

## Soil nitrogen cycling and nitrous oxide flux in a Rocky Mountain Douglas-fir forest: effects of fertilization, irrigation and carbon addition

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**Abstract.** Nitrous oxide fluxes and soil nitrogen transformations were measured in experimentally-treated high elevation Douglas-fir forests in northwestern New Mexico, USA. On an annual basis, forests that were fertilized with 200 kg N/ha emitted an average of 0.66 kg/ha of N<sub>2</sub>O-N, with highest fluxes occurring in July and August when soils were both warm and wet. Control, irrigated, and woodchip treated plots were not different from each other, and annual average fluxes ranged from 0.03 to 0.23 kg/ha. Annual net nitrogen mineralization and nitrate production were estimated in soil and forest floor using *in situ* incubations; fertilized soil mineralized 277 kg ha<sup>-1</sup> y<sup>-1</sup> in contrast to 18 kg ha<sup>-1</sup> y<sup>-1</sup> in control plots. Relative recovery of <sup>15</sup>NH<sub>4</sub>-N applied to soil in laboratory incubations was principally in the form of NO<sup>3</sup>-N in the fertilized soils, while recovery was mostly in microbial biomass-N in the other treatments. Fertilization apparently added nitrogen that exceeded the heterotrophic microbial demand, resulting in higher rates of nitrate production and higher nitrous oxide fluxes. Despite the elevated nitrous oxide emission resulting from fertilization, we estimate that global inputs of nitrogen into forests are not currently contributing significantly to the increasing concentrations of nitrous oxide in the atmosphere.

### Introduction

Nitrous oxide is a greenhouse gas whose atmospheric concentration is increasing at the rate of 0.2–0.3% per year (Watson et al. 1990). Early analyses of global nitrous oxide sources and sinks suggested that fossil fuel and biomass burning together could account for the annual increment in the atmosphere (Crutzen et al. 1985; Keller et al. 1986; McElroy & Wofsy 1986; Hao et al. 1987). However, recent studies have demon-

strated that those sources were greatly overestimated (Muzio & Kramlich 1988; Cofer et al. 1991). The real cause of the increase is unknown; increased emissions associated with tropical land use change may provide up to a quarter of the missing source (Matson & Vitousek 1990), and a number of other relatively small sources may also contribute to the atmospheric increase (Watson et al. 1990).

Temperate forests have been considered to represent a relatively minor global source of nitrous oxide (Keller et al. 1983; Bowden 1986; Schmidt et al. 1988; Bowden et al. 1990), despite the fact that they cover  $12 \times 10^6$  km<sup>2</sup> or 25% of forestlands globally (Bolin 1977). However, temperate forests are being altered by a number of disturbances that could increase N<sub>2</sub>O flux, including deliberate fertilization to increase growth or to dispose of sewage sludge, and inadvertent fertilization via acid deposition. In the Pacific northwest and the southeastern United States alone, over 140,000 ha of forests receive N fertilizers each year (Chappell et al. 1991; North Carolina State Forest Nutrition Cooperative 1991). Nitrogen deposition due to anthropogenic emissions is important across large areas of the temperate zone, especially in northeastern United States and northern Europe (Bonis et al. 1980; Melillo et al. 1989), and deposition from anthropogenic emissions is increasing even in relatively unpopulated areas of the tropics (Andreae et al. 1988). These additions of nitrogen may lead to increased rates of nitrogen transformations in the soil, possibly with concomitant increases in emissions of nitrous oxide and other trace gases from the soil to the atmosphere (Aber et al. 1989; Melillo et al. 1989).

The objective of this study was to examine the effect of increased nitrogen availability (via fertilization), decreased nitrogen availability (via application of woodchips), and increased water availability (via irrigation) on soil nitrogen transformations and annual nitrous oxide flux from a temperate coniferous forest in northwestern New Mexico.

## Methods

### *Site description and experimental design*

The study site is located at 2900 m above sea level on Mount Taylor in northwestern New Mexico (35°15'N, 107°34'W). During this study, average maximum daily air temperature was -10.5 °C in January, and 17 °C in July. Precipitation during the growing season (May–October) ranges from 640–720 mm, and the mean annual snowfall for the region is 1400 mm of snow (McWilliams 1986).

The site was located in 50-year-old mixed conifer forest. Douglas-fir (*Pseudotsuga menziesii* var *glauca* (Beissn.) Franco) comprised on average 91% of the total basal area in the plots; average total basal area across all plots was 52.6 m<sup>2</sup>/ha (Gower et al. 1992). Other species included aspen (*Populus tremuloides* Michx.), Engelmann spruce (*Picea engelmannii* Parry), and corkbark fir (*Abies lasiocarpa* var *arizonica* (Merriam) Lemon). Soil is less than 35 cm in depth to basalt bedrock, and is classified as a clayey-skeletal Mollic Paleoboralf of tertiary volcanic origin (McWilliams 1986).

Beginning in 1985, five treatments were randomly applied to two plots each; treatments included control, fertilization, irrigation, and woodchip amendment (to reduce nitrogen availability by stimulating microbial immobilization), and woodchip plus irrigation (Gower et al. 1992); for this study, the wood chip/irrigation treatment was not used. All plots were 15m × 15m, with a 5-m treated buffer zone. Fertilizer was applied in a single annual application of 200 kg N ha<sup>-1</sup> y<sup>-1</sup> in early June 1985, early May 1986, and mid-July 1987. Nitrogen was applied as NO<sub>3</sub>-N and NH<sub>4</sub>-N in equal proportions, and other essential macro- and micro-nutrients were added in appropriate proportions (see Gower et al. 1992).

The irrigation treatment consisted of a weekly application of water equivalent to 4 cm/week from June through September. The carbon amendment consisted of a one-time application of Douglas-fir/ponderosa pine woodchips, applied at a rate of 50,000 kg/ha with the goal of doubling the C:N ratio of the forest floor. For further details on the treatments, see Gower et al. (1992).

### *Soil sampling and analysis*

Soil samples were collected monthly from May–October 1986 and four times during the summer of 1987. Three soil cores (5 cm diameter and 20 cm depth) were taken at eight random locations in each plot; collections were always made four days after the irrigated plots were watered. Forest floor and mineral soil samples from one core at each sampling location were placed in separate bags and returned to the laboratory at Northern Arizona University (NAU) for measurement of initial NH<sub>4</sub> and NO<sub>3</sub> concentrations and moisture content (Gower et al. 1992).

Mineral soil and forest floor samples were processed in the laboratory within 48 h of collection. Forest floor samples were thoroughly mixed by hand, and mineral soil samples were sieved using a 2-mm mesh screen. Approximately 5 and 10 g subsamples of forest floor and mineral soil, respectively, were placed in 100 mL 2N KCl, shaken, and allowed to equilibrate for approximately 24 h. Aliquots of the extraction solution were

analyzed colorimetrically for  $\text{NH}_4$  and  $\text{NO}_3$  using an autoanalyzer. Chemical analysis of the forest floor extractant followed the method of White & Gosz (1981). Moisture content was measured gravimetrically after drying for 48 h at 70 °C.

The other two cores collected at each location were placed in separate gas-permeable polyethylene bags, tied, reburied, and incubated for 1 and 2-month periods; the 2-month incubations were prepared every other month. After incubation, the samples were returned to NAU for determination of  $\text{NH}_4$  and  $\text{NO}_3$  as above. Any incubated sample that was disturbed or damaged by animals was discarded. Net nitrogen mineralization was calculated as the difference between  $\text{NH}_4$  and  $\text{NO}_3$  concentrations of the incubated and initial samples. Net nitrification was calculated as the difference between  $\text{NO}_3$  concentrations in the incubated and initial samples. Annual net mineralization and nitrification were estimated by summing the 2-month incubation values for May 1986–May 1987.

Soil temperature was measured at 5 cm below forest floor in the control plots using a Partflow 30-d cycle thermograph (Gower et al. 1992); temperatures were not measured in 1987.

#### *Laboratory $^{15}\text{N}$ labelling experiment*

In late August 1987, three soil composite samples were collected from each treatment plot and used for an  $^{15}\text{N}$  labelling study. Samples were stored in plastic bags on ice for 18–24 h until processing at NASA-Ames Research Center (ARC). One 25 g subsample was removed from each composite sample and extracted immediately in KCl as described above; the extracted solution was analyzed colorimetrically for initial ammonium-N and nitrate-N concentrations. An additional large subsample was removed from each composite, weighed, and dried at 70 °C to a constant mass to determine water content.

Six 50 g soil subsamples from each composite were placed in cups that received 1 mL of a 20 ppm N solution containing 99%  $^{15}\text{N}$  as  $(\text{NH}_4)_2\text{SO}_4$ . These subsamples were placed in jars (two cups per jar) along with 10 mL of 1N NaOH to trap  $\text{CO}_2$ , and were incubated in the dark at 20 °C for 4, 10, and 30 day periods. At the end of each incubation period, one jar (with two soil subsamples and the NaOH vial) for each soil composite was removed. One subsample was extracted in 2N KCl as described above for measurement of ammonium and nitrate- $^{15}\text{N}$ . The second was fumigated with 1 mL chloroform, aired after 24 h, incubated in the dark for 10 days, extracted as described above, and used to calculate microbial biomass  $^{15}\text{N}$  (Vitousek & Andariese 1986). The NaOH was titrated with standard

HCl in the presence of excess  $\text{BaCl}_2$  to determine  $\text{CO}_2$  evolved during incubation.

Supernatant from the KCl extract solutions was used for  $^{15}\text{N}$  analyses according to the method of Vitousek & Andariese (1986). Supernatant was placed in a screw-cap flask along with a test tube containing 0.5 N HCl. The supernatant was made alkaline with concentrated NaOH, and the sealed flasks were held at 70 °C for 48 h, during which time ammonia diffused into the HCl. After the ammonium was diffused from the alkaline supernatant, Devarda's alloy was added to reduce  $\text{NO}_3$  to  $\text{NH}_3$ , and the samples were diffused again. The HCl solutions were evaporated to dryness, and the test tubes containing the solid  $\text{NH}_4\text{Cl}$  were shipped to Isotope Services, Inc. (Los Alamos, NM) for analysis of  $^{15}\text{N}$  using isotope ratio mass spectrometry.

Potential net nitrogen mineralization was calculated as ammonium-N plus nitrate-N concentrations at the end of the incubation period minus initial nitrate-N plus ammonium-N. Microbial biomass N was calculated from chloroform-labile N using a recovery coefficient ( $k_N$ ) derived from C and N release during incubation (Voroney & Paul 1984);  $k_N$  values for this study ranged from 0.227—0.297. Recovery of added  $^{15}\text{N}$  in each pool was calculated by multiplying the atom percent excess  $^{15}\text{N}$  times the amount of N in a particular form, then dividing by the amount of  $^{15}\text{N}$  added.

#### *Field measurements of nitrous oxide emissions*

Nitrous oxide was measured 5 times during the 1986 snow-free season, and 4 times in 1987. Gas samples were taken on the same day or within several days of soil collections except in May and September 1986, when soils were collected approximately 10 days prior to gas measurements. At each sampling time, flux measurements were made at six locations in each of two plots per treatment; all flux measurements were carried out within a six-hour period on a single day. At each sampling point, a sharpened polyvinyl chloride ring 25 cm in diameter was driven approximately 3 cm into the ground and left in place. At each sampling date, the ring was reinserted if necessary, allowed to air for 10 min, and capped tightly with a chamber top made of molded acrylonitrile-butadiene-styrene plastic; gas samples were withdrawn through an injection port at 0, 6, 12, 18 and 30 min after closure, and were injected into specially prepared Venoject™ evacuated vials (Matson et al. 1991). Any vial that did not draw a strong suction was discarded, and another sample was taken. Vials were sealed with Apiezon™ grease and shipped to ARC for analysis. Analysis took

place within 5 days of sampling; laboratory and field tests indicated that the evacuated vials lost less than 5% of an injected standard over a two-week period.

At the field site, nitrous oxide standards (0.5 and 1.0 ppmv N<sub>2</sub>O in N<sub>2</sub>) were injected into 5–10 vials for each set of 80 samples. The coefficient of variability for these standards averaged around 3%.

Nitrous oxide concentrations were determined by gas chromatography using a Hewlett Packard 5890 gas chromatograph equipped with a Porapak Q column maintained at 55 °C and a <sup>63</sup>Ni electron capture detector. N<sub>2</sub>O and CO<sub>2</sub> were separated, but only N<sub>2</sub>O concentrations were quantified. Fluxes were estimated as the change in N<sub>2</sub>O concentration over time corrected for the ratio of chamber volume to soil surface area covered.

For estimates of annual rates of nitrous oxide emission, each sampling date was considered a midpoint for a sampling period, average fluxes were weighted by the length of the sampling period, and the net annual flux was considered the sum of all weighted fluxes. The snow-free season was estimated to extend from May 15–September 30; in 1987, no measurements were taken until July, so May 15–June 30 was assigned a zero flux by default (probably not unreasonable, given the May 1986 values). Because no diel measurements were made, we assume a constant diel flux.

### *Statistical analyses*

Because samples variances were significantly different between the fertilizer treatment and the other treatments, all soil data were log-transformed before statistical analysis. One-way ANOVA's were conducted on log-transformed data to test for treatment differences; differences between means were analyzed using Tukey's least significant difference test. All significance levels are reported at  $p < 0.05$  unless otherwise noted.

Stepwise linear regressions between nitrous oxide flux and soil variables used non-transformed plot means. Data from the May and September 1986 collections were not included in the regressions, because soil sampling was carried out 10 days before nitrous oxide measurements.

## **Results**

Soil moisture generally was not different among the control, fertilized, irrigated and woodchip treatments (Fig. 1a); it tended to be highest during snow-melt and in mid-summer rainstorm periods. Moisture content in the

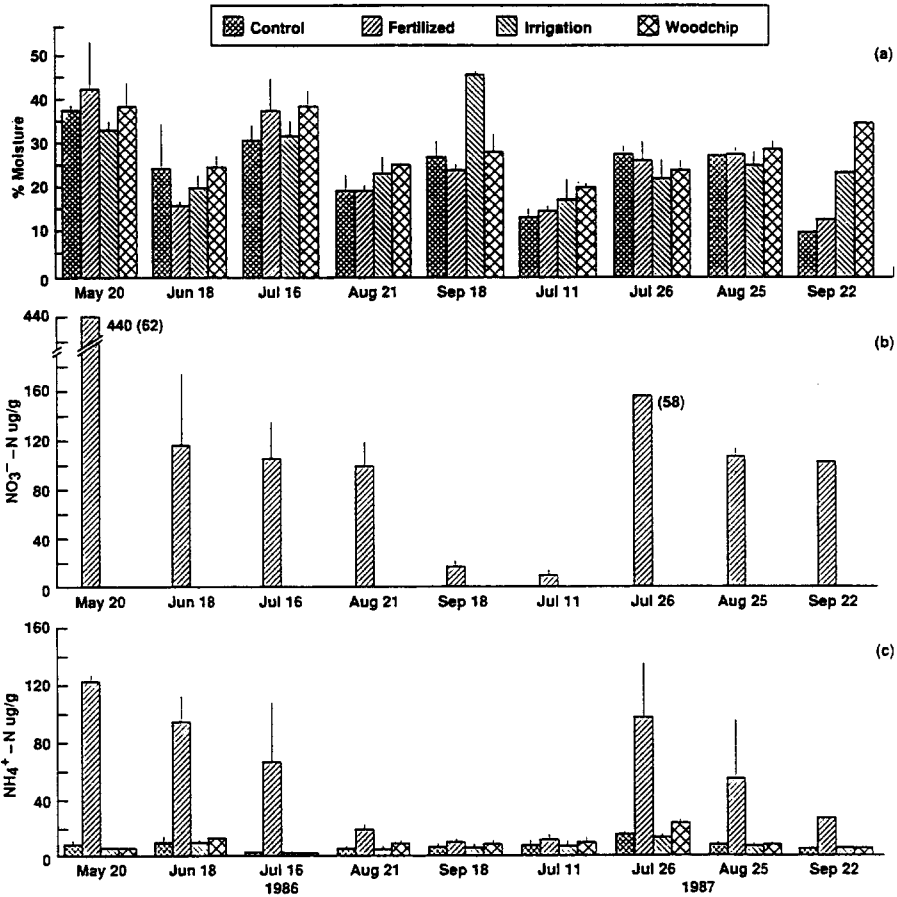


Fig. 1. Average percent moisture (a), nitrate (b), and ammonium (c) in mineral soil for the 4 treatments at 5 dates in 1986 and 4 dates in 1987. Fertilizer was applied prior to the May collection in 1986 and between the July 11 and July 26 collections of 1987. Values are averages of 2 replicates, each having 8 subsamples, except for September 22, when only 1 replicate plot of each treatment was sampled. One standard deviation of the mean is plotted or in parentheses.

forest floor was frequently significantly greater in the woodchip treatment than in the other plots (Fig. 2a).

Nitrate concentrations in soil and forest floor in the fertilized plots were elevated significantly throughout 1986 and 1987, and showed a gradual decline in the months following fertilization (Figs. 1b and 2b). Ammonium concentrations in mineral soil and forest floor of the fertilized plots were also generally significantly greater than those of the control,

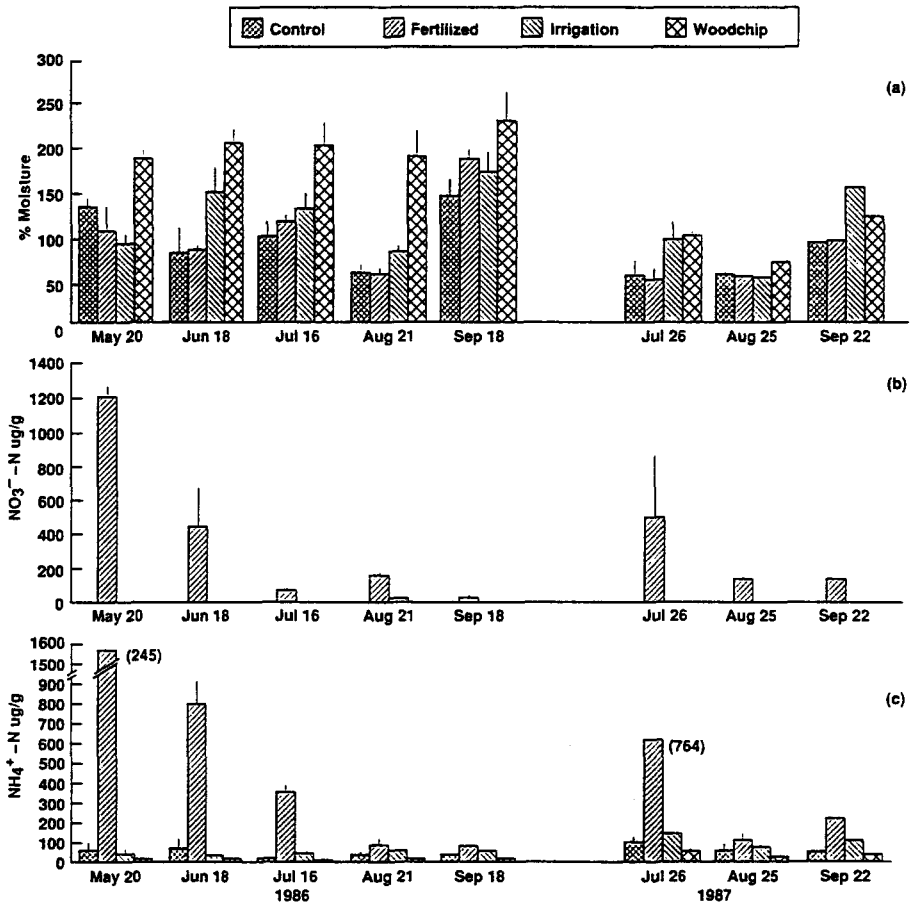


Fig. 2. Average percent moisture (a), nitrate (b), and ammonium (c) in forest floor for the 4 treatments at 5 dates in 1986 and 3 dates in 1987. Values are averages of 2 replicates, each having 8 subsamples, except for the September 22, when only 1 replicate plot of each treatment was sampled. One standard deviation of the mean is plotted or in parentheses.

irrigated and woodchip treatments immediately after fertilization, but decreased steadily throughout the year (Figs. 1c and 2c). Soil ammonium levels in the control, irrigated and woodchip treatments were not significantly different at any time, and showed no seasonal trends; forest floor ammonium was greater in the irrigated than woodchip plot in July 1986 (Fig. 2c).

Net nitrogen mineralization and nitrification rates in mineral soil and forest floor clearly responded to nitrogen fertilization (Table 1). In mineral soil, net nitrogen mineralization and nitrification rates were highest in the



*Table 1.* Annual net nitrogen mineralization (ug/g) and net nitrification (ug/g) per incubation interval (length of time in days) in the mineral soil and forest floor for each treatment during one year (May 1986–May 1987). Values are averages of 2 replicate plots per treatment, each with 8 subsamples. C = control; F = fertilized; I = irrigation; WC = woodchip.

Incubation period		Net mineralization (ug/g)				Net nitrification (ug/g)			
Interval	Days	C	F	I	WC	C	F	I	WC
<i>Mineral soil</i>									
May–Jun 86	56	-1.7	168.7	0.1	-0.5	1.8	21.1	0.8	2.6
Jul–Aug 86	71	11.5	-154.2	9.7	12.1	6.8	-84.7	2.6	10.0
Sept–Oct 86	64	2.8	139.1	5.5	5.6	0.1	133.2	-0.1	0.6
Nov–Dec 86	68	0.4	0.8	1.9	-3.3	1.6	2.6	0.8	0.1
Jan–Mar 87	92	-3.6	3.5	-0.9	-1.7	2.0	2.4	2.6	1.2
Apr–May 87	14	1.3	5.0	1.8	1.0	1.2	5.4	1.9	0.8
Annual total	365	10.7	162.9	18.1	13.2	13.5	80.0	8.6	13.7
<i>Forest floor</i>									
May–June 86	56	15.4	-786.5	7.8	100.8	9.1	-110.7	0.2	25.2
Jul–Aug 86	71	4.0	-21	0.3	26	9.8	310.9	0.4	17.2
Sep–Oct 86	64	17.6	83.8	-4.5	50.6	0.6	88.4	-0.5	1.5
Nov–Dec 86	68	-14.3	-10.9	-11.1	-13.8	1.4	16.4	2.4	1.5
Jan–Mar 87	92	12.8	-58.4	27.9	20.1	4.0	-42.0	3.0	4.6
Apr–May 87	14	11.8	2.5	-0.7	-2.1	0.2	5.6	0.6	0.1
Annual total	365	47.3	-790.5	19.7	181.6	25.1	268.6	6.1	50.1

fertilizer plots, although net immobilization occurred during the July–August period (Table 1). Differences among the other treatments were not significant. Expressed on an areal basis, annual net nitrogen mineralization in mineral soil was  $277 \text{ kg ha}^{-1} \text{ y}^{-1}$  in the fertilized treatment and 18, 30 and  $22 \text{ kg ha}^{-1} \text{ y}^{-1}$  in the control, irrigation and woodchip treatments, respectively.

Despite periods of net mineralization, annual net nitrogen mineralization in the forest floor was negative in the fertilizer plots (Table 1), suggesting that the applied fertilizer was being immobilized. Annual net nitrification in forest floor was much greater in the fertilized treatment than in the others, although periods of apparent immobilization did occur soon after application of the fertilizer (Table 1).

Net nitrogen mineralization and nitrification rates of the soils collected in August 1987 and incubated in the laboratory also clearly reflected the fertilization treatment. Net mineralization and net nitrification were on

average nearly an order of magnitude higher for soils from the fertilized plots than the other treatment plots (Table 2). Microbial biomass N was on average lower in the fertilized plots than in the other treatments; the woodchip treatment had significantly higher microbial biomass N concentrations than all other treatments (Table 2). Total nitrogen concentration in the soil did not differ between fertilized plots and controls; one of the woodchip plots, however, had significantly higher total N (t-test  $p < 0.05$ ).

Recovery of applied  $^{15}\text{N}$  in nitrate, ammonium, and microbial biomass nitrogen also showed a strong fertilization effect (Table 3). Even within 4 days after  $^{15}\text{N}$  application, more than 60% of the applied  $^{15}\text{N}$  was recovered in microbial biomass in the control, irrigated, and woodchip soils; in these treatments, almost no  $^{15}\text{N}$  was recovered as  $\text{NO}_3\text{-N}$ . In the fertilized soils, on the other hand, approximately 40% of applied  $^{15}\text{N}$  was recovered in microbial biomass, and an average of 42% was recovered in  $\text{NO}_3\text{-N}$  (Table 3). By the end of the 28-day incubation, control and irrigated plots had 2–3% recovery in  $\text{NO}_3\text{-N}$ , sawdust treated plots had 0.2% in  $\text{NO}_3\text{-N}$ , and fertilized plots had approximately 53% in  $\text{NO}_3\text{-N}$ . Fertilized plots also had less recovery of applied  $^{15}\text{N}$  in microbial biomass in the 28-day incubations than in the shorter incubations (in part because microbial biomass N dropped from the 4-day average of 199  $\mu\text{g/g}$  (Table 2) to 160  $\mu\text{g/g}$  at 28 days).

Nitrous oxide fluxes during the snow-free season in 1986 and 1987 are shown in Fig. 3. Fluxes from the control, irrigated and woodchip treatments were generally very low throughout the sampling periods. Fluxes from the fertilized plots were also very low early and late in the

Table 2. Soil net N mineralization and net nitrification ( $\mu\text{g g}^{-1} 28 \text{ days}^{-1}$ ), microbial biomass N ( $\mu\text{g/g}$ ) at the beginning (4th day) of the incubation, and total N ( $\text{mg/g}$ ) for each plot in August 1987. Values are plot means ( $\pm$  SE) of three subsamples.

Plot		Mineralization	Nitrification	Microbial biomass	Total N
Control	1	7.3 (5.4)	7.7 (4.5)	196 (45)	2.9 (0.2)
	2	3.5 (4.7)	8.5 (3.9)	259 (20)	3.3 (0.2)
Fertilized	1	37.6 (17.3)	54.8 (32.6)	200 (15)	2.9 (0.2)
	2	34.4 (0.7)	110.7 (74.1)	200 (32)	3.2 (0.4)
Irrigation	1	3.0 (2.0)	8.7 (1.5)	250 (22)	3.2 (0.3)
	2	3.4 (3.3)	6.5 (4.2)	178 (49)	2.4 (0.3)
Woodchip	1	-2.3 (1.3)	1.1 (0.4)	330 (28)	4.1 (0.6)
	2	-2.6 (0.9)	0.5 (0.4)	359 (79)	3.3 (0.5)

Table 3. Percent recovery of applied  $^{15}\text{N}$  in ammonium-N, nitrate-N and microbial biomass-N (MB-N) at the end of 4, 12, 28-day incubations of mineral soil collected in August 1987. Each value is the mean ( $\pm$  SE) of three samples in each plot.

Plot		Percent recovery of $^{15}\text{N}$											
		4-Day				12-Day				28-Day			
		$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	MB-N		$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	MB-N		$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	MB-N	
Control	1	2.6 (1.6)	0.2 (0.2)	68.6 (8.9)	1.3 (0.1)	0.1 (0.1)	59.5 (2.0)		2.1 (0.1)	2.9 (0.1)	59.5 (2.2)		
	2	2.1 (1.0)	0.1 (0.1)	60.8 (1.3)	1.0 (0.3)	0.5 (0.3)	55.2 (1.9)		0.9 (0.1)	2.7 (1.0)	62.5 (5.7)		
Fertilizer	1	7.0 (3.6)	56.1 (9.4)	34.6 (2.0)	7.7 (5.8)	39.7 (2.0)	35.2 (8.4)		1.5 (0.6)	52.3 (5.4)	19.8 (2.3)		
	2	26.8 (24.7)	28.2 (7.8)	44.3 (7.2)	10.9 (9.7)	34.5 (2.1)	43.9 (16.4)		0.6 (0.2)	54.0 (11.2)	17.2 (5.5)		
Woodchip	1	1.3 (0.5)	0 (0)	59.9 (2.8)	2.0 (1.1)	0 (0)	65.4 (4.3)		1.1 (0.1)	0.2 (0)	77.2 (1.8)		
	2	2.1 (0.6)	0 (0)	87.1 (20.6)	1.4 (0.2)	0 (0)	66.2 (1.8)		1.4 (0.1)	0.2 (0.1)	72.9 (2.5)		
Irrigation	1	1.4 (0.2)	0.3 (0.1)	65.9 (3.4)	1.4 (0.2)	0.5 (0.1)	63.3 (3.0)		1.3 (0.2)	1.9 (0.3)	76.1 (10.9)		
	2	4.2 (3.0)	0.2 (0.1)	65.1 (4.3)	1.3 (0.1)	0.2 (0.1)	57.2 (0.9)		1.5 (0.4)	2.0 (1.1)	78.8 (4.0)		

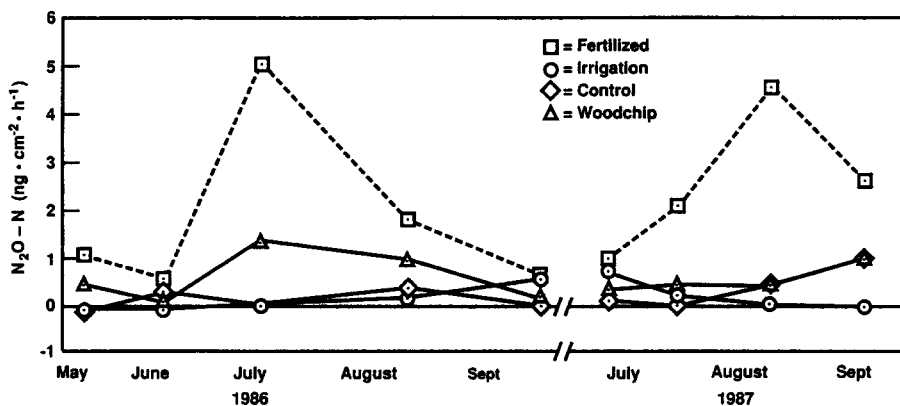


Fig. 3. Average nitrous oxide fluxes for 4 treatments at 5 dates in 1986 and 4 dates in 1987. Each value is the mean of two replicate plots, each having 6 subsamples within.

snow-free seasons, but were elevated during July and August of both 1986 and 1987. The analysis of variance of log-transformed treatment means indicated significant differences ( $p < 0.001$ ) in July 1986; the fertilizer treatment mean was significantly higher than all other treatments, and the woodchip treatment mean was higher than the control and irrigation means. Treatment mean differences for July 26, 1987 were significant at the 0.1 level; August differences were significant at  $p < 0.01$ , with the fertilizer treatment significantly higher than all other treatments. In addition, T-tests using chamber measurements as replicates ( $n = 12$ ) were significant at the 0.05 level in July 1986 and late July and August 1987. During these months, soil moisture (Figs. 1a and 2a) and temperature were both high relative to early and late season measurements; maximum daily soil temperatures in the control treatment, measured at the time of gas sampling in 1986, were 9 °C in June, 13.6 °C in July, 13.9 °C in August, and 12.7 °C in September. In a stepwise linear regression between plot means of nitrous oxide flux and soil  $\text{NO}_3$ ,  $\text{NH}_4$  and percent moisture,  $\text{NO}_3\text{-N}$  alone had a  $R^2$  of 0.40 ( $p < 0.001$ ); adding percent moisture increased the  $R^2$  to 0.48 ( $p < 0.01$ ); adding ammonium did not significantly improve the correlation. When stepwise linear regressions were carried out for nitrous oxide vs. forest floor variables, only  $\text{NH}_4\text{-N}$  was significant ( $R^2 = 0.11$ ,  $p < 0.05$ ).

Annual fluxes of nitrous oxide were substantially altered by fertilization. Average flux for the control treatment was 0.03 kg N ha<sup>-1</sup> y<sup>-1</sup> in 1986 (for the 139-day 'snow-free' season) and 0.09 kg N ha<sup>-1</sup> y<sup>-1</sup> in 1987 (for a 100-day 'snow-free' season). Average fluxes for the fertilized treatment were 0.67 and 0.65 kg N ha<sup>-1</sup> y<sup>-1</sup> for 1986 and 1987, respectively;

for the irrigated treatment, 0.03 and 0.05 respectively; and for the wood-chip treatment, 0.23 and 0.13 for 1986 and 1987, respectively.

## Discussion

Fertilization strongly affected soil nitrogen dynamics in this Douglas-fir forest. Soil inorganic nitrogen concentrations in the unfertilized plots were similar to those measured in mixed-fir forests in the Sierra Nevada (Schimel & Firestone 1989; Matson unpublished data), high elevation spruce-fir forests in New Mexico (Vitousek et al. 1982), mixed conifer forests in the southern Rocky Mountains (White et al. 1988), southern pine forests (Vitousek & Matson 1985), and a variety of other temperate forests (Robertson & Vitousek 1981; Vitousek et al. 1982). Fertilized plots, on the other hand, had greatly elevated ammonium and nitrate concentrations, similar to values for fertilized loblolly pine in Tennessee (Johnson et al. 1980) and fertilized Douglas-fir in Washington (Johnson 1979), but much greater than ammonium or nitrate concentrations in fertilized slash pine (DiStefano & Gholz 1989). Concentrations were also higher than those reported for fertile (but unfertilized) tropical forests (Robertson 1984; Vitousek & Matson 1988).

Fertilizer application altered nitrogen transformations as well as soil nitrogen pools. Net mineralization and nitrification rates measured in laboratory incubations of soils collected near the end of the growing season were on average nearly an order of magnitude higher in the fertilized treatment than in the other treatments. Results from the *in situ* incubations indicate apparent immobilization in fertilized soils in the period immediately following fertilization, followed by net mineralization in later months. Although the annual summation of 2-month buried bag incubations cannot be considered a direct measure of actual mineralization in these sites, it is interesting that our estimated net mineralization in the fertilized plot soils ( $277 \text{ kg ha}^{-1} \text{ y}^{-1}$ ) minus net immobilization in the forest floor ( $-29 \text{ kg ha}^{-1} \text{ y}^{-1}$ ) is comparable to the amount added in fertilizer. In contrast, annual net mineralization in the control soil was only  $18 \text{ kg ha}^{-1} \text{ y}^{-1}$ . The high rate in the fertilized treatment may result from mineralization of some of the previous year's fertilizer nitrogen that had been immobilized in forest floor and soil organic matter; it may also reflect the improved quality of above and below ground litter caused by increased N concentrations in plant tissues.

Differences in  $^{15}\text{N}$  recovery in the laboratory incubations in soils collected late in the growing season further illustrate the effects of fertilization. Microbial immobilization was the principal fate of added  $^{15}\text{N}$

in the non-fertilized forest soils, with very little remaining in the  $\text{NH}_4\text{-N}$  form and almost none being converted to  $\text{NO}_3\text{-N}$ , especially in the shorter-term incubations (Table 2). Schimel and Firestone (1989) reported similar results for a Sierran conifer forest soil. The high microbial demand for added N in our study supports other evidence that this Douglas-fir forest is strongly N limited, including the significant fertilization response in aboveground net primary production reported by Gower et al. (1992).

In contrast, relative immobilization of  $^{15}\text{N}$  in microbial biomass was far less important in the fertilized soils, with greatest recovery of label occurring in the  $\text{NO}_3\text{-N}$  pool. In absolute terms, the amount of N taken up by microbes from the very large inorganic N pool in the fertilizer treatment was probably greater than in the other treatments. However, the relatively low recovery of  $^{15}\text{N}$  in microbial biomass and substantial recovery of  $^{15}\text{N}$  in nitrate suggests that N availability in the fertilized plots was well in excess of the heterotrophic microbial demand, resulting in larger amounts available for use by nitrifiers. Vitousek and Matson (1988) found similar low  $^{15}\text{N}$  immobilization in microbial biomass and high recovery in nitrate in soils from fertile tropical forests, and the opposite result in tropical forest soils that were naturally deficient in nitrogen.

Given the high  $\text{NO}_3\text{-N}$  concentrations and the high rates of nitrification in the fertilized soils, it is not surprising that nitrous oxide emissions would also be elevated in the fertilized forests. Even in the fertilized plots, however, nitrous oxide fluxes were elevated only during periods with relatively high soil moisture and temperature. Results of the stepwise linear regressions between nitrous oxide and soil variables suggest that, while nitrate is the most critical variable, soil moisture is also significantly related to flux. In their review of denitrification in temperate forests, Davidson et al. (1990) also point out the apparent concomitant requirements for adequate soil moisture and available N.

Based on the annual extrapolation of nitrous oxide flux, losses of nitrogen via this pathway account for approximately 0.35% of the fertilizer N addition. Fertilizer losses via nitrous oxide flux are apparently small enough to be of little concern in terms of the nitrogen budget of this site.

The potential importance of nitrogen enrichment of forests to the atmospheric increase in  $\text{N}_2\text{O}$  is less clear. It has been suggested that such enrichment (from acid deposition or fertilizer application) could be an important source of the annual increase in atmospheric  $\text{N}_2\text{O-N}$  (Aber et al. 1989; Melillo et al. 1989). However, we find that the  $\text{N}_2\text{O-N}$  flux from our fertilized forest represents only 0.35% of added N. Bowden et al. (1991) similarly found large proportional increases in  $\text{N}_2\text{O-N}$  with forest fertilization, but that flux accounted for only 0.2% of the added N. Studies of sites altered by N deposition have conflicting results; Schmidt et al.

(1988) reported average  $\text{N}_2\text{O-N}$  fluxes of less than  $1 \text{ kg ha}^{-1} \text{ y}^{-1}$  in West German forests, but Papen et al. (in press) have reported larger losses. Brumme and Beese (1992) reported average  $\text{N}_2\text{O-N}$  fluxes of  $5.6 \text{ kg ha}^{-1} \text{ y}^{-1}$  from a West German forest in the Solling area of Germany that has received high long-term N deposition; fertilization in that forest resulted in a 39% increase in flux, accounting for 1.6% of the added fertilizer N. It is possible that long-term chronic additions via deposition may lead to increasing fluxes over time as the heterotrophic microbial demand for nitrogen is saturated.

Clearly, fertilization with N can lead to increased  $\text{N}_2\text{O}$  fluxes; however, the limited available evidence suggests that  $\text{N}_2\text{O-N}$  flux resulting from forest fertilization is not currently a globally significant source. If annual N fertilizer applications to forests add up to 1 Tg worldwide (a vast overestimate, considering that in the United States annual application rate is probably less than 0.05 Tg ( $200\text{--}400 \text{ kg ha}^{-1} \text{ y}^{-1} \times 140,000 \text{ ha}$ )) and  $17.8 \text{ Tg y}^{-1}$  of nitrogen are added to forest soils via atmospheric deposition (Melillo et al. 1989), even a loss rate of  $\text{N}_2\text{O-N}$  as high as 1% of added N would result in a global source of less than 0.2 Tg per year. Despite the fact that N additions can lead to large relative increases in  $\text{N}_2\text{O-N}$  fluxes from temperate forests, this source appears to be at present a minor contribution to the current 3.5 Tg annual increment in the atmosphere.

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