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DENITRIFICATION IN SURFACE SOILS OF A RIPARIAN FOREST: EFFECTS OF WATER, NITRATE AND SUCROSE ADDITIONS

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Summary—We studied the effects of adding water, nitrate and sucrose on denitrification in a riparian forest that receives nitrate in drainage from cornfields. We used large (3–6 m^2) flow-through chambers to sample N_2O emissions from surface soils. N_2 emissions were estimated by comparing N_2O efflux in chambers with or without acetylene addition. Water and solutions of nitrate or sucrose were sprinkled on the soil within the chambers and subsequent changes in N_2O efflux were observed continuously for several days. In 23 of 25 cases the soil beneath the chambers released N_2O at 0.19–17 μg N_2O-N m^{-2} h^{-1} prior to experimental treatments. In two chambers, there was consistent uptake of N_2O from the atmosphere at 2.2 and 1.0 μg N_2O-N m^{-2} h^{-1} prior to treatments. Release of N_2O was sometimes stimulated by adding water, usually stimulated by adding sucrose and always stimulated by adding nitrate. Stimulation by either nitrate and sucrose separately may reflect the heterogeneity of soil conditions that limit denitrification. After additions of nitrate or sucrose, N_2O efflux rates usually peaked at more than 10 times pre-treatment rates, but effects of adding water were less pronounced. Responses to treatments began within minutes or hours and usually did not persist for more than a day. The rapid response suggests that denitrifiers possess a stock of enzymes ready to exploit rapidly changing conditions in the soil. Acetylene added to the air in the chambers penetrated at least 40 cm into the soil within 10 h, reaching concentrations of 3–8 ml l^{-1} in the soil gas. Adding acetylene alone had no consistent effect on N_2O flux. However, adding acetylene and nitrate together resulted in greater stimulation of N_2O efflux than adding nitrate alone, presumably because acetylene inhibits N_2O reduction to N_2. The lack of effect of acetylene alone suggests that inhibition of N_2O reduction may not be effective *in situ* unless nitrate is also added. Based on the combined effects of acetylene and nitrate, we infer that 8.2–90% of the N_2O produced after adding nitrate without acetylene was converted to N_2. The amount of N_2O-N released after adding nitrate and acetylene was only 0.19–5.5% of the nitrate-N added. Published by Elsevier Science Ltd

INTRODUCTION

Agriculture has considerably increased the supply of biologically-available N in terrestrial ecosystems, but the fate of most of this anthropogenic N remains unknown (Jordan and Weller, 1996). Much of the N released from agricultural lands can be intercepted by adjacent downhill riparian forests (Lowrance *et al.*, 1984; Osborne and Kovacic, 1993; Hill, 1996; Correll, 1997). The effectiveness of riparian forests as N sinks may strongly influence watershed discharges of N (Jordan *et al.*, 1997). Maintaining forested buffers around streams has been advocated as a means of reducing N loads to downstream ecosystems (e.g. Lowrance *et al.*, 1995). However, the mechanisms of N uptake in riparian forests are not well understood (Hill, 1996). Riparian forests may convert intercepted N into gaseous forms or accumulate N in vegetation or soil (Weller *et al.*, 1994). Accumulation of N within forests would eventually saturate, but production of gaseous N, primarily through denitrification, could continue indefinitely (Hanson *et al.*, 1994; Weller *et al.*, 1994).

It is important to understand the factors controlling denitrification in riparian forests to evaluate their potential for N removal and their role in the production of nitrous oxide (N_2O) and nitric oxide (NO). Denitrification produces dinitrogen (N_2), N_2O and NO gases (Ye *et al.*, 1994). Recent increases in atmospheric N_2O (Rasmussen and Khalil, 1986) contribute to global warming (Abrahamson, 1989) and the destruction of stratospheric ozone (Bolin *et al.*, 1983). NO emissions contribute to acid precipitation (Cicerone, 1989) and to atmospheric delivery of available N to ecosystems (Galloway *et al.*, 1995). Better knowledge of the controls of denitrification could help in planning the use of riparian land to optimize N_2 production. This would maximize the benefits for water quality while minimizing the production of atmospheric pollutants.

Progress in understanding denitrification has been hampered by the technical difficulties of measuring

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denitrification in the field. Denitrification rates vary greatly in time and space and are very sensitive to physical and chemical conditions in the soil (Tiedje *et al.*, 1989). Also, production of N_2 gas cannot be measured directly against the background of atmospheric N_2. To address these problems, we developed a unique approach to measuring denitrification *in situ*. We sample gases emitted from the soil using tent-like flow-through chambers that minimize disturbance of the soil (Weller *et al.*, 1994). The unusually large size of the chambers (3–20 m^2) integrates spatial variability. Air in the chambers is replaced at a controlled rate and is continuously analyzed to observe temporal variations in N_2O concentration. Acetylene gas can be introduced into the chambers to block the conversion of N_2O to N_2. This enables us to infer the rate of N_2 production by comparing N_2O production with or without acetylene (Tiedje *et al.*, 1989).

In this study, we used our flow-through chambers to investigate some of the factors that control denitrification in a riparian forest that receives nitrate-N from adjacent corn fields. Denitrification may account for this forest's ability to take up most of the nitrate it receives, because biomass accumulation can account for only 33% of the nitrate uptake (Peterjohn and Correll, 1984). We had previously measured spatial and temporal variation of N_2O emissions in this forest (Weller *et al.*, 1994). In our present study, we used acetylene additions to infer N_2 production as well.

Many factors may influence denitrification rates in forests. Denitrification requires oxidized N (e.g. nitrate), available organic carbon and an absence of oxygen. The supply of nitrate or readily available organic C can limit denitrification rates in the surface soils of forests (Groffman *et al.*, 1991; Henrich and Haselwandter, 1991; Ineson *et al.*, 1991). Soil moisture exerts an important control on denitrification through its effects on oxygen diffusion into the soil. Hence, denitrification rates and potentials are often positively correlated with the proportion of water-filled pore space in the soil (Davidson *et al.*, 1993). We tested the effects of increasing soil moisture, nitrate and organic-C by sprinkling water, nitrate solutions or sucrose solutions on the soil within the gas-sampling chambers. Continuous monitoring of N_2O fluxes within the chambers allowed us to observe the dynamics of the responses to these experimental manipulations over several days.

MATERIALS AND METHODS

The riparian forest we studied surrounds a primary stream in the Rhode River drainage (38°51′N, 76°32′W) on the western shore of the Chesapeake Bay. The dominant tree species are tulip poplar (*Liriodendron tulipifera*) and sweet gum (*Liquidambar styraciflua*). The soils are fine sandy loams classified as Typic Hapludults (Kirby and Matthews, 1973). An underlying clay aquiclude forces groundwater from adjacent cornfields to flow laterally through the riparian forest before emerging in the stream (Chirlin and Schaffner, 1977). This riparian forest intercepts much of the nutrients entering from the cornfields (Peterjohn and Correll, 1984, 1986; Correll and Weller, 1989).

We used moveable flow-through chambers to sample N_2O emitted from the forest soil (Fig. 1). Methods for sampling and analyzing N_2O are detailed by Weller *et al.* (1994). Each flow-through chamber has an inner compartment (1 m wide × 0.35 m tall × 3–6 m long) nested within a slightly larger outer compartment. The compartments consist of PVC pipe frames covered with polyethylene sheeting that is pressed against the soil by 2 m long × 0.1 m dia sandbags. The outer compartment acts as a windbreak to prevent wind-driven air exchange in the inner compartment. We measured gas concentrations within the inner compartment. Inside the inner compartment, we suspended perforated hoses for sprinkling water or solutions within the chamber while monitoring N_2O fluxes.

An external blower continuously pumps air through a manifold into the inner compartment. The air inflow rate is monitored by a hot-wire anemometer (Kurz 435 DC, or TSI 8450) linked to a data logger (Campbell Scientific CR-21X). Air inside the chamber is mixed continuously by several fans. Air exits the chamber through gaps between the polyethylene cover and the soil surface. Flow-through rates of 0.4–4 $m^3\,h^{-1}$ were adjusted to maintain about a 10% difference in N_2O concentration between air within the chamber and inflowing air. This concentration difference sometimes increased to as much as 4-fold during brief periods of high N_2O efflux.

We made continuous-automated measurements of N_2O concentrations inside and outside the chambers using a custom-engineered tunable diode laser infrared-spectrophotometer (TDLS) system (Laser Photonics) as described by Weller *et al.* (1994). Our gas analysis system precisely quantifies small (±10 nl l^{-1}) variations around the normal atmospheric N_2O concentration (310 nl l^{-1}). Gas samples were drawn into the analyzer through polyethylene tubes linked to computer-controlled valves. Gas within the analysis cell was held at a regulated low pressure (10–20 Torr) to enhance resolution of particular absorbance peaks. Absorbance at precisely selected wavelengths was quantified continuously by derivative spectroscopy and recorded by a data acquisition system. We calibrate the instrument with mixtures of an N_2O standard and dry, N_2O–free N_2 that are prepared with high-precision gas-flow controllers (MKS Instruments Type

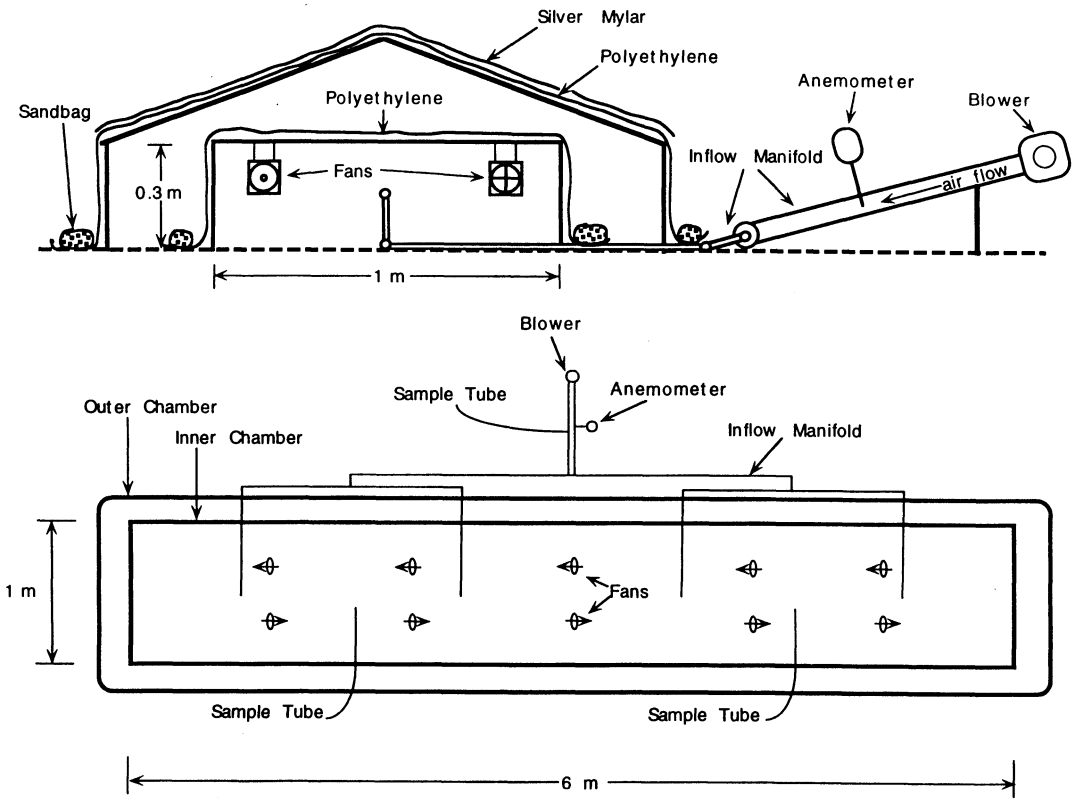

Fig. 1. Top: Cross section of flow-through chamber showing coverings of inner and outer chambers, the air inflow system and circulation fans blowing in opposite directions. Bottom: Top view of flow-through chamber used in 1993 experiments. Chambers used in 1994 were similar but 3 m long.

1359C). The gas analysis system is housed in a temperature-controlled building in the riparian forest.

The rates of N_2O emission were calculated as follows (Weller *et al.*, 1994): In a well-mixed inner compartment of volume V and ground area A with flow rate f, a constant soil emission m, N_2O concentration in inflowing ambient air C_a and N_2O concentration in the compartment C, the rate of change of N_2O in the compartment is:

$$V(dC/dt) = -fC + fC_a + mA.$$

We used the solution of this differential equation to calculate emission rate per unit area as a function of initial concentration C_0 and final concentration C_t at time t as:

$$m = \{f/[A(1 - e^{-(f/V)t})]\}[(C_t - C_a) - e^{-(f/V)t}(C_0 - C_a)]$$

We conducted a series of experiments from October 1993-September 1994 to investigate changes in N_2O emissions due to additions of acetylene, water, nitrate and sucrose to the soil. Each experiment used one or two pairs of sampling chambers, one chamber of each pair with and one without acetylene added. Treatments other than acetylene addition were identical for paired chambers. Having pairs of chambers helped us infer N_2 production based on the difference in N_2O emission in the presence or absence of acetylene.

There were some differences between experiments done in 1993 and those done in 1994. In 1993, we did three experiments with one pair of sampling chambers (2.0 m^3 volume, 5.7 m^2 area) at a time. In 1994, we ran five experiments with two pairs of chambers (1.1 m^3, 3.1 m^2) at a time. In the 1994 experiments, one pair of chambers received nitrate additions while the other pair usually received sucrose additions. In the 1993 experiments, there were no sucrose treatments. The 1993 experiments were located near the gas analysis laboratory, but the 1994 experiments were located about 150 m away, in an area where Peterjohn and Correll (1984, 1986) found high rates of nitrate uptake. In the 1994 site, the water table was usually closer to the surface than in the 1993 site. Based on unpublished measurements from nearby wells, the water table averaged about 0.7 m deep at the 1994 site and 1.5 m deep at the 1993 site. In both areas, the water table sometimes reached the soil surface, but experiments were not run when this happened in March–

Table 1. Experimental treatments for pairs of chambers, with one of each pair receiving acetylene. One pair of chambers was run for each date in 1993. Two pairs were run for each date in 1994, with one pair receiving nitrate solution and one pair receiving sucrose solution, except in April when both pairs received nitrate solutions. Water was added first (except in April, 1994) and solutions were added a day or more later in volumes and concentrations shown. Ranges of average air inflow rates and soil temperatures at 10 cm are also shown

Dates	Water ($l\ m^{-2}$)	Solution ($l\ m^{-2}$)	NO_3 ($mg\ N\ l^{-1}$)	Sucrose ($g\ C\ l^{-1}$)	Air flow ($m^3\ h^{-1}$)	Soil temp. (°C)
20–25 Oct. 1993	39	39	9	none	0.96–1.0	14–17
8–13 Nov. 1993	42	42	11	none	1.1–1.3	8.8–12
29 Nov.–5 Dec. 1993	42	42	8	none	1.0–1.2	8.7–11
18–28 Apr. 1994	0	39	5	none	3.4–4.4	13–18
3–11 Aug. 1994	19	19	14	3	0.39–1.0	21–24
29 Aug.–6 Sept. 1994	19	19	14	3	0.83–1.3	18–22
13–19 Sept. 1994	19	19	15	3	1.0–1.4	ND
19–30 Sept. 1994	19	19	15	3	0.96–1.3	18–20

early April, or after unusually heavy rains. Also, experiments were not run in January and February, when the soil was often frozen, or in May–July when there were equipment problems. After each experiment, the chambers were moved to new positions less than 20 m apart. In 1994, chambers of the same pair were less than 5 m apart.

Details of experimental treatments differed (Table 1), but we generally measured N_2O emission rates for about a day before any treatments. Then we introduced acetylene gas into the inflow manifold of one of each pair of chambers at a flow rate measured with a ball flow meter (Dwyer Instruments) and regulated to maintain an acetylene concentration of about 5–10 $ml\ l^{-1}$ throughout the experiment. We kept acetylene concentrations below 20 $ml\ l^{-1}$ in the chambers to prevent explosions. Acetylene flowing into the chambers was bubbled through 100 ml HCl l^{-1} to remove the acetone used as a solvent for commercially-bottled acetylene. By measuring N_2O flux in all the chambers before acetylene additions, we could compare the change in flux in each chamber before and after acetylene introduction began.

About a day after beginning acetylene additions, we began sequential additions of water and solutions of nitrate or sucrose. For all experiments, except the one in April 1994, we first sprinkled water in the chambers at rates comparable to a heavy rain (2–4 cm, Table 1). Water added to chambers receiving acetylene was sparged with acetylene to enhance acetylene delivery. Water added to chambers not receiving acetylene was sparged with N_2 to control for addition of deoxygenated water. About one or more days after the water additions, we sprinkled similar volumes of solutions of either sucrose or nitrate within the chambers (Table 1). These solutions were also sparged with either acetylene or N_2. Acetylene used in sparging was not bubbled through HCl solution, so it may have contained some acetone.

In the first two experiments, we assessed the penetration of acetylene into the soil by sampling soil gas through metal tubes inserted to different depths. Usually we used flexible aluminum tubing (1.9 mm ID, 3.2 mm OD) inserted vertically into the soil inside the chamber. The tubing extended out from under the chamber between the soil surface and the plastic cover. To block soil particles from clogging the tubing when it was being inserted, we placed a short (~1 cm) wire hook in the end of the tube. After the tubing was inserted to the desired depth,

Table 2. Response to adding acetylene alone: average efflux of N_2O ($\mu g\ N_2O\text{-}N\ m^{-2}\ h^{-1}$) and the change in efflux before and after acetylene addition. Data from paired chambers with and without acetylene are compared on each row. Thus, there are two rows for each date in 1994, when two pairs of chambers were run. The apparent increase in efflux due to acetylene was calculated by subtracting the change in efflux rate in chambers receiving acetylene from the change in chambers not receiving acetylene

	With acetylene ($\mu g\ N_2O\text{-}N\ m^{-2}\ h^{-1}$)			Without acetylene ($\mu g\ N_2O\text{-}N\ m^{-2}\ h^{-1}$)			Increase due to acetylene ($\mu g\ N_2O\text{-}N\ m^{-2}\ h^{-1}$)
Date	before	after	change	before	after	change	
20–25 Oct. 1993	8.7	20	12	11	11	0.19	12
8–13 Nov. 1993	−1.1	−5.0	−3.9	−2.2	−5.3	−3.1	−0.81
29 Nov.–5 Dec. 1993	1.4	4.3	2.8	7.0	5.5	−1.5	4.3
18–28 Apr. 1994	13	18	4.5	11	4.8	−6.5	11
18–28 Apr. 1994	17	8.1	−9.3	16	9.7	−6.1	−3.2
3–11 Aug. 1994	2.2	3.5	1.2	2.2	1.3	−0.90	2.1
3–11 Aug. 1994	13	11	−2.3	14	12	−2.2	−0.11
29 Aug.–6 Sept. 1994	6.2	8.5	2.2	5.6	5.8	0.21	2.0
29 Aug.–6 Sept. 1994	23	38	15	13	22	9.0	6.2
13–19 Sept. 1994	0.89	2.7	1.8	0.19	0.34	0.15	1.7
13–19 Sept. 1994	–	3.3	–	12	16	4.0	–
19–30 Sept. 1994	6.7	2.8	−3.9	3.7	0.98	−2.7	−1.2
19–30 Sept. 1994	2.9	0.14	−2.7	2.4	3.5	1.2	−3.9

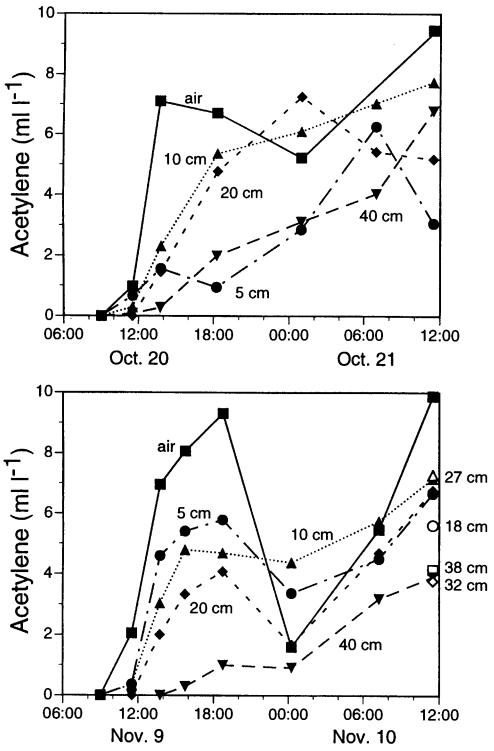

Fig. 2. Acetylene concentrations (ml l^{-1}) in chamber air and in soil air at different depths during two of the 1993 experiments. Data are just from the first day of acetylene inflow, which continued for the rest of the experiment. Open symbols plotted at 11:30, November 10 (lower graph), are for samples taken through stainless steel tubes that entered the soil outside the chamber and extended diagonally into the soil beneath the chamber to depths labeled to the right. Other samples were taken through aluminum tubes that penetrated the soil within the chamber and extended vertically to different depths as labeled.

it was withdrawn slightly to let the wire hook slip out and permit the flow of soil gases into the tube. Gas samples were withdrawn with syringes inserted through septa on the ends of the tubes. Acetylene concentrations were measured with a Perkin-Elmer model 8500 gas chromatograph equipped with Porapak QS column and a flame ionization detector.

We used a different sampling method to check the possibility that acetylene in the chamber air might flow down along the tubing through channels created during insertion. Rigid stainless steel tubing (1.4 mm ID, 3.2 mm OD) was inserted into the soil outside the chamber at an angle that would place one end beneath the soil within the chamber. Inserting the tubing in this way eliminated the possibility of gases within the chamber flowing down the sides of the tubing, but it was more difficult to place the ends of the rigid tubes at selected depths. To measure actual depths of the ends of the rigid tubes, we probed the tube position with a shovel after the experiment was over. We monitored soil temperatures at a depth of 10 cm using thermistors linked to a Campbell data logger.

RESULTS

In 23 of 25 cases, the soil beneath the chambers released N_2O prior to experimental additions of acetylene, water, nitrate or sucrose (Table 2). Average rates of N_2O emission prior to treatments ranged from 0.19 to 17 μg N_2O-N m^{-2} h^{-1}. In two chambers run from 8–13 November, 1993, there was consistent uptake of N_2O from the atmosphere at rates averaging 2.2 and 1.1 μg N_2O-N m^{-2} h^{-1} prior to treatments. The average pre-treatment flux of N_2O for all chambers run was an efflux of 7.6 μg N_2O-N m^{-2} h^{-1} (standard deviation = 6.5, n = 25).

Within 10 h after beginning inflow of acetylene into the chambers, the acetylene penetrated at least 40 cm into the soil (Fig. 2). Acetylene concentrations differed greatly among samples withdrawn from different tubes in the soil, possibly reflecting uneven distribution of acetylene or difficulties in sampling soil gas. However, similar concentrations were observed in samples drawn from tubes inserted vertically within the chambers and samples drawn from tubes inserted at an angle from outside the chamber (Fig. 2). This suggests that air from above ground did not flow down the sides of the vertical tubes during sampling so the samples from vertical tubes were representative of soil gas at the desired depths.

Comparison of fluxes before and after beginning acetylene introduction showed no consistent effect of acetylene alone on N_2O flux. In 7 out of 12 cases, acetylene seemed to increase N_2O flux, while in 5 cases it seemed to decrease flux (Table 2). Even in chambers where N_2O reduction was evidenced by uptake from the atmosphere into the soil, the N_2O influx seemed unaffected by acetylene (8–13 November, Table 2). Given the variability of N_2O flux, we can not conclude that acetylene alone had any effect at all.

Release of N_2O was sometimes stimulated by adding water, usually stimulated by adding sucrose and always stimulated by adding nitrate (Figs 3 and 4). After the additions of nitrate or sucrose, N_2O efflux rates usually peaked at more than 10 times pre-treatment rates, but effects of adding water were less pronounced. The effects began within minutes or hours and usually did not persist for more than a day. When peaks in N_2O efflux resulted from addition of water, the maxima occurred 2–14 h after water addition (Figs 3 and 4). Maxima in N_2O efflux followed sucrose additions in 3–15 h and followed nitrate additions in 2–32 h (Figs 3 and 4).

To compare the magnitude of experimental effects, we calculated how much more N_2O production occurred after the experimental amendments than would have occurred if fluxes had

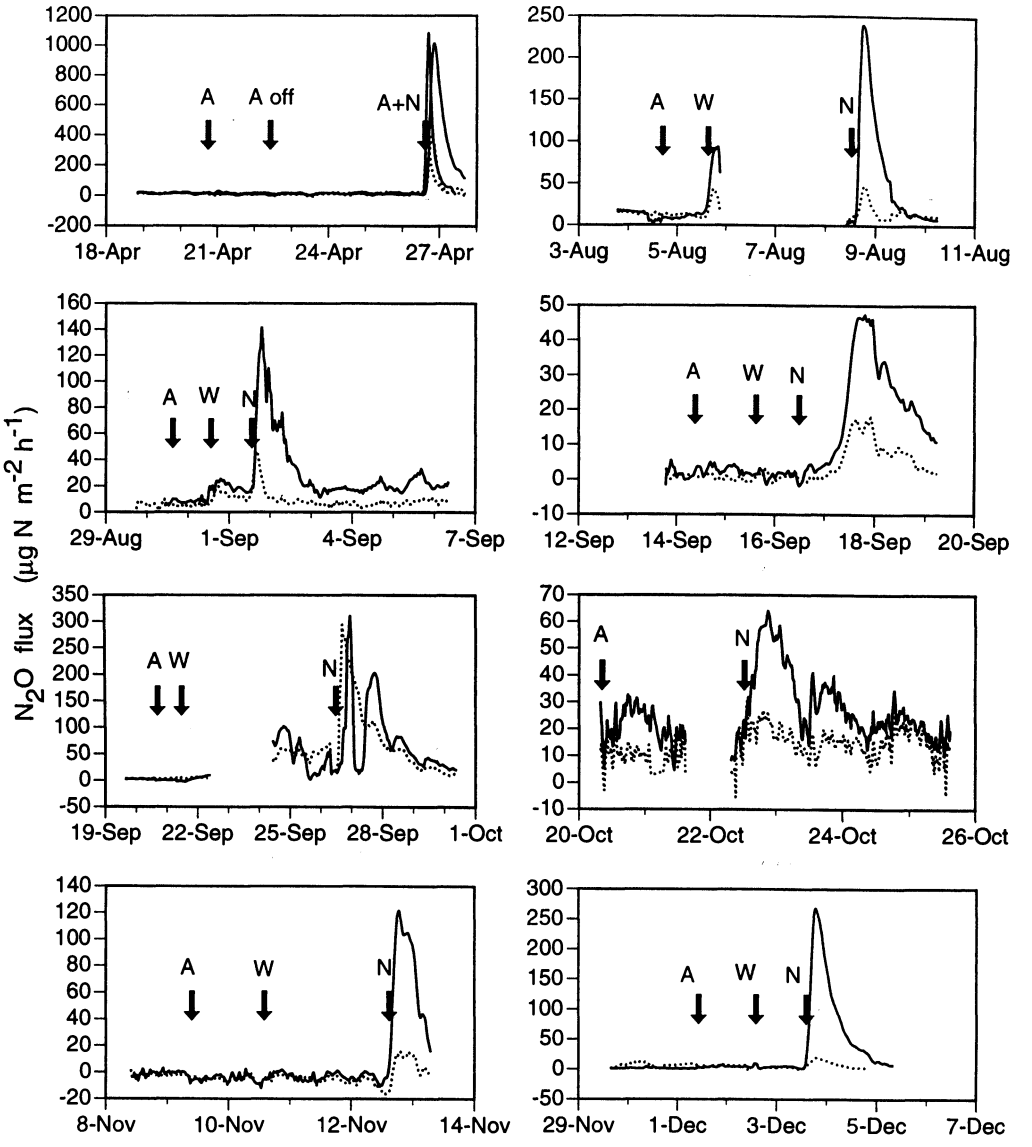

Fig. 3. N_2O flux (μg N m^{-2} h^{-1}) during experiments with nitrate additions. Dates are labeled at 0 h. Dates from April–September are from 1994. Dates from October–December are from 1993. Solid lines are for chambers with acetylene inflow beginning at the times labeled "A". Dotted lines are for nearby chambers without acetylene. The graph for April shows results for 2 pairs of chambers. In that experiment the acetylene was turned off ("A off") and on again. Times marked "W" and "N" are when water or nitrate solutions (respectively) were sprinkled inside the chambers. Note differences in vertical axes among experiments.

remained at the pre-treatment rates. According to this calculation, adding water resulted in a release of 0–4.3 mg N_2O-N m^{-2} more than would have been released if N_2O fluxes had remained at the pre-treatment rates (Table 3), adding sucrose resulted in releases of 0–17 mg N_2O-N m^{-2} additional N_2O-N (Table 4) and adding nitrate resulted in releases of 0.1–11 mg N_2O-N m^{-2} additional N_2O-N (Table 5).

The magnitude of the experimental effects varied greatly among experiments, but some patterns are revealed by comparing paired chambers that were close together and run at the same time. Chambers that received acetylene always showed greater responses to nitrate additions than paired chambers that did not receive acetylene (Table 5). Acetylene may have enhanced N_2O release by blocking conversion of N_2O to N_2 in the soil, but only when nitrate was added. Acetylene by itself had no consistent effect, nor did acetylene in combination with water or sucrose additions (Tables 2–4). Based on the amount of additional N_2O production in the presence of acetylene after nitrate addition, we estimate that 8.2–90% of the N_2O produced after add-

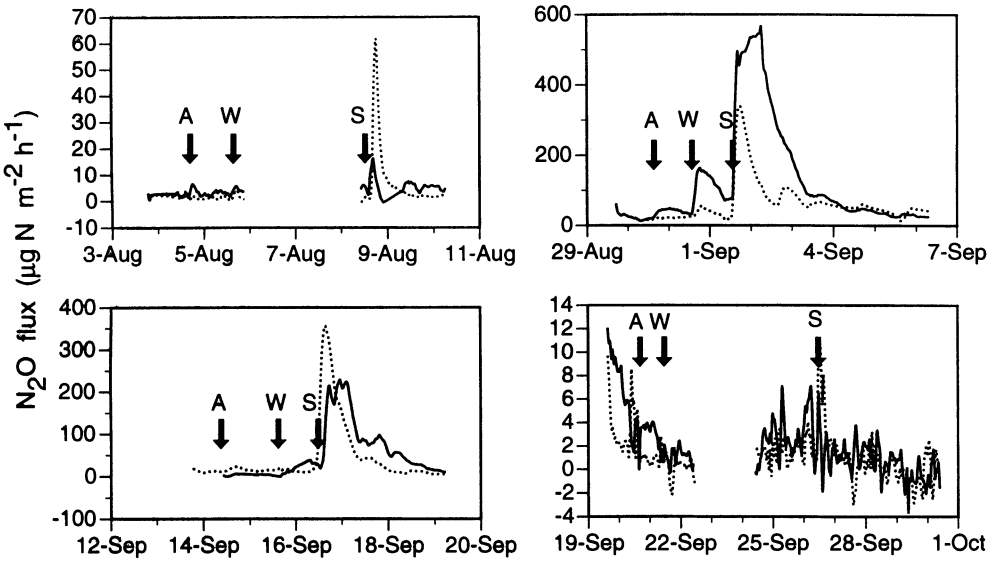

Fig. 4. N_2O flux ($\mu g\ N\ m^{-2}\ h^{-1}$) during experiments with sucrose additions. Dates are labeled at 0 h. Solid lines are for chambers with acetylene inflow beginning at the times labeled "A". Dotted lines are for nearby chambers without acetylene. Times marked "W" and "S" are when water or sucrose solutions (respectively) were sprinkled inside the chambers. Note differences in vertical axes among experiments.

ing nitrate without acetylene was converted to N_2 (Table 5). The amount of N_2O-N released after addition of nitrate and acetylene was only 0.19–5.5% of the nitrate-N added. The amount released without acetylene was only 0.029–2.1% of the nitrate-N added (Table 5).

DISCUSSION

Effects of acetylene

It is not clear why adding acetylene without adding nitrate had little or no effect on N_2O efflux. However, the lack of response to acetylene does suggest that the N_2O efflux was not due to nitrification near the surface, because nitrification is completely inhibited by acetylene concentrations above 0.1 ml l^{-1} (Robertson and Tiedje, 1987). N_2O efflux may depend on nitrification to replenish the supply of nitrate for denitrification, but in our system this dependence was evidently not strong enough to cause declines in N_2O efflux after several hours of exposure to acetylene.

It is possible that we did not add enough acetylene to the soil to inhibit conversion of N_2O to N_2. In our experiments, acetylene concentrations in the soil reached 5–10 ml l^{-1} within a day after starting acetylene influx, which continued throughout the experiment (Fig. 2). Some investigators have found that 1–10 ml l^{-1} acetylene is sufficient to cause inhibition (Yoshinari *et al.*, 1977; Ryden *et al.*, 1979; Duxbury and McConnaughey, 1986), but others suggest that more is needed (Smith *et al.*, 1978). Laboratory studies often use 100 ml acetylene l^{-1} to ensure complete inhibition of N_2O reduction, but additions of so much acetylene in the field could greatly disturb soil gas profiles (McConnaughey and Duxbury, 1986) and even risk explosions. Although

Table 3. Response to adding water with or without acetylene. Additional N_2O flux after adding water was estimated by calculating the total flux during the period following water addition and subtracting the flux that would have occurred if the rate had stayed the same as before the addition of water

	Additional N_2O-N flux ($mg\ m^{-2}$) after addition of water	
Dates	no acetylene	acetylene
8–13 Nov. 1993	−0.021	0.087
29 Nov.–5 Dec. 1993	−0.064	−0.0072
3–10 Aug. 1994	0.0047	0.0038
3–10 Aug. 1994	0.12	0.38
29 Aug.–6 Sept. 1994	0.16	0.27
29 Aug.–6 Sept. 1994	0.29	1.8
13–19 Sept. 1994	0.0006	−0.043
13–19 Sept. 1994	−0.052	0.41
19–30 Sept. 1994	0.023	−0.025
19–30 Sept. 1994	4.1	4.3

Table 4. Response to adding sucrose with or without acetylene. Additional N_2O flux ($mg\ m^{-2}$) after adding sucrose was estimated by calculating the total flux during the period following sucrose addition and subtracting the flux that would have occurred if the rate had stayed the same as before additions of water or sucrose

	Additional N_2O-N flux ($mg\ m^{-2}$) after adding sucrose	
Dates	no acetylene	acetylene
3–10 Aug. 1994	0.26	0.099
29 Aug.–6 Sept. 1994	6.7	17
13–19 Sept. 1994	3.6	5.2
19–30 Sept. 1994	−0.047	−0.20

Table 5. Response to adding nitrate with or without acetylene. Additional N_2O flux (mg m^{-2}) after adding nitrate was estimated by calculating the total flux during the period following nitrate addition and subtracting the flux that would have occurred if the rate had stayed the same as before additions of water or nitrate. The additional N_2O-N flux is also expressed as a percentage of the nitrate-N added. The percentage of additional N gas released as N_2O is inferred by assuming that N_2O flux in the presence of acetylene is equivalent to the sum of N_2 plus N_2O flux in the absence of acetylene

Dates	Additional N_2O-N flux (mg m^{-2}) after adding NO_3		% of added NO_3 released as N_2O		% additional N gas released as N_2O
	no acetylene	acetylene	no acetylene	acetylene	
20–25 Oct. 1993	0.36	0.66	0.10	0.19	55
8–13 Nov. 1993	0.18	1.2	0.039	0.25	15
29 Nov.–5 Dec. 1993	0.10	1.2	0.029	0.36	8.4
18–27 Apr. 1994	2.6	11	1.5	5.5	25
18–27 Apr. 1994	2.4	5.3	1.1	2.8	45
3–10 Aug. 1994	0.17	2.1	0.067	0.71	8.2
29 Aug.–6 Sept. 1994	0.40	2.6	0.15	0.99	15
13–19 Sept. 1994	0.40	1.2	0.14	0.43	34
19–30 Sept. 1994	6.5	7.2	2.1	2.7	90

we observed rapid and deep penetration of added acetylene into pore spaces in the soil (Fig. 2), we do not know the concentrations of acetylene within the anaerobic microzones where denitrification is likely to occur. However, if N_2O can diffuse out from the sites of production, then acetylene can probably diffuse in. Theoretical arguments suggest that acetylene in soil gas should penetrate soil aggregates within hours (Ryden *et al.*, 1979).

If we did add enough acetylene to block reduction of N_2O to N_2, then the lack of effect would suggest that N_2O rather than N_2 was the primary gaseous end-product of denitrification. However, we observed steady uptake of N_2O from the atmosphere in two chambers run in November, 1993 (Fig. 3). This N_2O uptake, which was probably due to reduction of N_2O to N_2, did not change after adding acetylene gas or water saturated with acetylene (Fig. 3). This suggests that the acetylene did not block N_2O reduction that was apparently occurring early in the November experiment.

In most experiments, unlike those in November, there was efflux of N_2O before addition of water, nitrate or sucrose. The lack of effect of acetylene on this efflux of N_2O might suggest a source of N_2O from groundwater or subsoil below the reach of acetylene additions. However, N_2O efflux resulting soon after surface additions of water, nitrate or sucrose was apparently generated near the surface. If the pretreatment efflux also originates from the surface soils, then either there was no N_2 production or the acetylene failed to inhibit N_2O reduction.

Reduction of N_2O might not have been effectively inhibited by acetylene in our soils because nitrate was limiting. Simarmata *et al.* (1993) found that acetylene concentrations of 10 ml l^{-1} inhibited reduction of N_2O to N_2 until nitrate became depleted. After nitrate depletion, N_2 was produced in the presence of acetylene (Simarmata *et al.*, 1993). This result is consistent with our observation that N_2O efflux was enhanced by acetylene only when we added nitrate. The observed response to the combination of acetylene and nitrate also suggests that we did achieve acetylene concentrations sufficient to at least partly block reduction of N_2O to N_2 in the surface soil. The amount of additional N_2O flux suggests that N_2 rather than N_2O was usually the primary gaseous end-product of denitrification after we added nitrate. The implied ratio of N_2-to-N_2O produced after nitrate addition ranged from 0.12–30 and was above 1.0 in 7 out of 9 cases (Table 5). The proportion of N_2 produced may have been even higher before we added nitrate, because adding nitrate can increase the proportion of N_2O produced (Firestone *et al.*, 1980; Weier *et al.*, 1993). The effect of adding nitrate on the proportion of N_2O produced can result in part from increase in osmotic potential (Weier *et al.*, 1993), but the concentration of nitrate we added was too low to raise the osmotic potential high enough to change the proportion of N_2O produced (Smith and Doran, 1996). Many laboratory and field studies of denitrification rely on acetylene to inhibit N_2O reduction (Tiedje *et al.*, 1989). Therefore, more research is needed on the effectiveness of acetylene inhibition, especially *in situ* when nitrate may be limiting.

Effects of nitrate and sucrose

The large increases in N_2O efflux after adding nitrate suggest that the supply of nitrate limits N_2O production by denitrification in the surface soil. However, the large increases in efflux after adding sucrose suggest that the supply of labile organic carbon limits N_2O production. These seemingly contradictory results probably reflect micro-scale heterogeneity in the distribution of oxygen and nitrate in the soil.

In unsaturated soil, the rapid diffusion of oxygen through gas-filled pore spaces can confine denitrification to anaerobic microzones (Myrold and Tiedje, 1985). Anaerobic microzones may consist of particles of organic detritus or aggregates of soil particles within which microbial respiration has depleted the oxygen (Sexstone *et al.*, 1985; Parkin, 1987). Nitrate diffusing into these microzones can be sequentially reduced via denitrification to nitrite, NO, N_2O and finally N_2. However, nitrate may become depleted within the microzones thereby limiting denitrifica-

tion. Our experimental additions of nitrate probably increased denitrification rates by increasing delivery of nitrate to anaerobic microzones.

Sometimes adding just water increased the N_2O efflux (Figs 3 and 4). Filling soil pores with water would have restricted oxygen diffusion and increased the volume of anaerobic zones within the soil. Nitrate that had been in oxygenated zones and therefore unavailable for denitrification could be engulfed by expanding anaerobic zones after water is added (Sexstone *et al.*, 1988; Nelson and Terry, 1996). Others have likewise found that denitrification rates increase as the proportion of water-filled pore space increases (Ambus and Christensen, 1993; Davidson *et al.*, 1993; Weier *et al.*, 1993).

Adding sucrose solutions may have also expanded the anaerobic zones in the soil. Added water restricts oxygen diffusion and sucrose stimulates oxygen consumption. The addition of sucrose would also increase the amount of organic carbon available to directly support denitrification. Adding readily-available organic substrates, such as glucose and ethanol, can increase emissions of nitrogenous gases (Groffman *et al.*, 1991; Ineson *et al.*, 1991; Weier *et al.*, 1993; Weier *et al.*, 1994), as we sometimes observed after adding sucrose (Fig. 4). It is not clear how much of the effect of sucrose is from consuming oxygen in the soil or from supplying denitrifying bacteria with organic matter. However, even if the nitrate supply within anaerobic microzones limits denitrification, adding sucrose solutions could increase denitrification by expanding anaerobic conditions into formerly aerobic, nitrate-rich zones. Denitrification depends on the micro-scale co-occurrence of nitrate, labile organic matter and anaerobic conditions. Therefore, bulk properties of the soil, such as nitrate content, may not accurately reflect conditions at the sites of denitrification.

Efflux of N_2O increased very rapidly after adding nitrate or sucrose, often reaching rates over 100 times the pretreatment rates within 2 or 3 h (Figs 3 and 4). The rapid response suggests that the microbial community in the surface soil has a large, normally-unused capacity for denitrification supported by a ready stock of denitrification enzymes. *De novo* synthesis of denitrification enzymes does not begin until 4 or 5 h after favorable conditions for denitrification have been established (Smith and Tiedje, 1979; Dendooven and Anderson, 1994). Therefore, denitrifying bacteria seem to follow an opportunistic strategy, maintaining a readiness to exploit transient favorable conditions for denitrification. Such a strategy may be advantageous when denitrification depends on the unpredictable co-occurance of nitrate, labile organic matter and anaerobic conditions. Although denitrification rates increased rapidly after addition of nitrate, only a small proportion of the added nitrate-N (0.2–6%, Table 5) was converted to gaseous products. The transience of the experimental responses suggests that added sucrose and nitrate were quickly consumed or drained from the soil, returning conditions for denitrification to their pre-treatment state, usually within a day. Others have found similarly transient responses to addition of water, N fertilizers and organic-C (Conrad *et al.*, 1983; Ineson *et al.*, 1991; Weier *et al.*, 1994; Qian *et al.*, 1997).

Because denitrification rates can change rapidly as soil conditions change, measurements of denitrification may be especially sensitive to disturbances caused by the measurement technique. Our flow-through chambers minimize disturbance because the soil is left *in situ* and not penetrated by the edges of the chambers. The flow-through system also allows us to track rapid changes in N_2O efflux brought about by experimental treatments. Our chambers coupled with our TDLS gas analysis system could also be used to measure changing emissions of methane, carbon dioxide and other infra-red absorbing gases.

The magnitudes of responses to experimental treatments differed greatly among experiments run at different times but did not follow a clear seasonal pattern (Figs 3 and 4). The variability among experiments may reflect spatial as well as temporal variability because the chambers were moved to new locations for each experiment. Pairs of chambers that received similar treatments with or without acetylene were located < 5 m apart to improve comparability, but locations for different experiments were sometimes > 100 m apart. The experiments run from October–December 1993 were in a different part of the forest than the 1994 experiments. Compared to the area of the 1993 experiments, the area of the 1994 experiments received drainage from a larger area of cornfield and was waterlogged more often. Despite these contrasts, the two areas were not systematically different in their responses to water and nitrate additions (Tables 3 and 5, Fig. 3). More data would be needed for a conclusive comparison.

Importance of denitrification

Based on the decrease in groundwater nitrate with increasing distance from the cornfield, Peterjohn and Correll (1984, 1986) concluded that the area of our 1994 experiments was a sink for nitrogen entering from the cornfield. Only 33% of the apparent nitrate uptake could be explained by tree growth (Peterjohn and Correll, 1984), suggesting that much of the intercepted nitrogen may be consumed by denitrification. Although many studies have suggested that riparian forests may be important sinks for N, the mechanisms of N uptake are not well understood (Hill, 1996). Nitrate disappearance from groundwater in riparian forests has been well documented (e.g. Peterjohn and Correll, 1984, 1986; Lowrance, 1992; Simmons *et al.*, 1992; Jordan *et al.*, 1993; Nelson *et al.*,

1995), but denitrification rates below the water table often seem too low to account for the uptake (e.g. Groffman *et al.*, 1992, 1996; Lowrance, 1992). Nitrate uptake by vegetation in riparian forests is limited by the net accumulation of biomass (Peterjohn and Correll, 1984), the timing of the growing season (Groffman *et al.*, 1992) and the depth of the rooting zone.

More research would be needed to determine whether denitrification is a major sink for N in our riparian forest. This study and previous measurements of N_2O flux in the same forest (Weller *et al.*, 1994) suggest that N_2O efflux is not a major sink. Rates of N_2O efflux without adding water, nitrate or sucrose were similar to those previously reported, which account for at most 1% of the N uptake that was not incorporated into wood (Weller *et al.*, 1994). However, it is still possible that N_2 is the main product of denitrification in this forest. When we added nitrate, our acetylene additions increased N_2O efflux by 2–12 times (Table 5). If we assume that the total efflux of gaseous N from denitrification is normally 2–12 times the N_2O flux, then that would suggest that total denitrification could account for at most 2–12% of the apparent N uptake in the forest. However, we cannot confidently infer *in situ* N_2 production from the responses to our acetylene additions. Adding nitrate can increase the proportion of N_2O produced relative to N_2 (Firestone *et al.*, 1980; Weier *et al.*, 1993) and our data suggest that acetylene might have failed to block N_2O reduction at ambient nitrate concentrations. Moreover, denitrification may occur deep in the soil or in the groundwater below the depth affected by our acetylene additions. N_2O released into our chambers could have been produced at any depth, but the responses to our additions of acetylene, water, nitrate and sucrose must reflect processes in the surface soil.

For processes in surface soil to remove nitrate from groundwater, the nitrate must first be brought to the surface. In our study site, the water table can come close to the surface after heavy rains and capillary action can further enhance delivery of groundwater to surface soils. Groundwater nitrate could also be transported to the surface by deep roots. Nitrogen taken up by roots could be deposited on surface soils in leachate from leaves or in organic detritus and eventually denitrified. Despite possible enrichment of surface soil with nitrogen, N_2O flux is apparently nitrogen-limited (i.e. stimulated by nitrate additions). Nevertheless, depletion of nitrate in anaerobic micro-sites of denitrification could explain nitrate limitation in a soil otherwise replete with nitrate. Although denitrification in our forest was limited by lack of nitrate and possibly lack of organic-C or over-abundance of oxygen, the soils show a large potential for rapid increases in denitrification rates when conditions become favorable. This suggests that denitrification could occur in brief difficult-to-observe bursts after heavy rain. Quantifying denitrification may be even more difficult if acetylene inhibition of N_2O reduction is ineffective *in situ* due to low nitrate availability.

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