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Influence of atmospheric CO_2 enrichment on nitrous oxide flux in a temperate forest ecosystem

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Abstract. Long-term exposure of native vegetation to elevated atmospheric carbon dioxide (CO_2) is expected to increase the water content and the input of labile carbon (C) to soil, which could stimulate nitrification and denitrification and enhance nitrous oxide (N_2O) emissions. We measured N_2O fluxes for 2 years in a *Pinus taeda* forest that was continuously enriched 200 μL L^{-1} CO_2 above the ambient atmospheric CO_2 concentration (~560 μL L^{-1}) beginning 16 months prior to our study. Soil treated with elevated CO_2 showed higher N_2O emissions at low winter temperatures than the ambient CO_2 control. Conversely, soil treated with elevated CO_2 showed lower N_2O emissions at high summer temperatures than the control soil. Annual N_2O fluxes, however, were similar between treatments (~6600 μg m^{-2}). Factors that influence denitrification and N_2O production were investigated in the laboratory using intact soil core incubations. Nitrate additions (0.17 mg KNO_3–N g^{-1}) to intact soil cores during laboratory incubations stimulated total N_2O production as well as denitrification in both treatments, whereas glucose additions lowered N_2O production in both treatments. These experiments demonstrated that N_2O production is strongly limited by available nitrogen (N) and that the addition of labile C is likely to reduce the amount of N_2O produced by nitrification. Our results collectively suggest that CO_2 enrichment of this N-limited ecosystem may reduce N_2O flux during the growing season, when soil C inputs and plant-microbial competition for NH_4^+ are high. Alternatively, elevated CO_2 may enhance N_2O flux in the winter, when conditions are moist and cold and plants are less active. The potential indirect effects of CO_2 enrichment (greater soil moisture and labile C inputs) could reduce N_2O flux from nitrification in summer and enhance N_2O flux from denitrification in winter, resulting in no net change in total ecosystem N_2O flux at the soil-atmosphere interface.

1. Introduction

The atmospheric N_2O concentration has been increasing at a rate of 0.3% per year, primarily as a result of anthropogenic emissions from agriculture, energy, industry, and animal waste management systems [*Khalil*, 1999]. Food production systems, in general, are largely responsible for enhanced biogenic emissions [*Kroeze et al.*, 1999]. The global warming potential of N_2O is ~300 times greater than that of CO_2, and enhanced N_2O emissions contributed 6% to the change in radiative forcing of Earth's atmosphere between 1980 and 1990 [*Houghton et al.*, 1997]. Nitrous oxide resides in the atmosphere 120 years, so small increases in atmospheric N_2O can have long-lasting effects [*Khalil*, 1999]. Soils are a major source of atmospheric N_2O, contributing 10 of the 16 Tg emitted annually from all sources [*Schlesinger*, 1997], yet environmental controls, interactions, and feedbacks that affect in situ N_2O production and emission are not well understood.

Denitrification and nitrification are the microbial processes primarily responsible for N_2O production [*Knowles*, 1982]. Denitrification is conducted by facultative anaerobes and is regulated by O_2, NO_3^-, and labile C availability. Nitrification is an aerobic process governed by NH_4^+ availability [*Knowles*, 1982]. Both processes are influenced by soil moisture, temperature, and O_2 [*Knowles*, 1982]. At an ecosystem scale, substrate supply for nitrification and denitrification is directly or indirectly determined by rates of N mineralization and N assimilation by plants and microbes and by diffusional constraints [*Firestone and Davidson*, 1989]. These processes drive N_2O production, and they are strongly controlled by factors expected to change as a result of elevated atmospheric CO_2. Alterations in soil physical or chemical properties, such as lower O_2 status or greater C and N availability, could lead to a greater flux of N_2O at the soil surface and an enhanced greenhouse effect.

The atmospheric carbon dioxide concentration has increased 30% since the beginning of the Industrial Revolution and is expected to rise from the present concentration of 360 to 460–560 μL L^{-1} during this century [*Schlesinger*, 1997]. It is uncertain how forest ecosystems will respond to the anthropogenic increase in atmospheric CO_2. High concentrations of atmospheric CO_2 have been

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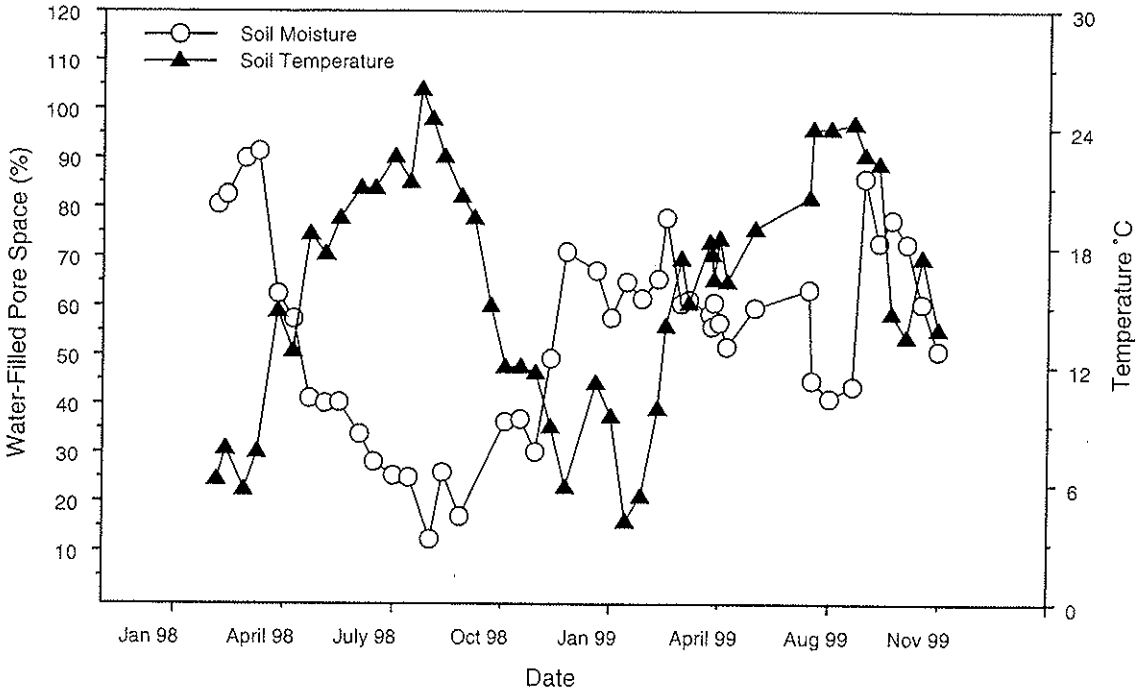

Figure 1. Mean soil moisture in the 0- to 30-cm zone and soil temperature averaged from the 1- to 20-cm zone on each sampling date.

shown to enhance plant productivity [*DeLucia et al.*, 1999], fine root turnover [*Pregitzer et al.*, 1995], and nutrient and water use efficiency [*Hungate et al.*, 1997b; *Jackson et al.*, 1994]. These plant responses to elevated CO_2 may, in turn, influence soil microbial activity [*Sadowsky and Schortmeyer*, 1997; *Zak et al.*, 1993].

The effects of extended, large-scale CO_2 enrichment on forest ecosystem function, including soil biotic processes, are not well defined [*Schimel and Gulledge*, 1998]. Few studies have investigated N_2O emissions under CO_2 enrichment [*Hungate et al.*, 1997a; *Ineson et al.*, 1998; *Ambus and Robertson*, 1999], and none have investigated N_2O in a free-air CO_2-enriched forest ecosystem. Forests contribute roughly 25% of the total N_2O annually emitted to the atmosphere [*Schlesinger*, 1997], and these ecosystems occupy one third of the Earth's land surface [*Leith and Whittaker*, 1975]. Consequently, a detailed understanding of the impact of elevated CO_2 on net N_2O flux from forest soils is critical to predicting the future source strength of these ecosystems in the atmospheric N_2O budget.

The Duke Forest Free-Air CO_2 Enrichment (FACE) project is an integrated, multidisciplinary experiment designed to assess the response of a temperate forest to projected future atmospheric concentrations of CO_2. Open, experimental plots of a sufficient size (30 m) to encompass ecosystem-level feedbacks and interactions between the atmosphere and plant-soil systems are directly and continuously fumigated with CO_2 to maintain a concentration 560 uL L^{-1}. For each CO_2-enriched plot, there is a similar control plot that is continuously fumigated with ambient air only.

Our study was conducted at the Duke Forest FACE site with the overall objective of assessing the influence of elevated CO_2 on N_2O production and emission in a temperate forest soil. To meet this objective, we conducted a 2-year time series for changes in N_2O emission, soil temperature, and soil moisture at permanently established locations within CO_2-enriched plots and companion plots amended with ambient air only (controls). In addition, we conducted laboratory studies assessing denitrifier enzyme activity, chemical limitations (NO_3^- and labile C) to denitrification, and the relative importance of nitrification and denitrification to N_2O production for homogenized soil samples and soil cores from CO_2-enriched and control plots.

2. Methods

2.1. Field Site

The study site is located in Orange County, North Carolina, United States (35°58′N, 79°05′W), where a 90-ha parcel of even-aged loblolly pine (*Pinus taeda* L.) was planted on a clay loam soil in 1983. Topography is flat, and soils are Ultic Alfisols of the Enon Series, (Ultic Hapludalf) [*Allen et al.*, 2000]. Average monthly air temperature ranges from 1°C in January to 36°C in July. Annual precipitation averages 1150 mm and is evenly distributed throughout the year [*State Climate Office of North Carolina*, 1999].

Six circular, 30-m-diameter plots (herein referred to as "rings") were established within the Duke forest to fumigate trees with CO_2. Each of the six rings was divided into four pie-shaped sections for a total of 24 individual sectors. Three rings served as a control to which ambient air was added, whereas the remaining three rings were fumigated continuously to maintain atmospheric CO_2 levels at ~560 μL L^{-1}. Treatment began on August 27, 1996, and has continued with minimal interruptions. Carbon dioxide concentration was tracked continuously with sensors within plots. Actual mean enrichments ranged from 199 to 203 μL L^{-1} above ambient CO_2 (365 μL L^{-1}), with concentrations inside the CO_2-treated rings varying from 550 to 570 μL L^{-1} [*Hendrey et al.*, 1999].

2.2. Soil Sampling Methods

Soil cores were collected to a depth of 15 cm using a hammer-driven core device containing stainless steel inserts (5-cm diameter by 30-cm height). During each sampling session a single core was

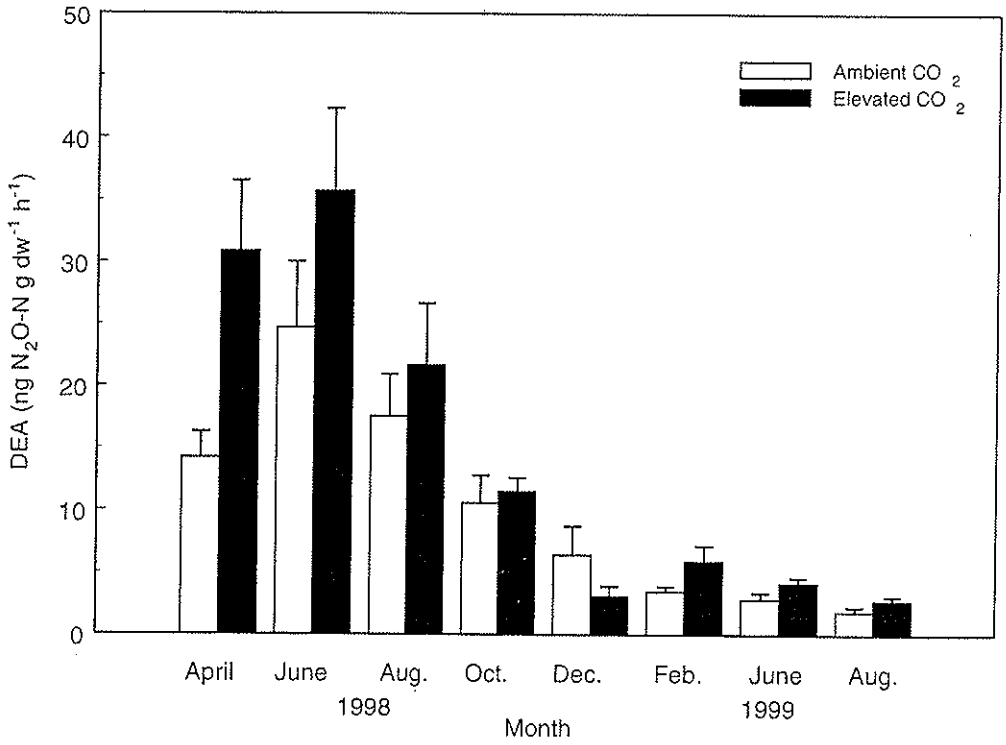

Figure 2. Mean (±standard error of the mean (SEM) and $n = 3$) denitrifier enzyme activity (DEA) for CO_2-enriched and control plots.

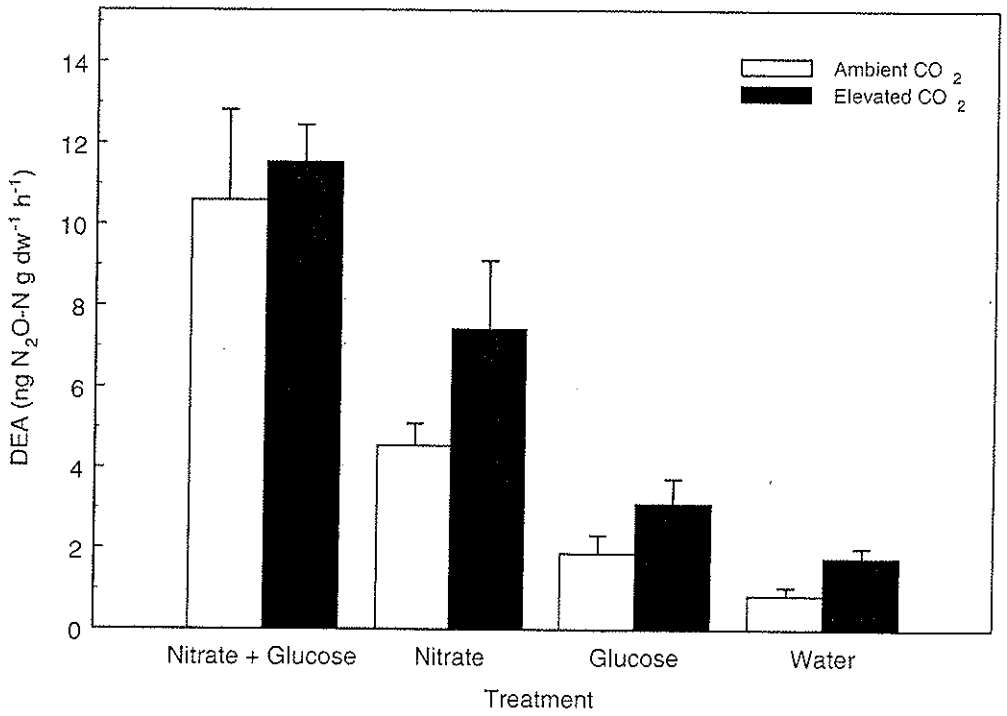

Figure 3. Mean (±SEM and $n = 4$) denitrifier enzyme activity (DEA) in homogenized soil composites in response to glucose and nitrate addition in October 1998.

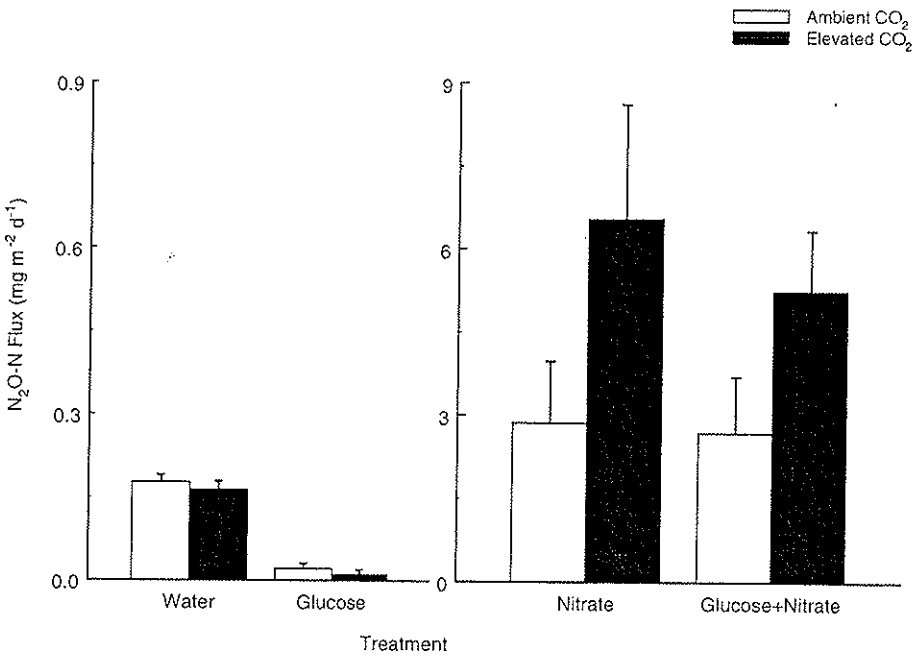

Figure 4. Mean (±SEM and $n = 12$) N_2O flux by intact soil cores (15-cm length) collected from CO_2-enriched and control plots. Cores were amended with glucose, nitrate, glucose plus nitrate, or water only (control). Note the change in scale between ordinal axes.

randomly collected within each sector of each plot for a total of 24 cores. The bottom of each soil core was covered with a plastic cap. Each covered core was placed in cold storage and transported to the laboratory within 6 hours of collection. Core sections from the 0- to 7.5-cm depth interval were composited by ring and sieved (through 4-mm mesh) for experiments requiring homogenized soil samples. Most soil sampling was performed at ~8-week intervals during the March through November growing season.

2.3. Determination of Soil Physical Properties

In each ring, organic content for homogenized soils in the 0- to 7.5-cm and 7.5- to 15-cm zones was determined by loss on ignition (550°C for 4 hours) for triplicate, oven-dried (105°C for 24 hours) subsamples. Soil particle density was measured pycnometrically, and bulk density was computed as the quotient of oven-dried mass divided by field volume. Soil pH was measured potentiometrically on 1:2 soil-deionized H_2O paste. All determinations of soil physical properties follow *Carter* [1993].

2.4. Laboratory Experiments With Homogenized Soil Samples

Laboratory experiments with homogenized samples were used to assess potential denitrification, controls on denitrification, and sources of N_2O (nitrification or denitrification). Experiments were conducted in 133-mL mason jars equipped with O-seal fittings and septa to allow introduction and withdrawal of headspace gas. As required, sealed jars were amended with liquid medium (described below) and made anaerobic. Anaerobiosis was achieved by repeated evacuation of the headspace, introduction of N_2, and vigorous shaking to equilibrate headspace gases in the dissolved phase. In all experiments involving the addition of H_2SO_4-scrubbed acetylene, samples were vigorously shaken by hand to equilibrate headspace C_2H_2 with the aqueous phase. Jars were incubated on a rotary shaker (175 rpm), and 2-mL headspace samples were analyzed for N_2O after 0.5 hour and at 0.5-hour intervals thereafter for 2 hours.

Denitrification enzyme activity (DEA) was determined in 1998 and 1999 according to *Tiedje* [1994]. Triplicate field-moist soil samples (25 g each) from each ring on each collection date were amended with 25 mL of the standard medium (40 mg glucose-C L^{-1}, 100 mg NO_3^--N L^{-1}, and 10 mg chloramphenicol L^{-1}), and the headspaces were adjusted to 10 kPa C_2H_2 prior to time course determination of headspace N_2O. A modification of this experiment was used to assess factors limiting DEA for soils collected in October 1998. Quadruplicate 25-g field moist soil samples from each ring were amended with 25 mL of one of four solutions: (1) deionized water (control), (2) 40 mg glucose-C L^{-1}, (3) 100 mg NO_3^--N L^{-1}, or (4) 40 mg glucose-C L^{-1} plus 100 mg NO_3^--N L^{-1}. Acetylene was added (10 kPa), and the time course for accumulation of headspace N_2O was determined.

A selective inhibition technique [*Klemedtsson et al.*, 1988] was used to assess the relative contribution of nitrification and denitrification to N_2O production for soils. We added deionized water to achieve 49% and 94% of water-filled pore space (WFPS) because these values for the percentage of WFPS bracketed most of the field observations made during 2 years of study. Quadruplicate (35 g) samples from each ring were adjusted to each percentage of WFPS and acclimated overnight at 20°C. Samples were equilibrated with the ambient atmosphere, and N_2O production was measured every 0.5 hour over a 2-hour time period. Headspaces were adjusted to 10 Pa C_2H_2, samples were then incubated for 5 hours, headspaces were reequilibrated with the ambient atmosphere, and time courses for N_2O accumulation were again assessed. The initial rate of N_2O production represents the contribution of both nitrifiers and denitrifiers, while the C_2H_2-amended rate indicates N_2O production by denitrifiers because C_2H_2 at this concentration eliminates autotrophic nitrifier activity but has no influence on the reduction of N_2O to N_2 by denitrifiers. The difference between the two rates gives the contribution of nitrifiers only.

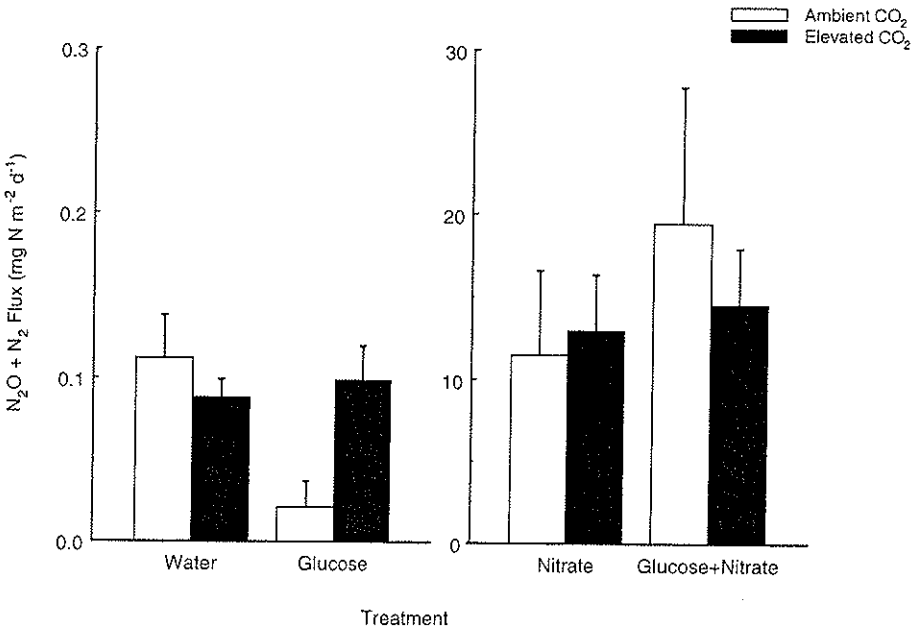

Figure 5. Mean (±SEM and $n = 12$) rate of total denitrification ($N_2 + N_2O$) by intact soil cores (15-cm length) from CO_2-enriched and control plots. Cores were amended with glucose, nitrate, glucose plus nitrate, or water only (control). Note the change in scale between ordinal axes.

2.5. Laboratory Experiments With Intact Soil Cores

Factors limiting N_2O flux and total denitrification ($N_2O + N_2$) were investigated using 15-cm-depth cores collected in June 1999. These remained intact inside 30-cm stainless steel insert tubes used for sample collection. Twenty-four cores (12 ambient and 12 elevated CO_2) were amended with 60 mL of one of four solutions: (1) deionized water (control), (2) 0.4 g glucose-C L^{-1}, (3) 1 g KNO_3-N L^{-1}, or (4) 0.4 g glucose-C L^{-1} plus 1 g KNO_3-N L^{-1}. Solutions were applied with a spinal syringe to evenly distribute the solution throughout the soil profile with a volume (60 mLs) sufficient to raise soil moisture to the typical June level of 55% WFPS, and cores were incubated for 12 hours. Each insert tube was sealed at both ends with plastic stainless-steel-lined caps equipped with O-seal fittings and septa, and the time course for N_2O accumulation in the headspace was determined. Headspaces and air-filled pore spaces were then adjusted to 10-kPa C_2H_2 using a 20-cm-long, 1-mm-diameter stainless steel needle perforated to distribute C_2H_2 evenly in the soil profile. After 5 hours each core was vented and capped, and the time course for N_2O accumulation was revaluated. The first flux measurement estimates community N_2O production, while the latter gives total denitrification ($N_2O + N_2$), because C_2H_2 at this concentration inhibits the reduction of N_2O to N_2 by denitrifiers.

2.6. Field Studies of N_2O Emission

Nitrous oxide fluxes within each ring sector were determined bimonthly from January 1998 through December 1999 using the static chamber technique [*Whalen et al.*, 1990]. One polyvinyl chloride collar (20-cm diameter by 11-cm height) was randomly located within each sector prior to initiation of the study for a total of 12 per treatment. Polyvinyl chloride covers equipped with butyl O-rings were placed on the collars during each N_2O flux measurement. Covers included a capillary bleed to equalize pressure and an O-seal fitting and septa for syringe sampling of the headspace gas at 0.5-hour intervals during a 2-hour time course.

Soil moisture and temperature were determined in conjunction with N_2O flux measurements. Depth-integrated (0–30 cm) soil moisture determinations were made continuously with time domain reflectometry (TDR) probes installed within each sector. Soil temperature was measured at 3-cm intervals from 1 to 16 cm with a multithermistor probe during each site visit, and the average temperature for this depth interval was calculated.

2.7. Gas Analysis

Headspace gas was sampled with 5- or 10-mL SESI nylon syringes equipped with pistons modified to accept a larger-diameter sealing O-ring. Samples were analyzed for N_2O with a Shimadzu GC-14A ^{63}Ni electron capture gas chromatograph fitted with a 0.5-mL sample loop. Gases were injected onto a 1-m precolumn and a 3-m analytical column (both Porapak-Q) operated at 40°C with a 5% CH_4 to 95% Ar carrier flowing at 25 mL min^{-1}. The gas chromatograph was calibrated with commercial N_2O-air blends (Scott Specialty Gases) following verification of stated concentrations with standards from the National Institute of Standards and Technology. The precision of analysis expressed as a coefficient of variation for 10 replicate injections of 301- and 360-nL N_2O-N L^{-1} standards was <2%. The minimum detectable concentration change was ±7 nL L^{-1}. Values that were within 7 nL L^{-1} of each other were considered to be no different from zero. Gas samples were stored <8 hours in the SESI syringes before analysis. Tests showed no change in N_2O concentration during storage.

2.8. Calculations and Statistics

Rates of N_2O production in field and laboratory experiments were calculated from the time linear change in headspace concentration and headspace volume of the static chamber or incubation vessel. Annual N_2O fluxes from field sites were calculated by time integration of the daily N_2O flux from each chamber. The integrated N_2O flux was numerically approximated with the midpoint rule [*Berkey and Blanchard*, 1992] using the set of flux observations

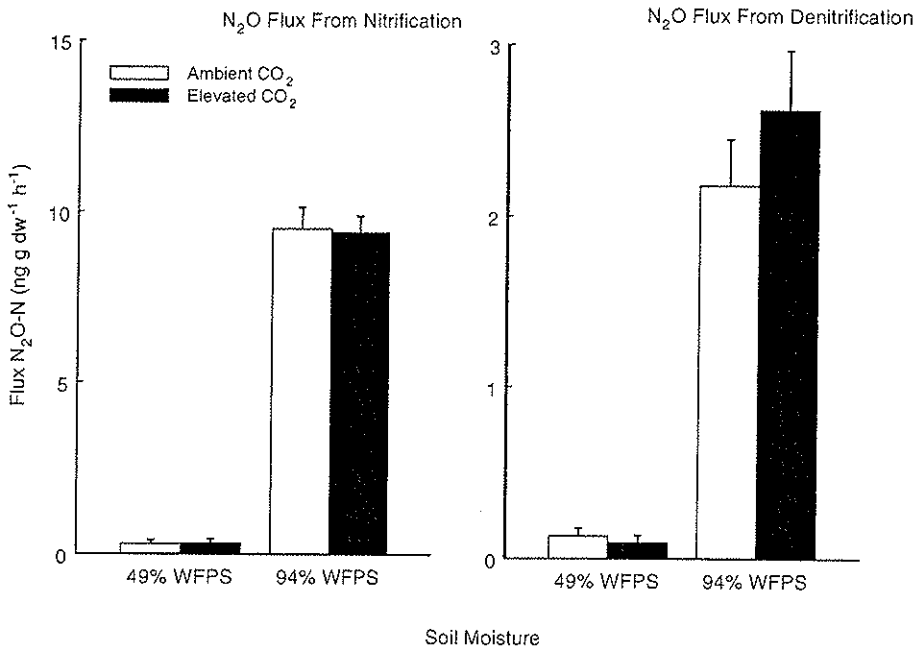

Figure 6. Mean (±SEM and $n = 24$) N_2O flux before and after C_2H_2 addition (10 Pa) at two levels of soil moisture (in percent water-filled pore space (WFPS)). Nitrous oxide flux prior to C_2H_2 addition is due to both nitrification and denitrification, while the flux following C_2H_2 addition is due to denitrification only.

over time (Figure 7) for each year. A t test was used to determine differences between treatment and year for annual N_2O flux.

Field flux data were analyzed using a mixed linear model with repeated measures. Carbon dioxide treatment was the main effect, with moisture, temperature, and season as covariates. This was necessary to determine the effect of treatment (given soil moisture variability among plots) and if this effect varied with moisture, temperature, or season. A nested hierarchical model was used with sectors nested inside plots and treatments. Data collection intervals were unequal, so a time series covariance structure was used where correlations decline as a function of time. All interactions were tested; only significant three-way interactions remained in the model along with all lower-order terms and interactions.

All laboratory data were analyzed using a mixed, hierarchical linear model. Modified versions of this model were used to test the effect of CO_2 enrichment on DEA, sources of N_2O, N_2O flux from intact cores, and denitrification. DEA data collected in 1998–1999 were analyzed with a repeated measures model that included CO_2 treatment and year and month of soil collection. Limitations to DEA were tested in a model that included CO_2 treatment and media amendment. Experiments assessing the contribution of nitrification and denitrification to N_2O production were analyzed separately for each moisture level. Nitrous oxide fluxes from intact soil cores were analyzed separately from the $N_2O + N_2$ flux produced by denitrification only, and these models were tested for both the effect of CO_2 treatment and media amendment. Only significant interactions remained in each model. A significance level of $\alpha = 0.05$ was used for all tests, and data were transformed (natural log) when necessary to homogenize variances.

3. Results

3.1. Soil Properties

Soil temperatures varied from 4° to 26°C, with an overall mean of 16°C (Figure 1). The lowest soil temperature was 4°C in February 1999, and the highest was 26°C in August 1998. Individual soil moisture content by sector, expressed as percent WFPS, varied from 1 to 120% with an overall mean of 60%. Soil particle density was 2.5 g cm^{-3}, and pH was 6.0. Bulk density was 1.05 g cm^{-3} in the 0- to 7.5-cm zone and 1.3 g cm^{-3} in the 7.5- to 15-cm zone. Soil organic matter ranged from 6.4 to 9.0% in the 0- to 7.5-cm depth interval and from 3.6 to 6.3% in the 7.5- to 15-cm depth interval. These soil properties measured on the dates shown during this study period (Figure 1) were similar between treatments except for percent soil organic matter, which was slightly higher for CO_2-enriched plots in June 1999 [*Phillips et al.*, 2001].

3.2. Denitrifier Enzyme Activity

Denitrifier enzyme activity ranged from 1 to 95 ng N_2O-N g_{dw}^{-1} h^{-1} (where dw is dry soil mass), with the highest DEA at the beginning of the study in June 1998 and the lowest DEA at the end of the study in August 1999 (Figure 2). Average DEA in 1998 (19 ng N_2O-N g_{dw}^{-1} h^{-1} with a standard error of the mean (SEM) of 2 and $n = 60$) was higher ($p < 0.05$) than in 1999 (3.2 ng N_2O-N g_{dw}^{-1} h^{-1} with SEM = 0.2 and $n = 60$) for both elevated and ambient CO_2 plots. Overall, mean DEA for CO_2-enriched plots was 13 ng N_2O-N g_{dw}^{-1} h^{-1} (SEM = 2 and $n = 60$), while mean DEA for controls was 9 ng N_2O-N g_{dw}^{-1} h^{-1} (SEM = 1 and $n = 60$). The effect of CO_2 on DEA depended upon the month of soil collection, with a significant ($p < 0.05$) treatment by month interaction (Figure 2).

3.3. Organic C and NO_3^--N Limitations of DEA

The chemical composition of the medium added to homogenized soil composites significantly ($p < 0.05$) affected DEA for October 1998 soils (Figure 3). Samples amended with both NO_3^- and glucose showed the highest DEA, which was ~1 order of magnitude greater than control soils amended with water only. Denitrifier enzyme activity for the NO_3^- addition was ~5 times

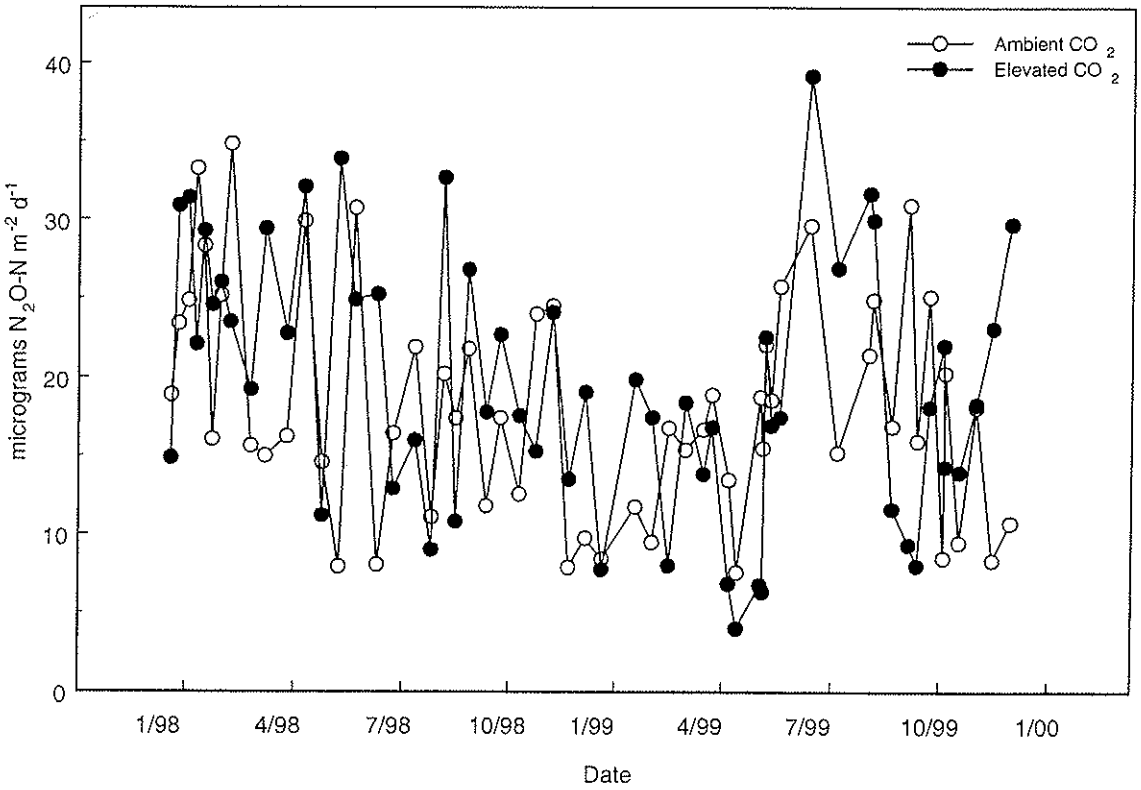

Figure 7. Mean ($n = 12$ for each date) N_2O flux from static chambers in CO_2-enriched and control forest plots. Error bars are eliminated for clarity.

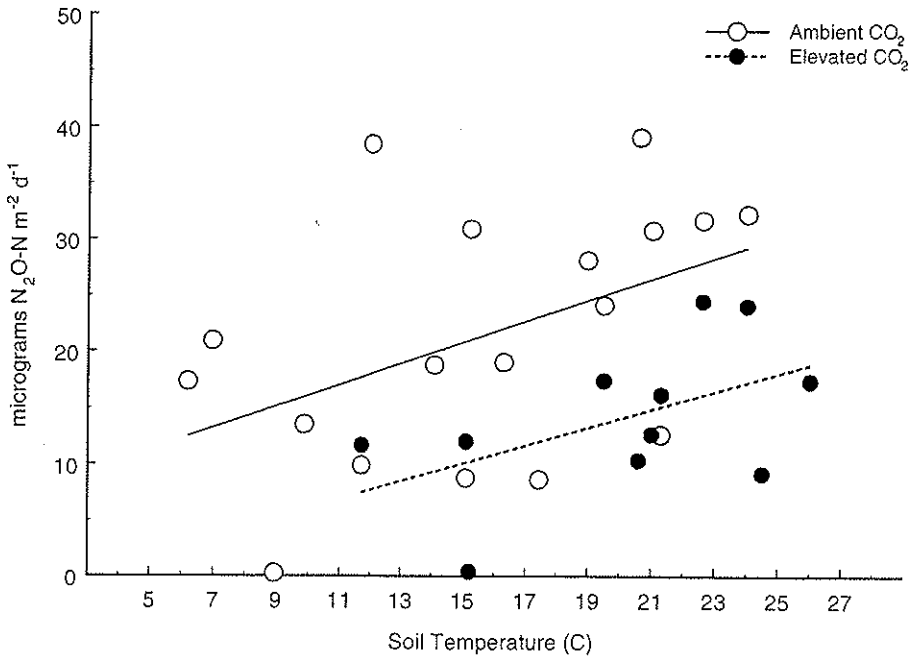

Figure 8. Mean N_2O flux from field plots by soil temperature where soil moisture was $\leq$30% WFPS (mean by date for 159 observations). Linear regression lines illustrate trends for CO_2-enriched and control plots.

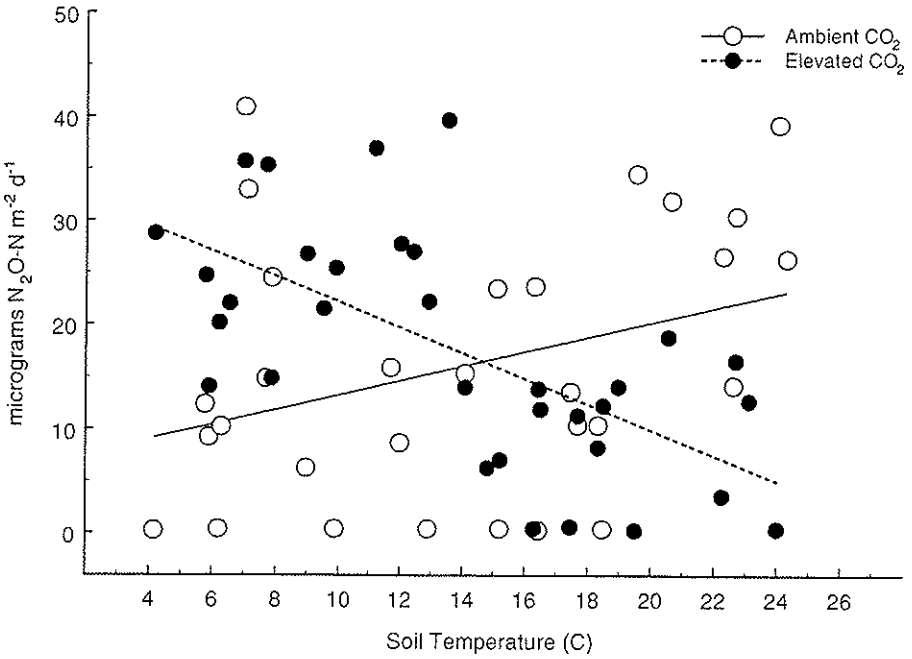

Figure 9. Mean N_2O flux from field plots by soil temperature where soil moisture was ≥70% WFPS (mean by date for 320 observations). Linear regression lines are plotted to illustrate trends for CO_2-enriched and control plots.

greater than the control, whereas DEA for the glucose-C medium was only twice that of the control. Soils from elevated CO_2 plots tended to respond to NO_3^-, glucose, and water-only media with greater DEA as compared with ambient CO_2 soils, but this trend was not statistically significant. Soils from CO_2-enriched plots consistently showed higher within-treatment DEA than soils from control plots, but the differences were not significant.

3.4. Intact Soil Core Experiments

Nitrous oxide fluxes for cores collected from CO_2-enriched plots were similar to ambient CO_2 control cores for each substrate addition (Figure 4). However, N_2O flux was significantly affected by the composition of the medium when compared with the flux from water-only media. Nitrous oxide fluxes from cores amended with NO_3^--N or glucose-C + NO_3^--N were significantly higher than N_2O flux from water-only media. However, cores amended with glucose-C only showed significantly lower N_2O flux with respect to water-only media.

Mean total N (N_2O + N_2) flux from denitrification for cores collected from CO_2-enriched soils was similar to the flux for ambient control soils for each chemical treatment (Figure 5). However, total N flux from denitrification was significantly affected by substrate addition when compared with flux from treatments that received water only. Total N fluxes for the NO_3^--N and NO_3^--N + glucose-C treatments were significantly higher than the flux from the water-only treatment. However, addition of glucose-C alone did not significantly alter the total N flux relative to the addition of water only.

3.5. Sources of N_2O

An increase in soil moisture significantly increased total N_2O production in homogenized soil composites (Figure 6). The average N_2O-N production rate of 18 ng g_{dw}^{-1} h^{-1} (SEM = 1 and n = 24) at 94% WFPS exceeded the mean of 0.79 ng g_{dw}^{-1} h^{-1} (SEM = 0.11 and n = 24) at 49% WFPS by a factor of ~20. Addition of 10 Pa of C_2H_2, to inhibit only nitrification, reduced the rates of N_2O-N production at 94% WFPS and 49% WFPS by 73% and 80%, respectively. The average rate of N_2O produced by denitrifiers at 94% WFPS (3.6 ng N_2O-N g_{dw}^{-1} h^{-1} with SEM = 0.2 and n = 24) exceeded the mean at 49% WFPS (0.21 ng N_2O-N g_{dw}^{-1} h^{-1} with SEM = 0.05 and n = 24) by a factor of ~17. Nitrous oxide production was not significantly different between CO_2-enriched and control soils at similar moisture contents, both before and after C_2H_2 addition. Thus denitrification and nitrification accounted for ~25% and 75%, respectively, of the total N_2O

Table 1. Model Estimates of N_2O Flux at Mean Soil Moisture and Temperature Values for Each Season[a]

Season	CO_2	CO_2 by Temperature	CO_2 by Moisture	CO_2 by Season	CO_2 by Temperature by Moisture	CO_2 by Temperature by Season
Winter	−32.4	2.1	137.8	−34.6	−8.6	3.5
Spring	−32.4	2.1	137.8	−3.1	−8.6	−0.03
Summer	−32.4	2.1	137.8	23	−8.6	−1.1
Fall	−32.4	2.1	137.8	0	−8.6	0

[a] All units are in μg m^{-2} d^{-1}; see Figure 10.

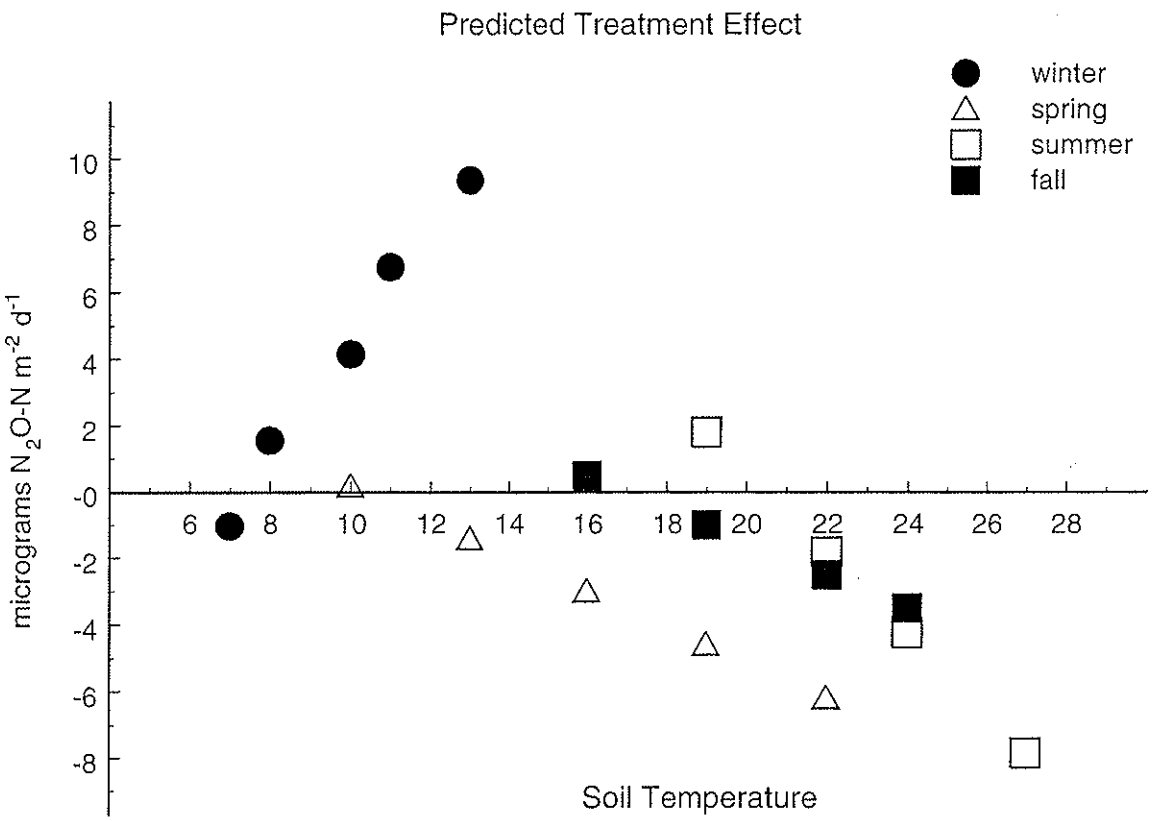

Figure 10. Predicted effect of CO_2 enrichment on N_2O flux for the winter (December through February), spring (March through May), summer (June through August), and fall (September through November) as a function of soil temperature. Model estimates (in $\mu g\ N_2O\ m^{-2}\ d^{-1}$) shown determined the predicted effect at mean soil moisture for the seasonal range of soil temperature.

produced at both moisture levels, and CO_2 enrichment did not alter these proportions.

3.6. Field N_2O Fluxes

Nitrous oxide fluxes in 1998 and 1999 varied from 0 to 110 μg N_2O-N m^{-2} d^{-1} with an overall mean of 19 μg m^{-2} d^{-1} (SEM = 0.6 and n = 1300). The average N_2O flux for CO_2-enriched plots was 20 μg m^{-2} d^{-1} (SEM = 0.9 and n = 650), while the average flux for control plots was 18 μg m^{-2} d^{-1} (SEM = 0.9 and n = 650) (Figure 7). The average annual integrated N_2O-N flux for CO_2-enriched plots was 7900 μg m^{-2} (SEM = 950 and n = 12) in 1998 and 5900 μg m^{-2} (SEM = 560 and n = 12) in 1999. The average annual integrated flux for control plots was 6900 μg m^{-2} (SEM = 810 and n = 12) in 1998 and 5500 μg m^{-2} (SEM = 780 and n = 12) in 1999. Within-treatment annual fluxes were not significantly different between years, and annual N_2O fluxes were not significantly different between CO_2-enriched and control plots. The overall mean integrated N_2O flux was 6600 μg N_2O-N m^{-2} yr^{-1} or 0.066 kg N ha^{-1} yr^{-1}.

Analysis of field data showed a significant CO_2 treatment by moisture by temperature interaction with respect to N_2O emission, indicating that the effect of CO_2 enrichment on N_2O flux varied, depending upon in situ soil moisture and temperature conditions. When soil moisture content was $\leq$30% WFPS, flux in both enriched and ambient plots responded similarly to increases in soil temperature (Figure 8). However, when soil moisture exceeded 70% WFPS, N_2O flux from CO_2-enriched plots declined with increasing temperature (Figure 9), while flux from control plots increased with increasing temperature in a pattern similar to the low-moisture conditions (Figures 8 and 9). CO_2-enriched plots showed greater N_2O flux than control plots under soil conditions of high moisture and low temperature, coinciding with the winter season. Conversely, flux for CO_2-enriched plots was lower than controls when both soil moisture and temperature were high, conditions more commonly found in summer.

The statistical model also showed a significant CO_2 treatment by season by temperature interaction, which complements the CO_2 treatment, moisture, and temperature interaction indicated above. The predicted effect of CO_2 treatment on N_2O flux did not vary between spring, summer, and fall, but the predicted effect in the winter was significantly different from the warmer seasons when moisture was held constant (Table 1, Figure 10). This corresponds to the enhanced N_2O emissions found for CO_2-enriched plots at high moisture and low temperature (Figure 9), which is correlated with typical winter conditions (Figure 1). In winter the positive effect of CO_2 enrichment on N_2O flux also increased with increasing soil temperature. The predicted treatment during the spring, summer, and fall for a range of soil temperatures indicated that the negative effect of CO_2 enrichment on N_2O flux increased with increasing temperature at these times of the year.

4. Discussion

Nitrous oxide is highly effective as a greenhouse gas, given its radiative properties and long atmospheric lifetime (120 years). The contemporary increase in N_2O will clearly influence future cli-

mates. Soils represent the largest single source of atmospheric N_2O, so the global response of microbial N_2O production to a CO_2-enriched atmosphere is critical to future climate model predictions. Our investigation of a temperate forest ecosystem shows that soil biota responsible for N_2O production respond to elevated CO_2 in a complex, interactive manner. No single factor was sufficient to explain how N_2O flux varied with CO_2 treatment. Both nitrification and denitrification govern N_2O flux, and each varies concomitantly with soil conditions. Our results demonstrate that CO_2 enrichment interacts with soil moisture, temperature, and N availability to alter N_2O emissions. These key components need to be factored into CO_2 enrichment models to make better predictions of N_2O fluxes from temperate forests.

4.1. Denitrification

Nitrate availability was the most important individual factor affecting denitrification in soils from both CO_2-enriched and control plots. Denitrifying enzyme activity for homogenized soils amended with NO_3^- was about twice that for soils amended with glucose-C (Figure 3). Total denitrification was >2 orders of magnitude higher in NO_3^--amended cores than in watered controls (Figure 5). Nitrate limitation of denitrification here is corroborated by the low rates of nitrification and small pools of NO_3^- found in this forest [*Allen et al.*, 2000]. This is also consistent with reports of NO_3^- stimulation of denitrification in other forest soils [*Davidson and Swank*, 1987], for NO_3^- is typically the limiting nutrient in forest soils [*Vitousek et al.*, 1982].

Although DEA tended to be slightly higher in CO_2-enriched plots during the warmer months (treatment by month interaction), rates of total denitrification in intact cores were not significantly different between CO_2-enriched and control plots within a chemical treatment (e.g., NO_3^- addition) (Figure 5). Similarly, *Ambus and Robertson* [1999] found slightly higher DEA in summer but similar rates of N_2O production for intact cores when CO_2-enriched soils were compared with control soils in an open-top chamber CO_2 experiment. These results may be explained by differences in soil O_2 status, as denitrification requires anaerobic conditions. Anaerobiosis was achieved during DEA assays, whereas aerobic conditions prevailed during field and core experiments. The full potential for denitrification was probably not achieved in situ, although the presence of DEA suggests that anaerobic conditions occasionally occur. Collectively, these results at CO_2-enriched sites suggest that soil O_2 status ultimately dictates whether changes in the denitrifying microbial community resulting from CO_2 fertilization will be manifested as increased denitrification at an ecosystem level.

4.2. Laboratory N_2O Fluxes

Our results indicate that nitrification is the primary microbial process responsible for N_2O production in soils from both CO_2-enriched and control plots. We found that inhibition of nitrification reduced N_2O flux by 70–80% in laboratory incubations at both moderate (49% WFPS) and high (94% WFPS) soil moisture levels. The enhanced N_2O emission at 94% WFPS, relative to 49% WFPS, was due to proportional increases in both nitrification and denitrification. These moisture values represent the typical range of field conditions (Figure 1), which demonstrate that these soils are generally well drained. Additionally, over 98% of the individual field observations showed soil moisture contents of <110% WFPS, indicating that nitrification is the dominant source of N_2O-N at typical in situ soil moisture conditions. Finally, in experiments assessing chemical influences on denitrification, reduced N_2O production was observed in watered controls following addition of 10 kPa C_2H_2 to inhibit nitrification entirely [*Klemedtsson et al.*, 1988] (Figures 4 and 5), which indicates a strong nitrification component to total N_2O flux. This is corroborated by other studies that show nitrification as the dominant source of N_2O in well-drained terrestrial environments, including an early successional temperate forest [*Robertson and Tiedje*, 1987], a tropical forest [*Davidson et al.*, 1993], and a shrub-steppe ecosystem [*Mummey et al.*, 1994].

Glucose-amended cores showed significantly reduced N_2O flux relative to watered controls (Figure 4). Reduced N_2O: N_2 ratios from denitrification were expected in response to an increase in the labile C supply [*Sahrawat and Keeney*, 1986]. However, since nitrification clearly dominated N_2O production in both CO_2-enriched and control soils, it is more likely the observed response is largely attributable to enhanced N immobilization, which is a process highly competitive with nitrification [*Davidson et al.*, 1992]. Therefore soil C enrichment may enhance immobilization and reduce N_2O flux from nitrification.

4.3. Field N_2O Fluxes

We estimated the annual N_2O flux from this ecosystem to be 0.066 kg N_2O-N ha^{-1} yr^{-1}, which is similar to estimates reported for a shortgrass steppe [*Mosier et al.*, 1996; *Parton et al.*, 1988], Wisconsin prairies [*Goodroad and Keeney*, 1984], and a northern temperate forest [*Bowden et al.*, 1990]. Comparable annual, time-integrated N_2O flux data for soils exposed to free-air CO_2 enrichment, however, are lacking. We found fluxes of N_2O ranging from 5 to 40 μg N_2O-N m^{-2} d^{-1} (Figure 7). Other investigators at CO_2 enrichment sites report N_2O fluxes during the summer ranging from 2.4 to 50.4 μg N_2O-N m^{-2} d^{-1}, depending upon nutrient and water additions [*Ambus and Robertson*, 1999; *Ineson et al.*, 1998].

Results of the statistical analysis for field N_2O flux measurements showed that at a given level of soil moisture and temperature, N_2O emissions were significantly different between treatments. Carbon dioxide enrichment enhanced N_2O emissions under conditions of high soil moisture and low temperature (Figure 9). An increase in soil temperature, however, resulted in a more negative treatment effect. This was consistent with statistical model predictions of greater N_2O emissions under elevated CO_2 conditions in the winter (when soil temperatures were lowest) and lower N_2O emissions in the spring, summer, and fall (when soil temperatures were highest) (Figure 10). The strong interaction with season indicated that while holding moisture and temperature constant, N_2O emission under elevated CO_2 conditions tended to be lower than the control from spring through fall. This effect was most apparent at high temperatures. In winter, higher N_2O emissions were found under elevated CO_2 conditions than in control soil. The net result on an annual basis was that N_2O flux from both treatments was similar in 1998 and 1999.

Both nitrification and denitrification were expected to increase in response to greater soil moisture and temperature irrespective of CO_2 treatment, with a proportionately greater flux of N_2O from denitrification as soils reached saturation [*Maag and Vinther*, 1996]. When soils were closer to saturation, N_2O flux from elevated CO_2 plots responded negatively to an increase in temperature, suggesting that one or both of these processes may have been inhibited when soils were moist and temperatures were >15°C. Since a positive temperature response was evident only during the cold, wet season, differences in denitrification may have contributed to this interaction between CO_2 enrichment and temperature. Denitrification contributed ~25% to the total N_2O flux during laboratory incubations and may be an important source of N_2O during the wet season [*Davidson et al.*, 1993]. Additionally, NO_3^- uptake by loblolly pine is greater under elevated CO_2 [*BassiriRad et al.*, 1997], so competition for NO_3^- during the warm growing season may have limited denitrification, resulting in lower N_2O emissions.

Carbon dioxide enrichment negatively impacted N_2O emission during the summer months (Figure 10) when soils were both very

warm and moist (60% WFPS). This may be linked to lower nitrification, as nitrification is the primary source of N_2O during the dry season [*Davidson et al.*, 1993]. Although N_2O flux from nitrification was similar for both treatment groups in laboratory incubations (Figure 6), reductions in N_2O emissions were found when cores were enriched with glucose (Figure 4). This may have resulted from enhanced N immobilization [*Hungate et al.*, 1997b] and reduced soil NH_4^+ availability under elevated CO_2 [*Matamala*, 1997]. Greater plant NH_4^+ uptake and reduced gross nitrification have been reported for CO_2-enriched plots during the growing season when organic C input from autotrophic production was high [*Hungate et al.*, 1997b], and this CO_2 effect on gross nitrification may have also depended upon soil moisture. Summers 1998 and 1999 were exceptionally dry, so the extent that elevated CO_2 lowered N_2O emission was limited by low summer rainfall. Our analysis and the literature suggest, however, that lower N_2O emission during the growing seasons at higher temperature and moisture indicates processes operating at an ecosystem level that may have lowered N_2O flux by altering denitrification, nitrification, or both.

Environmental factors regulating N_2O emission are clearly complex and interactive. Results from this 2-year investigation infer that changes in soil physicochemical properties and biology as a result of FACE did not alter total annual N_2O emission in this temperate forest, at least in the time frame of the observation period. We lack comparable N_2O data from other FACE sites, and results from short-term studies are mixed. Nitrous oxide fluxes measured in a California grassland FACE site in October [*Hungate et al.*, 1997a] and in a Michigan forest open-top chamber experiment in July [*Ambus and Robertson*, 1999] were similar for both ambient and elevated CO_2. However, emissions were higher in elevated plots following NH_4NO_3 application to a Swiss grassland in July [*Ineson et al.*, 1998]. Cumulative information from these enriched sites, excluding those that were fertilized with N, show that the many positive and negative feedbacks and interactions associated with CO_2 enrichment and N limitation may result in largely similar N_2O fluxes at an ecosystem level.

5. Conclusions

Our study demonstrates that the effect of elevated CO_2 on soil N_2O emissions varies with N availability and soil conditions. Our statistical analysis controlled for soil moisture and temperature differences between sites and collection times, so we conclude that interactions with treatment are driven by changes in N_2O production processes. At mean soil moisture levels, microorganisms under CO_2 enrichment responded to warm soil temperatures (such as those found in the spring, summer, and fall) with lower N_2O emissions than the control. When conditions were relatively wet and cold, microorganisms under CO_2 enrichment produced more N_2O than the control. Both nitrification and denitrification are strongly controlled by N availability, so seasonal differences in N_2O emission may be due to variation in plant and microbial competition for N. Lower N_2O production during the growing season suggests that greater plant demand for N under CO_2 enrichment may slow these processes and reduce N_2O flux. Conversely, this may be counteracted by increased N_2O flux during the winter, when soils are moist and plant and microbial competition for N has subsided and when enhanced C availability could stimulate denitrification.

Our results collectively suggest that conditions favorable to denitrification are rare in this well-drained, N-limited forest and that nitrification is the primary source of N_2O. Nitrate limitation and aerobic conditions preclude this ecosystem from responding to C enrichment with enhanced denitrification during most of the year, although further studies in winter are needed. We suspect that with greater C inputs over time, enhanced N immobilization and plant uptake will lead to seasonal reductions in nitrification and N_2O emission under FACE. Reductions may be offset, however, if the microbial community under elevated CO_2 more actively nitrify and denitrify during the winter, which may result in no change to annual N_2O flux.

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