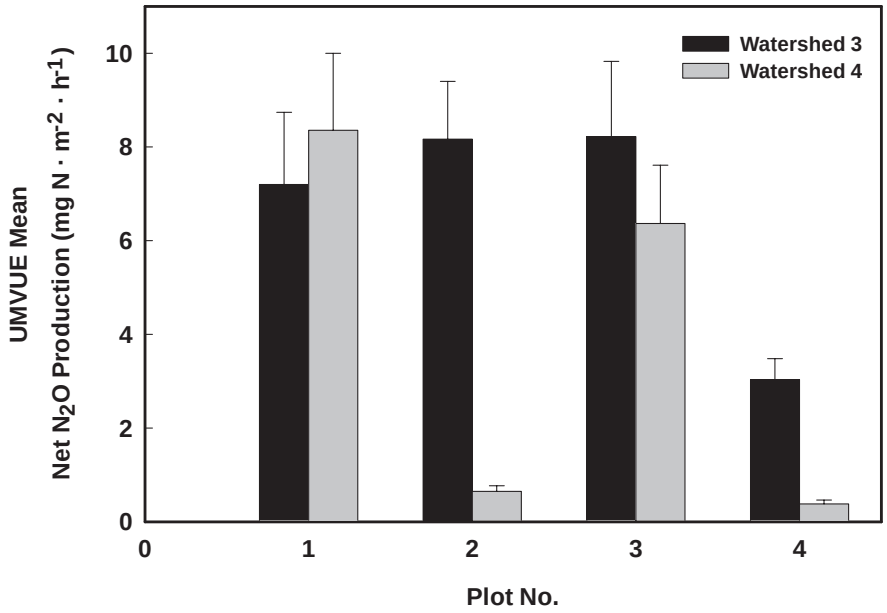
Our estimates of the mean rate of net N_2O production in WS 3 (6.6 μg $N \cdot m^{-2} \cdot h^{-1}$) and WS 4 (5.4 μg $N \cdot m^{-2} \cdot h^{-1}$) appear to be consistent with rates from other forests on well-drained soils, including forests located in regions of high N deposition and sites receiving N fertilizer. In Europe, for example, measurements from central and northern Germany range from 3.00 to 10.6 μg $N \cdot m^{-2} \cdot h^{-1}$ (Schmidt et al. 1988; Mogge et al. 1998), and measurements from southern Sweden range from 0.81 to 2.90 μg $N \cdot m^{-2} \cdot h^{-1}$ (Klemedtsson et al. 1997). In the eastern United States, similar measurements range from –1.12 to 5.24 μg $N \cdot m^{-2} \cdot h^{-1}$ (Keller et al. 1983; Bowden et al. 1990, 1991; Castro et al. 1993, 1994; Peterjohn et al. 1994).

The addition of N fertilizer typically increases the rate of N_2O emissions from well-drained forest soils (Bowden et al. 1991; Castro et al. 1993; Klemedtsson et al. 1997). However, even when fertilized, the amount of N lost as N_2O is quite small, typically ≤1 kg $N \cdot ha^{-1}$ per year (Bowden et al. 1990, 1991; Klemedtsson et al. 1997; Mogge et al. 1998).

The highest measurements of N_2O flux appear to be associated with N-rich sites that have wet soils or a periodically shallow groundwater table. For example, Castro et al. (1994) measured an average N_2O flux of 38.7 μg $N \cdot m^{-2} \cdot h^{-1}$ from a fertilized (180 kg N per year) slash pine (*Pinus elliottii* Engelm.) plantation in Florida with poorly drained soils and a water table that was periodically at the surface. Even higher rates are reported for poorly drained sites in Europe. For example, Mogge et al. (1998) measured N_2O emissions of 55.9 μg $N \cdot m^{-2} \cdot h^{-1}$ in a black alder (*Alnus glutinosa* (L.) Gaertn.) forest adjacent to Lake Belau in northern Germany, and Tietema et al. (1991) report emissions of 228 μg $N \cdot m^{-2} \cdot h^{-1}$ for a N saturated oak–beech forest in the Netherlands that periodically has groundwater within 15–45 cm of the surface.

Although net N_2O production in upland forests experiencing N saturation may be small, the results reported in the literature suggest that N saturation could still significantly

Fig. 5. Uniformly minimum variance unbiased estimates of the mean N_2O production rates for each plot sampled during the study period (June–October 1994). Error bars are SE.



increase N_2O emissions to the atmosphere if the NO_3^- exported from these sites in stream water is subsequently reduced to N_2O in more anaerobic downstream habitats.

For the entire study period, our measurements of net N_2O production in WS 4 were more variable (CV = 338%) than those measured in WS 3 (CV = 148%). In part, the greater variability in WS 4 was due to large among-plot differences in net N_2O production rates. In WS 4, our measurements of net N_2O production in plots 1 and 3 were similar to each other and ca. 10 times greater than measurements made in plots 2 and 4 (Fig. 5). These among-plot differences in WS 4 are especially interesting because plots with similar rates of net N_2O production are located on slopes with a similar aspect (Fig. 2). In general, plots located on more south-facing slopes (plots 2 and 4) had lower rates of N_2O production. A similar spatial pattern between the plots in WS 4 was not observed in our measurements of soil temperature, air temperature, WFPS, or soil pH. This suggests that the aspect-related differences in N_2O production are controlled by other factors.

In both watersheds, chemoautotrophic nitrification made a significant contribution to net N_2O production: 60% in WS 3 and 49% in WS 4. These values fall within the wide range (0–64%) reported for other forest soils (Robertson and Tiedje 1987). The greater contribution of chemoautotrophic nitrification in WS 3 is also consistent with the fact that this watershed has been fertilized with NH_4SO_4 since 1989.

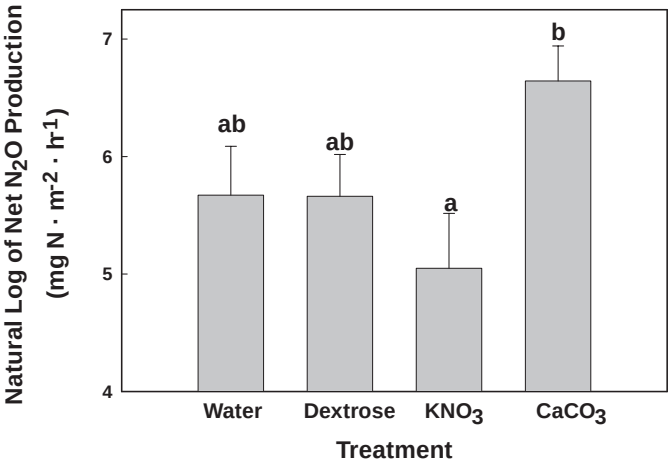
The portion of net N_2O production that is not due to nitrification is often attributed solely to denitrification (Davidson et al. 1986; Klemedtsson et al. 1988). This is probably the case in our study, however we cannot unequivocally associate net N_2O production in the presence of C_2H_2 solely to the process of denitrification (Bleakley and Tiedje 1982). For example, Robertson and Tiedje (1987) found that unknown biotic processes, which may have been due to fungi, accounted for up to 77–100% of the N_2O produced in some forest soils in Michigan. For most forest soils, however, the potential contribution of processes other than nitrification and denitrification to N_2O production remains largely unknown.

Amendment experiment

Compared with our estimates of net N_2O production under field conditions (Fig. 4), the mean rates of net N_2O production were more than 100 times greater in our amendment experiment. This difference was probably the response of denitrification to anaerobic conditions created by a greater WFPS. Denitrification increases dramatically when O_2 concentrations are <0.1% (Parkin and Tiedje 1984), and it becomes increasingly important as WFPS exceeds 60% (Linn and Doran 1984; Davidson 1991; Groffman and Tiedje 1991; Scholefield et al. 1997). Furthermore, under conditions of high WFPS, the contribution of denitrification to net N_2O production has been found to be one to two orders of magnitude greater than the contribution of chemoautotrophic nitrification (Davidson 1991; Rudaz et al. 1991). The influence of O_2 concentrations on denitrification rates in a temperate forest were clearly shown by Davidson and Swank (1987), who measured rates that were 1000 times greater when soils were wet and experienced high NO_3^- availability. From the observations in our study, the greater production of N_2O under wetter conditions suggests that on-site losses of N_2O from upland forests experiencing N saturation will be severely constrained by aerobic conditions typical of well-drained soils.

The response of soil cores from WS 3 to the various treatments suggests that conditions for net N_2O production were nearly optimal with respect to the availability of C and NO_3^-. For the soils sampled in this watershed, the primary constraint on net N_2O production, other than soil moisture, may be the low soil pH. The average soil pH in cores receiving only deionized water was 3.56, and the pH increased to 5.36 in cores receiving a $CaCO_3$ solution. In addition to increasing soil pH, amending soils with $CaCO_3$ more than doubled

Fig. 6. Treatment effects on N_2O production rates when averaged across all plots sampled in watershed 3. Error bars are SE, and different letters indicate significant differences ($p < 0.1$). Values are the means and standard errors of log-transformed data. A simple back transformation of these means yields the geometric mean of the untransformed data.



the geometric mean rate of net N_2O production: 290 μg $N \cdot m^{-2} \cdot h^{-1}$ for water alone and 767 μg $N \cdot m^{-2} \cdot h^{-1}$ for soils treated with $CaCO_3$. In general, an increase in soil pH can affect N_2O production by increasing the overall rates of nitrification and denitrification or by decreasing the ratio of N_2O to N_2 produced by denitrifying organisms (Focht and Verstraete 1977; Firestone et al. 1980; Knowles 1982). Since the conditions of our experiment (i.e., high WFPS) favored denitrification, the response we observed was probably caused by an overall increase in the activity of denitrifying bacteria. More specifically, the increase in net N_2O production suggests that N_2O-producing microbes have not completely adapted to ambient levels of soil pH (Parkin et al. 1985; Martikainen and DeBoer 1993) or that acid-sensitive and acid-tolerant microbes coexist in these soils (DeBoer et al. 1990). In either case, it appears that liming this watershed might increase N_2O emissions to the atmosphere.

The response of soil cores from WS 4 to the various treatments indicated that the factors controlling net N_2O production depended on the plot being sampled. More east-facing plots (plots 1 and 3) had higher rates of net N_2O production and conditions that were nearly optimal for net N_2O production with respect to the availability of C and NO_3^-. Rates of net N_2O production in these plots were also insensitive to additions of $CaCO_3$. This suggests that the organisms responsible for net N_2O production in these plots may have a broad pH optimum, occupy protected microsites, and (or) form protective microbial aggregates (DeBoer et al. 1990, 1991).

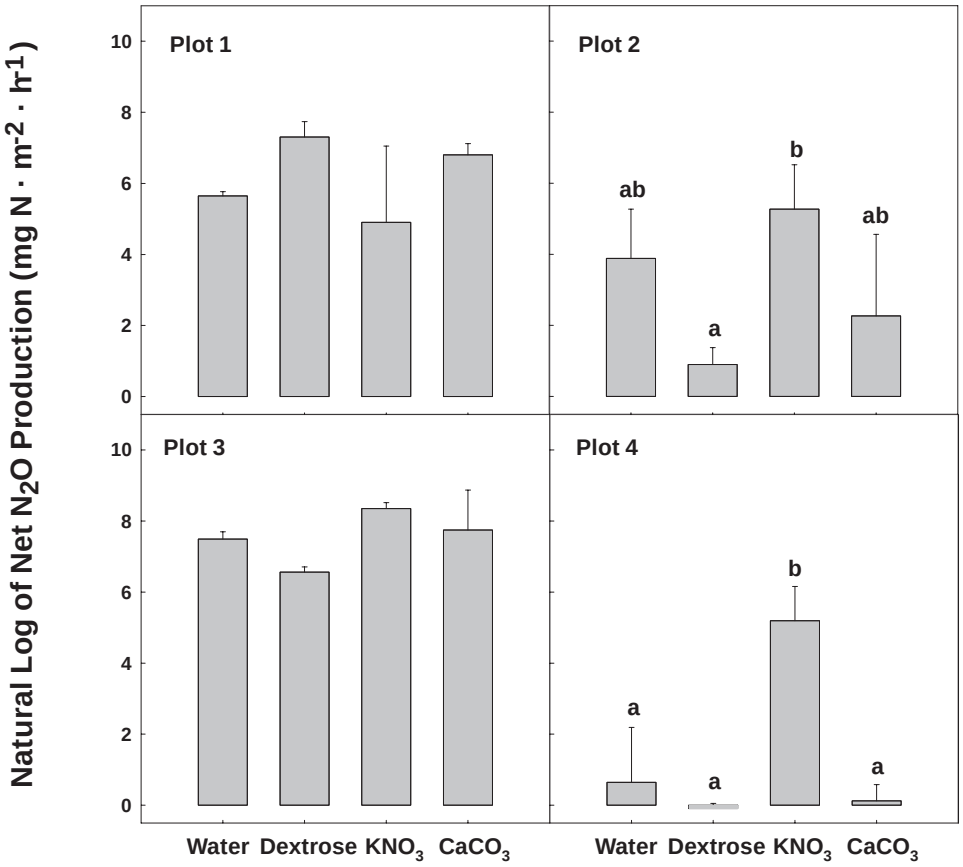
In contrast to soils from the more east-facing slopes, soil cores from south-facing slopes (plots 2 and 4) responded to the various treatments in a manner suggesting that net N_2O production in these plots is constrained by NO_3^- availability. More specifically, lower rates of net N_2O production were measured in cores receiving additions of dextrose, and higher rates were measured in cores receiving additions of NO_3^-. Greater C availability can either increase or decrease the rate of denitrification. Added C can enhance denitrification by stimulating the growth of denitrifying bacteria or by increasing the supply of the electron donor required by this process. In contrast, and consistent with the results from this experiment, added C can reduce the rate of denitrification by stimulating NO_3^- immobilization which, in turn, enhances the extent of NO_3^- limitation. In agreement with our observations for the south-facing slopes of WS 4, denitrification in temperate forest soils is often enhanced by the addition of NO_3^- (Davidson and Swank 1987; Robertson et al. 1987; Groffman and Tiedje 1989). The evidence for NO_3^- limitation in plots 2 and 4 is strengthened by a recent study of KCl-extractable soil NO_3^- concentrations and buried-bag estimates of net nitrification rates in the upper 10 cm of soil in WS 4 (Foster 1997). In this study, mean soil NO_3^- concentrations from plots 1 and 3 were ca. 0.05 mg NO_3^--$N \cdot cm^{-2}$, and mean net nitrification rates were 3.4 μg $N \cdot cm^{-2}$ per day. In contrast, soil NO_3^- concentrations in plots 2 and 4 averaged ca. 0.001 mg NO_3^--$N \cdot cm^{-2}$, and mean net nitrification rates were ca. 0.2 μg $N \cdot cm^{-2}$ per day. Thus, it appears that plots on the more south-facing slopes of WS 4 are not showing symptoms of N saturation and that this is reflected in lower rates of net N_2O production.

The ultimate cause for the apparent differences in the extent of N saturation between plots on east- and south-facing slopes in WS 4 cannot be determined by this study. However, a striking dissimilarity in the vegetation on the two sides of WS 4 suggests that the species composition of woody vegetation may be important. More specifically, plots on the more east-facing slopes (1 and 3) are characterized by a greater importance of *A. saccharum*, *Quercus prinus* L., and *Tilia americana* L. (M.J. Christ, unpublished data). In contrast, plots on the south-facing slopes (2 and 4) are characterized by a greater importance of *Nyssa sylvatica* Marsh., *F. grandifolia*, *Oxydendrum arboreum* (L.) DC, *A. rubrum*, and *Q. rubra*. Therefore, in the absence of large differences in our measurements of soil temperature, air temperature, WFPS and soil pH, we hypothesize that differences in species composition can alter the susceptibility of forest communities to the process of N saturation.

Conclusions

We draw three main conclusions from this study. First, the process of N saturation appears to have increased the rate of net N_2O production at our sites. This is consistent with theoretical expectations and supported by the higher rates of net N_2O production on the east-facing slopes of WS 4 relative to the very low rates we measured on the N-poor, south-facing slopes of the same watershed. The magnitude of such an increase, however, appears to be severely constrained by the aerobic conditions typical of well-drained forest soils. Substantial losses of N_2O are more likely from N-rich sites with poorly drained soils or from more anaerobic habitats located downstream from N-saturated forests. Second, chemoautotrophic nitrification can make a significant contribution to net N_2O production at normal values for WFPS. In this study, it accounted for 60 (WS 3) and 40% (WS 4) of the total net N_2O production. Finally, the distinctive spatial pattern in WS 4 of net N_2O production, substrate limitations,

Fig. 7. Treatment effects on N_2O production rates for each plot sampled in watershed 4. Error bars are SE, and different letters indicate significant differences ($p < 0.1$). Values are the means and SEs of log-transformed data. A simple back transformation of these means yields the geometric mean of the untransformed data.



and indices of N availability, suggests that aspect-related differences in vegetation may influence N cycling rates and susceptibility to N saturation. On the more east-facing slopes of WS 4, a greater abundance of *A. saccharum* and *T. americana*, species whose leaf litter is rapidly decomposed, appears to support higher rates of N cycling and a greater susceptibility to the early onset of N saturation. We are currently examining this hypothesis, but if it proves true, then knowing the species composition of a forest stand may significantly improve our ability to identify a forest's vulnerability to the effects of chronic N deposition.

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