

Ecological Applications, 16(6), 2006, pp. 2168–2176
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N FERTILIZATION EFFECTS ON DENITRIFICATION AND N CYCLING IN AN AGGRADING FOREST

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Abstract. We investigated N cycling and denitrification rates following five years of N and dolomite amendments to whole-tree harvested forest plots at the long-term soil productivity experiment in the Fernow Experimental Forest in West Virginia, USA. We hypothesized that changes in soil chemistry and nutrient cycling induced by N fertilization would increase denitrification rates and the $N_2O:N_2$ ratio. Soils from the fertilized plots had a lower pH (2.96) than control plots (3.22) and plots that received fertilizer and dolomite (3.41). There were no significant differences in soil %C or %N between treatments. Chloroform-labile microbial biomass carbon was lower in fertilized plots compared to control plots, though this trend was not significant. Extractable soil NO_3^- was elevated in fertilized plots on each sample date. Soil-extractable NH_4^+ , NO_3^- , pH, microbial biomass carbon, and %C varied significantly by sample date suggesting important seasonal patterns in soil chemistry and N cycling. In particular, the steep decline in extractable NH_4^+ during the growing season is consistent with the high N demands of a regenerating forest. Net N mineralization and nitrification also varied by date but were not affected by the fertilization and dolomite treatments. In a laboratory experiment, denitrification was stimulated by NO_3^- additions in soils collected from all field plots, but this effect was stronger in soils from the unfertilized control plots, suggesting that chronic N fertilization has partially alleviated a NO_3^- limitation on denitrification rates. Dextrose stimulated denitrification only in the whole-tree-harvest soils. Denitrification enzyme activity varied by sample date and was elevated in fertilized plots for soil collected in July 2000 and June 2001. There were no detectable treatment effects on N_2O or N_2 flux from soils under anaerobic conditions, though there was strong temporal variation. These results suggest that whole-tree harvesting has altered the N status of these soils so they are less prone to N saturation than more mature forests. It is likely that N losses associated with the initial harvest and high N demand by aggrading vegetation is minimizing, at least temporarily, the amount of inorganic N available for nitrification and denitrification, even in the fertilized plots in this experiment.

Key words: denitrification; dinitrogen; forest; N fertilization; N saturation; nitrous oxide; soil.

INTRODUCTION

Nitrogen deposition has increased in many regions of the United States, including the Central Appalachian region, due to emissions from agro-industrial activities (Galloway et al. 1995, Vitousek et al. 1997). Forests are an important resource in this region, and the interactions of forest management with N deposition is a critical area of study (Adams et al. 2000). Of particular concern are the effects of acidic deposition on long-term soil productivity. In 1996, an experiment to study these long-term effects was established at the Fernow Experimental Forest in West Virginia (Adams 1999). This

study examines treatment effects on N cycling and denitrification following five years of chronic N additions and dolomite treatments in a young regrowth forest.

The high rates of N deposition in the Central Appalachians can result in N saturation, a suite of changes in soil chemistry and nutrient cycling that are incurred as N inputs exceed N demand (Ågren and Bosatta 1988, Aber et al. 1989, 1998). At the Fernow Experimental Forest, a mature forested watershed and a fertilized young mixed hardwood forest exhibit signs of N saturation (Gilliam et al. 1996, Peterjohn et al. 1996). For example, nitrification rates are ~100% of net N mineralization, and NO_3^- leaching into streams has been increasing.

As nitrogen inputs to forest ecosystems increase, nitrogen may be lost through NO_3^- leaching or as gaseous emissions from nitrification and denitrification. Increased rates of N_2O efflux have been observed in N-

Manuscript received 27 September 2004; revised 28 July 2005; accepted 29 September 2005. Corresponding Editor: A. R. Townsend. For reprints of this Invited Feature, see footnote 1, p. 2055.

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saturated environments (Tietema and Verstraten 1991, Peterjohn et al. 1998, Papen and Butterbach-Bahl 1999), and under nitrogen fertilization (Bowden et al. 1991, Brumme and Beese 1992, Klemmedtsson et al. 1997). Mohn et al. (2000) detected increased annual total denitrification rates in fertilized spruce forests ($2.9 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$) compared to unfertilized plots ($1.7 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$), and they attributed most of the increased flux to periods of maximum denitrification activity. However, no study to our knowledge has measured N_2O and N_2 fluxes simultaneously to assess the effects of increasing N inputs on both the total rate of denitrification and the ratio of its products. The relative amount of N_2O and N_2 generated from denitrification has important implications for the global N cycle and for climate change, since N_2O is a potent greenhouse gas, while N_2 is relatively inert in the atmosphere.

Many of the changes in soil chemistry that can be induced by increased N inputs are known to affect denitrification rates in laboratory studies. The relative proportion of N_2O and N_2 produced by denitrification is affected by numerous factors including soil moisture, available carbon, pH, and nitrate (Weier et al. 1993). For example, as nitrate availability increases in laboratory incubations, total nitrogen gas emissions increase and the relative proportion of N_2O increases (Firestone et al. 1979, Letey et al. 1980, Weier et al. 1993, Thomas et al. 1994, Jordan et al. 1998). Laboratory studies also suggest that the rate of denitrification decreases and the $\text{N}_2\text{O}:\text{N}_2$ ratio increases as pH decreases (Naegele and Conrad 1990, Thomas et al. 1994, Stevens et al. 1998).

We evaluated the effects of N fertilization and dolomite applications on soil chemistry, denitrification enzyme activity (DEA), and production of N_2O and N_2 by denitrification on whole-tree harvested plots. We anticipated that N-fertilization would induce N saturation and that subsequent changes in soil chemistry would drive increased total denitrification rates as well as an increase in the $\text{N}_2\text{O}:\text{N}_2$ ratio. We also hypothesized that the dolomite application would counteract some of the effects of N fertilization and thereby decrease denitrification rates. As dolomite application is a common forest management technique, it is important to evaluate its role in mitigating N gas emissions from soils.

METHODS

Study area and project description

This study was conducted at the Fork Mountain Long-Term Soil Productivity (LTSP) experiment located within the 1900-ha Fernow Experimental Forest, West Virginia ($39^{\circ}03' \text{ N}$, $79^{\circ}29' \text{ W}$). A thorough site characterization is available in Adams et al. (2004). In 1996, 12 0.2-ha plots with buffer areas were whole-tree harvested and randomly assigned to two treatments and a control within four blocks (Adams 1999). Another set of four plots were unharvested control plots, but were

not included in most aspects of this study. The treatments are unharvested forest (control), unfertilized whole-tree harvest (WT), whole tree-harvest with $(\text{NH}_4)_2\text{SO}_4$ fertilization (WTN; $40 \text{ kg S}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1} + 36 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$), and fertilized whole-tree harvest plus dolomite application (WTNCA; $10 \text{ kg Ca}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1} + 10 \text{ kg Mg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$).

Soil horizons are poorly developed in most locations, and precluded separation of soil samples by horizon. The soils have an average depth of $<1 \text{ m}$ and are mapped as predominantly a Calvin channery silt loam (loamy-skeletal, mixed, mesic Typic Dystrochrept) that is derived from the acidic sandstone and shale of the Hampshire formation (Adams et al. 2004). Annual precipitation averages about 146 cm which is uniformly distributed throughout the year. Mean monthly air temperatures range from about -2°C in January to about 20°C in July and the mean annual temperature is about 9°C (Adams et al. 1994). Inputs of inorganic N in bulk precipitation average about $11.3 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$, and 55% of these inputs are in the form of NO_3^- (Adams et al. 1997).

In-situ net N mineralization and net nitrification rates

Net N mineralization and nitrification rates were measured using in-situ incubations of intact open-bottom cores (Raison et al. 1987). On 18 June and 16 July 2000, and on 28 September and 25 October 2001, five pairs of cores were sampled to a depth of 7.6 cm in each plot. One set of cores was removed from the soil and stored on ice during transport to the lab for extraction. The other set of cores was left in the ground and the top of the core was sealed using plastic film sealed with a rubber band. These cores remained in the ground for ~ 28 days before removal. Soils were extracted by shaking for 1 h in a 2 mol/L KCl solution (1:5 w:v) and filtered using prerinsed Whatman #1 filters. The extracts were analyzed for extractable NO_3^- and NH_4^+ on a TRAACS colorimetric analyzer using standard laboratory methods.

Substrate addition experiment

The response of denitrification rates to substrate and nutrient additions was evaluated in a laboratory experiment. From each plot, ten random samples were collected to a depth of 7.6 cm (excluding the Oi layer) and were composited and homogenized. Four sets of triplicate soil samples ($\sim 5 \text{ g}$) were weighed into 60-mL vials and each set received 5 mL of one of the following solutions: dH_2O , 1 mmol/L KNO_3 , $1 \text{ mmol/L dextrose}$, or 50 g/L CaCO_3 . The vials were sealed and then stored at 4°C for 24 h to acclimate. After acclimation, the vials were evacuated and flushed with Ar four times, and 5 mL of C_2H_2 and 10 mL of Ar were added to produce an overpressure. The samples were then incubated anaerobically at 22°C for 24 h in the dark. Gas samples (5 mL each) were drawn after 1.5 h and 24 h. N_2O concentrations were measured using a Shimadzu 14A gas

TABLE 1. Results of mixed-effects ANOVA for denitrification-related activity and soil chemistry at the Fernow Long-Term Soil Productivity site for three treatments.

Effect	Denitrification enzyme activity			log(N ₂ O)			N ₂			Microbial biomass C		
	F	df	P	F	df	P	F	df	P	F	df	P
Date	69.04	2, 149	<0.0001	160.77	2, 152	<0.0001	600.49	2, 155	<0.0001	15.93	2, 155	<0.0001
Block	5.37	1, 6	0.059	1.16	1, 6	0.32	1.82	1, 6	0.22	3.32	1, 6	0.12
Treatment	1.84	2, 6	0.23	1.95	2, 6	0.22	0.06	2, 6	0.93	1.51	2, 6	0.29
Date × block	12.69	2, 149	<0.0001	2.41	2, 152	0.09	1.49	2, 155	0.22	24.46	2, 155	<0.0001
Date × treatment	8.37	4, 149	<0.0001	1.56	4, 152	0.18	0.08	4, 155	0.98	1.91	4, 155	0.11
Block × treatment	0.31	2, 6	0.73	0.86	2, 6	0.46	0.35	2, 6	0.71	0.18	2, 6	0.84
Date × block × treatment	1.81	4, 149	0.13	0.85	4, 152	0.49	0.26	4, 152	0.89	0.38	4, 155	0.82

Notes: Treatments are unfertilized whole-tree harvest (WT), whole tree-harvest with (NH₄)₂SO₄ fertilization (WTN), and fertilized whole-tree harvest plus dolomite application (WTNCA). Effects with *P* values <0.05 are highlighted in boldface text.

chromatograph (Shimadzu, Kyoto, Japan) equipped with a ⁶³Ni-electron capture detector. The oven temperature was 40°C, the detector temperature was 300°C, and the carrier gas was 5% CH₄ in Ar flowing at 11.6 mL/min through a 10-ft (1 ft. = 0.305 m) stainless-steel column packed with Porapak Q (80:100 mesh; Alltech, Deerfield, Illinois, USA).

Total N₂O production rates were calculated by the slope of the difference between samples collected at 1.5 h and 24 h.

Denitrification enzyme activity

Soil samples were collected on 18 June 2000 and three sampling dates in 2001 (10 May, 15 July, and 31 October) from five random points within each plot. Soil samples were collected to a depth of 15 cm using a 2-cm diameter corer after removing the Oi layer. Samples were kept intact and placed on ice. Within 24 h of collection, soils were sieved (4 mm) and the water-holding capacity (WHC) was calculated as the water held after saturating a subsample and filtering through a Whatman #41 filter for 2 h with plastic wrap on top of the filter to minimize evaporation. Denitrification enzyme activity (DEA) was measured using the technique of Tiedje et al. (1989). Triplicate ~10-g samples were weighed into incubation vials and 10 mL of the DEA solution (1 mmol/L glucose, 1 mmol/L KNO₃, and 1 g/L chloramphenicol) was added. The vials were evacuated and flushed with Ar four times, and returned to 1 atm pressure. C₂H₂ was injected to achieve 10 kPa and the samples were mixed before taking the initial gas samples (DEA₀). The vials were incubated at 22°C on a shaker table for 1.5 h before taking a second gas sample (DEA_{1.5}). DEA was determined by the accumulation of N₂O between DEA₀ and DEA_{1.5}.

Denitrification

On three sampling dates in 2001 (10 May, 15 July, and 31 October), five samples were collected from random points within each plot as described in *Denitrification enzyme activity*. Denitrification was measured by laboratory incubations of field soil. Subsamples (10 g) were

placed in 60-mL incubations jars fitted with septa. Distilled water containing 1 mg of K¹⁵NO₃ was mixed into each sample to bring it to 60% WHC. The headspace of each jar was flushed and filled with Ar to create anaerobic conditions. The samples were incubated for 24 h at room temperature, and a 20-mL gas sample was collected and stored in pre-evacuated Exetainers (LABCO Ltd., High Wycombe, UK). N₂O was analyzed on a Shimadzu gas chromatograph equipped with a ⁶³Ni Electron Capture Detector. Samples were analyzed for N₂ at the University of California at Davis Stable Isotope Facility using a Europa Scientific 20/20 Mass Spectrometer (Europa Scientific, Crewe, UK). Flux was determined by direct measurement of the ³⁰N₂:²⁹N₂ and ²⁹N₂:²⁸N₂ mass ratios using mass spectrometry using equations derived by Mulvaney and Boast (1986). The ratio data were used to calculate values for the mole fraction of ¹⁵N in the N pool from which the N₂ was derived (¹⁵X_N) and the micrograms of N as labeled N₂. This calculation is based on the assumption that the NO₃ undergoing denitrification existed in a single pool that is isotopically uniform. Headspace volume was measured by applying a known gas volume and measuring pressure with a transducer (Parkin et al. 1984).

Soil chemistry

To measure total C and N, soils were air dried at 60°C for 48 h, then ground and measured using a Carlo Erba elemental analyzer (Carlo Erba Instruments, Milan, Italy). Soil pH was measured using a 1:1 dH₂O slurry.

Chloroform-labile microbial biomass carbon (MBC) was determined by the chloroform fumigation-extraction method (Vance et al. 1987). For each sample, one replicate was extracted in 0.5 mol/L K₂SO₄ by shaking for 1 h and another replicate was exposed to chloroform for 5 days before extraction. Six 3-g (approximate dry mass) subsamples of soil were weighed into 50-mL centrifuge tubes for each replicate. Three subsamples were extracted by shaking for 1 h in 30 mL of dH₂O and three were exposed to CHCl₃ vapor for 5 days in a vacuum desiccator prior to extraction. Following cen-

TABLE 1. Extended.

pH			NH ₄			NO ₃			%C			%N		
<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
39.71	2, 155	<0.0001	104.57	2, 155	<0.001	101.89	2, 155	<0.001	4.79	2, 154	0.009	0.84	2, 154	0.43
1.28	1, 6	0.30	0.62	1, 6	0.46	0.52	1, 6	0.50	1.50	1, 6	0.26	8.23	1, 6	0.02
7.32	2, 6	0.024	0.40	2, 6	0.68	3.22	2, 6	0.11	1.09	2, 6	0.39	0.37	2, 6	0.70
1.05	2, 155	0.35	0.75	2, 155	0.56	0.27	2, 155	0.76	3.34	2, 154	0.03	3.57	2, 154	0.03
3.23	4, 155	0.014	1.48	4, 155	0.21	2.59	4, 155	0.03	1.64	4, 154	0.16	0.80	4, 154	0.52
2.08	2, 6	0.20	0.75	2, 6	0.51	0.71	2, 6	0.52	0.03	2, 6	0.97	0.08	2, 6	0.91
0.81	4, 155	0.52	3.42	4, 155	0.01	1.97	4, 155	0.10	0.39	4, 154	0.81	0.39	4, 154	0.80

trifugation at 3200 rpm for 5 min, the extracts were filtered through prerinsed Whatman #1 filters. The extracts were acidified using HCl to pH 2.0 and analyzed with a Shimadzu TOC-5000 for dissolved organic carbon.

Statistics

Differences among treatments, sample dates, and blocks were analyzed with mixed-effects multiple analysis of variance (ANOVA) measures. N₂O and N₂ measurements were log-transformed prior to analyses. Replicates within plots were treated as random effects. Differences are considered significant when *P* < 0.05 unless otherwise reported. All statistical analyses were performed using S-Plus 2000 Professional (Insightful Corporation, Seattle, Washington, USA).

RESULTS

Most properties and activities measured in this study differed between sampling dates (Table 1). Only pH had a significant treatment effect, though several variables had date × treatment interactions.

Soil chemistry

The effect of the various treatments on soil pH depended on the sampling date. When averaged across sample dates, N-fertilization in combination with SO₄²⁻ additions decreased soil pH to 2.96 compared to 3.22 in the unfertilized WT plots, and 3.41 in the WTNCA plots (Table 2). Chloroform-labile MBC varied by date when averaged across the blocks and did not vary significantly with treatment, although there was a trend of lower MBC in the WTN plots compared to the WT plots on all three sample dates (Fig. 1). Extractable NH₄⁺ decreased

strongly from May through October, but did not differ by treatment (Fig. 2), and NO₃⁻ concentrations were much lower in October than in May and June and were higher in the fertilized plots compared to the WT plots on all sample dates (Fig. 2).

In-situ net N mineralization and net nitrification rates

In-situ N mineralization rates varied between the two sample years (*P* = 0.02) but were not affected by treatment (*P* = 0.55; Fig. 3). Net nitrification rates also varied between years (*P* < 0.001) but were not affected by treatment (*P* = 0.20; Fig. 3).

Substrate addition experiment

The effects of soil amendments on N₂O flux varied by treatment (Fig. 4). In the unharvested control plots, KNO₃ additions increased N₂O flux, but dextrose and CaCO₃ additions decreased N₂O production. KNO₃ additions also increased N₂O flux in WT, WTN, and WTNCA soils, though the effect was strongest in the WT soils. Dextrose additions increased N₂O flux only in the WT soils. CaCO₃ additions did not affect N₂O flux in WT, WTN, or WTNCA soils.

Denitrification enzyme activity

DEA varied with date and block, and there were also date × treatment and date × block interactive effects (Table 1). When averaged across the block, soils collected in October 2001 had a strongly decreased DEA compared to all other sample dates (Fig. 5). DEA was increased slightly in WTN plots compared to WT plots in July 2000 and June 2001. DEA was also

TABLE 2. Values (mean with SE in parentheses) of soil %N, %C, C:N ratio, and pH by treatment.

Treatment	N (%)	C (%)	C:N	pH
WT	0.648 (0.0112)	9.73 (0.195)	15.25 (0.305)	3.22 (0.117)
WTN	0.694 (0.0159)	10.92 (0.603)	16.09 (0.426)	2.96 (0.139)
WTNCA	0.716 (0.0311)	11.16 (0.830)	15.78 (0.468)	3.41 (0.044)

Note: Treatments are unfertilized whole-tree harvest (WT), whole tree-harvest with (NH₄)₂SO₄ fertilization (WTN), and fertilized whole-tree harvest plus dolomite application (WTNCA).

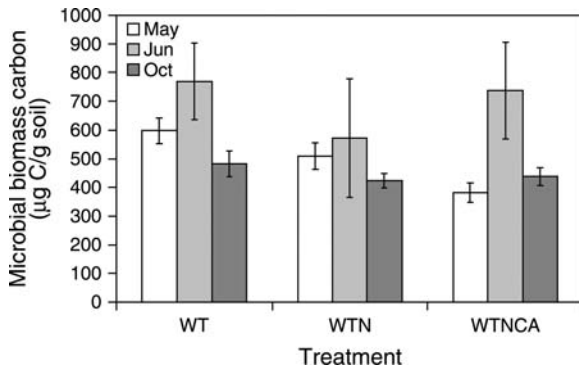


FIG. 1. Chloroform-labile microbial biomass carbon by treatment (mean \pm SE). Treatments are unfertilized whole-tree harvest (WT), whole tree-harvest with (NH₄)₂SO₄ fertilization (WTN), and fertilized whole-tree harvest plus dolomite application (WTNCA).

positively correlated with soil NO₃⁻, %C, and %N (Fig. 6).

Denitrification

Both N₂O and N₂ emissions under anaerobic conditions varied strongly with sampling date being greatest in June 2001 (Fig. 7). There were no treatment effects on either N₂O or N₂ emissions (Table 1).

DISCUSSION

Five years after whole-tree harvesting and the initiation of chronic (NH₄)₂SO₄ applications and

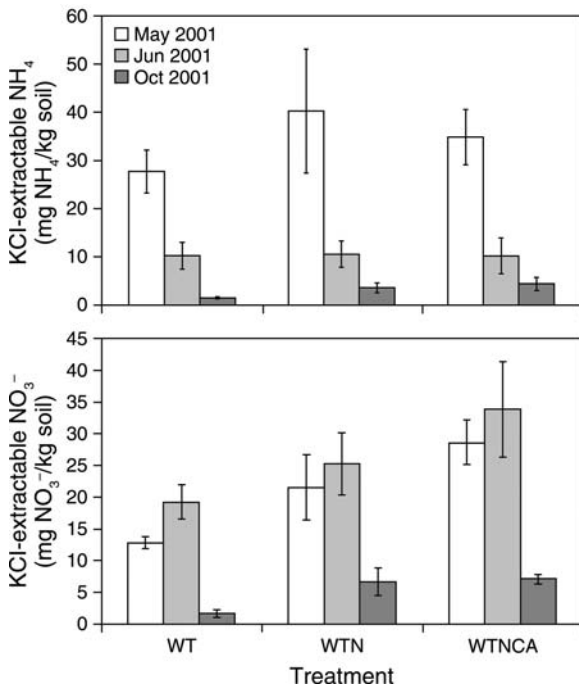


FIG. 2. KCl-extractable NH₄⁺ and NO₃⁻ by treatment (mean \pm SE). Treatments are as in Fig. 1.

dolomite treatments, we found few treatment effects on soil chemistry and soil N cycling in this experiment. The only significant treatment effect was a decreased pH in the WTN plots, which varied by sample date. Decreases in pH can result from nitrification, plant uptake of NH₄⁺, and from base cation loss associated with NO₃⁻ leaching and reactions with the added SO₄. It appears that the dolomite addition has alleviated this effect at the LTSP experiment.

In the first two years of this experiment, net nitrification rates were elevated in the fertilized plots (WTN and WTNCA) relative to unfertilized WT plots; however, there was no difference in nitrification rates by year three (F. Gilliam, *unpublished data*). The results of the present study suggest that nitrification rates remained unaffected by N fertilization for several years into this experiment. It is likely that by year 3, demand for NH₄⁺ by the rapidly recovering vegetation limited its availability to nitrifiers. This is supported by the large decline observed in the extractable NH₄⁺ pool during the growing season (Fig. 2), and the observations that plant biomass appears to be accumulating more rapidly in fertilized plots (M. B. Adams, *personal communication*), which may be limiting the amount of excess N in soils. Although there were no differences in nitrification rates between treatments, the fertilized plots exhibited con-

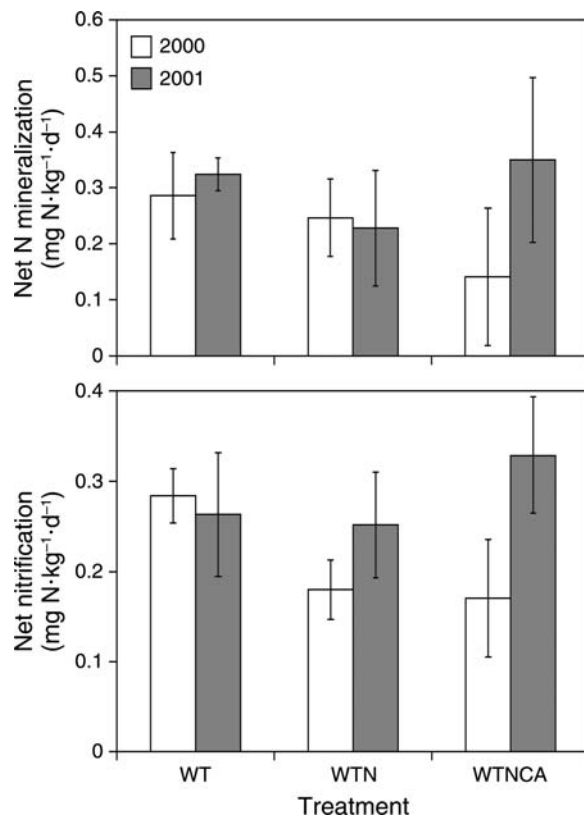
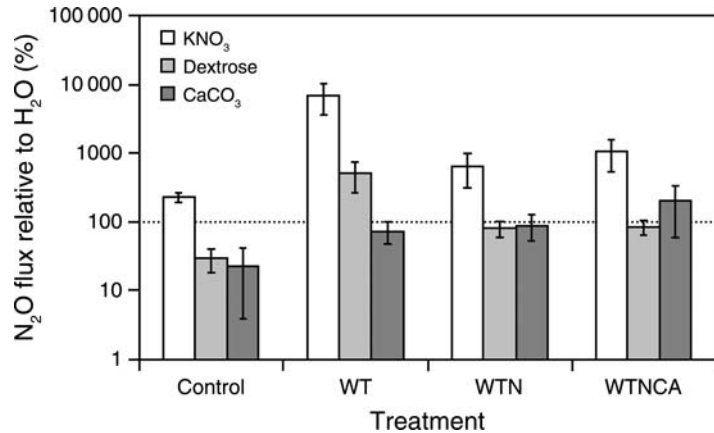


FIG. 3. Net N mineralization and nitrification rates by treatment (mean \pm SE). Treatments are as in Fig. 1.

FIG. 4. Response of denitrification rates (mean \pm SE) to three soil amendments in a laboratory incubation. N_2O flux is reported as a percentage of the rate achieved with H_2O additions for soils collected from the same treatment. Note the log scale. Treatments are as in Fig. 1.

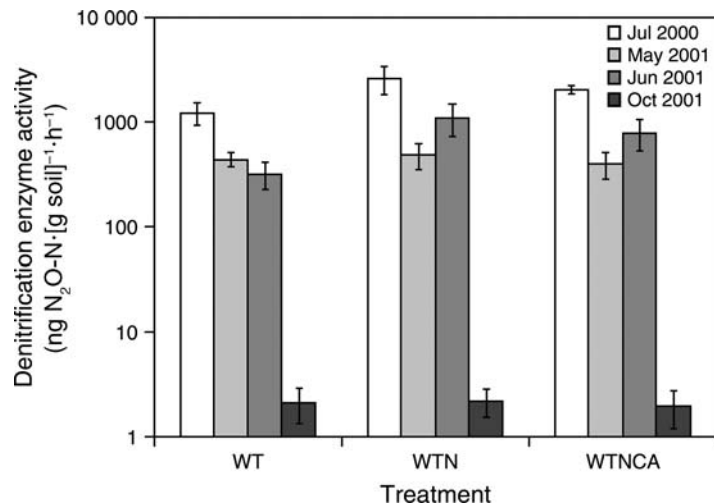


sistently higher NO_3^- concentrations than the unfertilized plots. This could be due to preferential uptake of NH_4^+ by plants in the fertilized plots, resulting in a decreased demand for NO_3^- . Consistent with this mechanism, increased NO_3^- leaching to streams has been observed in a whole-watershed fertilization experiment at the Fernow experimental forest despite no measurable increase in net nitrification rates (Gilliam et al. 1996).

The substrate addition experiment provided an important insight into the factors which limit rates of denitrification under strongly anaerobic conditions in these soils. The addition of KNO_3 increased N_2O flux in all harvested plots, and this effect was strongest in the unfertilized WT plots, suggesting that denitrification rates at this site are tightly coupled to the supply of NO_3^- by nitrification in the absence of oxygen. Dextrose additions resulted in increased N_2O only in the WT plots, suggesting that denitrification was not limited by C in fertilized plots. Finally, $CaCO_3$ additions had no effect on denitrification rates, suggesting pH does not impact enzyme activity, though it remains possible that it affects the denitrifying community in the longer term.

DEA is a commonly used index that is known to correlate with annual rates of denitrification (Groffman and Tiedje 1989). In this study, DEA varied with sampling date, and there was a significant date \times treatment interaction (Fig. 6). In July 2000 and June of 2001, DEA was elevated in fertilized plots compared to unfertilized WT plots. This suggests that denitrification may be increased in the fertilized plots in mid-summer. The availability of NO_3^- also seems to peak in midsummer (Fig. 2), which could drive increased denitrification rates during periods of high soil moisture. There was a strong correlation between extractable NO_3^- levels and DEA rates (Fig. 6), adding further support to the hypothesis that rates of denitrification and nitrate production are tightly linked under anaerobic conditions in these soils. DEA rates were also correlated with soil C and N (Fig. 6) which may indicate that soil C also affects denitrification enzyme activity. Increased soil C is likely to be correlated with changes in the soil physical environment, and could result in conditions more favorable for denitrification enzyme production. The October 2001 samples exhibited particularly low DEA (Fig. 5). The soil collected on this date

FIG. 5. Denitrification enzyme activity by treatment (mean \pm SE) on four sampling dates. Note the log scale. Treatments are as in Fig. 1.



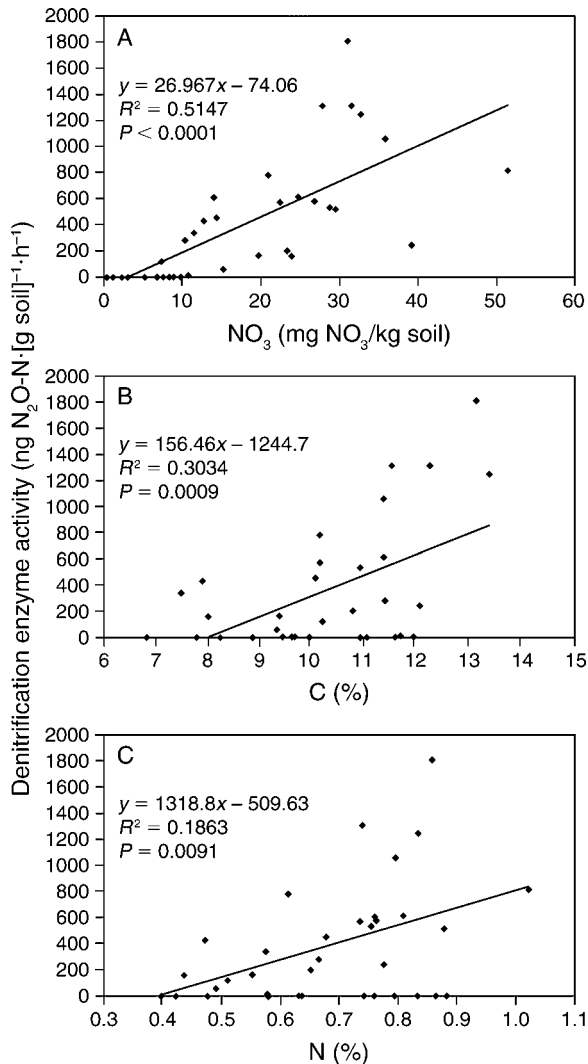


FIG. 6. Relationship of denitrification enzyme activity with (A) extractable NO_3^- , (B) soil %C, and (C) soil %N. Points represent mean plot values. Lines are best-fit linear regressions.

was very dry, and may have caused a decrease in the size or enzyme production rate of the denitrifier community. In addition, extractable NO_3^- was very low on this date (Fig. 2), which may have suppressed enzyme production rates.

We had expected that N_2O and N_2 emission rates would be affected by the fertilization treatments in this experiment. However, it appears that in-situ denitrification rates are not affected by the experimental treatments at this stage of the long-term experiment, despite evidence of accumulating NO_3^- pools and higher DEA in the fertilized plots. This suggests that denitrification is thermodynamically constrained in these upland soils during the summer, though there remains a potential for high activity under favorable conditions.

The denitrification assay used in this study was designed to minimize the effects of temporal variability in soil moisture and temperature on denitrification rates by incubating the samples under standardized conditions. We reasoned that differences between sample dates could then be attributed to differences in substrate availability and the antecedent size and physiological state of the denitrifying community. Denitrification rates varied by several orders of magnitude temporally. In this study, we did not sample frequently enough to elaborate on the fine-scale temporal dynamics of denitrification at this site. It appears, however, that denitrification rates are highly affected by seasonal changes in soil moisture and NO_3^- availability.

There are few reports in the literature of $\text{N}_2:\text{N}_2\text{O}$ ratios in forest soils due to the technical difficulties in measuring N_2 and a focus on N_2O emissions (Naegle and Conrad 1990, Bergsma et al. 2001, Wolf and Brumme 2003). The mean $\text{N}_2:\text{N}_2\text{O}$ ratio measured in this study was 5.18, which is within the range of other literature values. Under similar incubation conditions to those used here (laboratory incubation of sieved soil), Wolf and Brumme (2003) measured mean $\text{N}_2:\text{N}_2\text{O}$ ratios of 4.2 in a beech forest soil with a pH of 5.8, and a mean ratio of 0.32 in another beech forest with a pH of 3.2.

The LTSP experiment is somewhat unusual compared to most other long-term forest N fertilization experi-

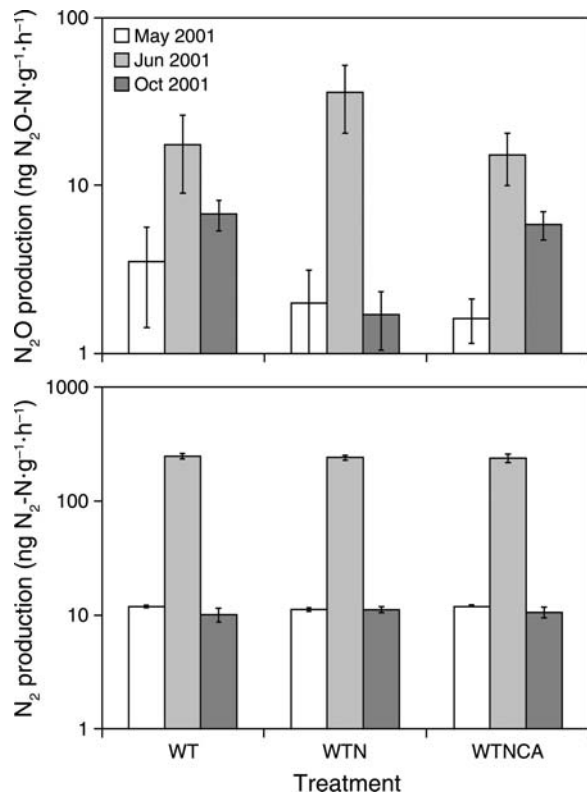


FIG. 7. N_2O and N_2 production by treatment (mean \pm SE). Note the log scales. Treatments are as in Fig. 1.

TABLE 3. Mean values of net N mineralization, nitrification, denitrification enzyme activity (DEA), and denitrification in summer 2000.

Treatment	Net N mineralization (mg N·kg ⁻¹ ·d ⁻¹)	Net nitrification (mg N·kg ⁻¹ ·d ⁻¹)	DEA (ng N ₂ O-N·g ⁻¹ ·h ⁻¹)	Denitrification (ng N ₂ O-N·g ⁻¹ ·h ⁻¹)
Control	0.42	0.43	1524	852
WT	0.29	0.28	1235	292
WTN	0.25	0.18	2614	783
WTNCA	0.14	0.17	2044	1063

Notes: Total denitrification was measured in a soil slurry using the acetylene inhibition technique (Mosier and Klemmedtsson 1994). Treatments are unfertilized whole-tree harvest (WT), whole tree-harvest with (NH₄)₂SO₄ fertilization (WTN), and fertilized whole-tree harvest plus dolomite application (WTNCA).

ments in that this forest was harvested at the initiation of the experiment. Thus, the data presented here are the result of interactions between forest harvesting and increased N inputs. The Fernow Experimental Forest receives some of the highest rates of N deposition in the United States (Gilliam and Adams 1996), and previous studies of a paired-watershed experiment in this forest indicate that it is in the latter stages of N saturation (Peterjohn et al. 1996, 1999). Prior to harvest, pretreatment data indicated that experimental plots at the LTSP site were also exhibiting signs of N saturation. For example, the ratio of net nitrification to net N mineralization was ~1 (F. Gilliam, *unpublished data*). During the first two years following harvest, nitrification increased and the plots continued to exhibit signs of N saturation, which was further enhanced in fertilized plots. However, by years 5 to 6, the plots appear to have reverted to stage 0–1 of N saturation, supported by very low rates of NO₃⁻ leaching (M. B. Adams, *unpublished data*) and greater mineralization and nitrification rates in the unharvested control plots in 2000 compared to the whole-tree harvested plots (Table 3). Denitrification measurements taken from the unharvested control plots in 2000 also suggest that forest harvesting has resulted in decreased N gaseous loss at this stage (Table 3).

The results of this study suggest that forest harvesting has temporarily alleviated symptoms of N-saturation through N losses, decreased C input, and other soil physical and chemical changes associated with the harvest, and probably also due to increased N demand by rapidly aggrading vegetation. Therefore, many of the effects of N fertilization that have been observed in more mature forests have not been observed in this experiment to date. As this project was designed as a long-term experiment, the results presented here are an important snapshot of N cycling at this early stage. Future studies should follow-up on this research to gain further insight into longer-term changes in N cycling and soil chemistry that may impact forest productivity.

ACKNOWLEDGMENTS

The authors thank the Department of Biology at West Virginia University and the Department of Biology and Nicholas School of Earth and Environmental Sciences at Duke University for their support. M. Wallenstein was supported by

a NASA Earth System Science graduate fellowship and a NSF dissertation improvement grant.

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