Pergamon

Soil Biol. Biochem. Vol. 26, No. 2, pp. 279–286, 1994
Elsevier Science Ltd. Printed in Great Britain
0038-0717/94 $6.00 + 0.00

NITROUS OXIDE FLUX FROM A SHRUB-STEPPE ECOSYSTEM: SOURCES AND REGULATION

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(Accepted 4 August 1993)

Summary—The semi-arid shrub-steppe is the largest grassland-type ecosystem of North America and may make significant contributions to the global atmospheric N_2O budget. However, little information is available concerning sources and regulation of N_2O flux in this ecosystem. Experiments were made to determine the relative importance of nitrification, denitrification and abiotic sources to total N_2O flux and to investigate the factors regulating N_2O flux rates from an undisturbed shrub-steppe ecosystem. The contributions to N_2O flux by nitrification and denitrification were estimated using acetylene (10 Pa) to selectively inhibit N_2O production by nitrifiers. Abiotic sources of N_2O were evaluated using sterilized soil. Factors limiting N_2O production were evaluated by monitoring N_2O flux rates from soil-cores amended with combinations of NO_3^--N, NH_4^+-N, soluble C and water. The effect of wet–dry cycles on N_2O flux was determined by wetting field dry soil to field capacity and monitoring N_2O flux rates, soil NH_4^+-N, NO_3^--N and water content throughout a drying period. Our results showed that nitrification accounts for 61–98% of the N_2O produced from soil at water contents below saturation and that denitrification is the primary N_2O source at saturated water contents. No detectable N_2O was produced by abiotic sources. In intact soil cores N_2O flux rates were found to be most limited by water and N availability. Wetting of dry soil resulted in a pulse of N_2O flux due to increased N availability. It is likely that this ecosystem exhibits relatively low N_2O flux rates for much of the year due to low soil moisture and inorganic N contents. Since soil moisture content is generally well below field capacity in this ecosystem, nitrification must be the dominant N_2O source. These results suggest that conditions favorable for substantial N_2O production in shrub-steppe ecosystems probably exist only at times following precipitation events.

INTRODUCTION

Gaseous nitrous oxide (N_2O) is one of the chemically-reactive greenhouse gases in the atmosphere responsible for the catalytic destruction of stratospheric ozone (Dickenson and Cicerone, 1986; Cicerone, 1987). Interest in identifying sources and regulation of N_2O flux rates from various ecosystems has been stimulated by the finding that atmospheric N_2O has increased from the time monitoring began in the 1970s (Rasmussen and Kahlil, 1986). Research has recently shifted from N_2O emissions due to fossil fuel combustion and the use of nitrogen fertilizers, which make relatively small contributions to the global N_2O budget (Davidson, 1991), to emissions from undisturbed terrestrial ecosystems. N_2O production from soil in undisturbed natural ecosystems is the least well quantified of the known N_2O sources (Wuebbles and Edmonds, 1988). Undisturbed arid and semi-arid ecosystems have received less attention than any of the other major ecosystems but may contribute as much as 30% of the total gaseous N emissions to the atmosphere from terrestrial ecosystems (Bowden, 1986).

Denitrification and nitrification are considered to be the most important sources of N_2O from soils. The denitrification process is regulated by several factors including the availability of nitrate (NO_3^-), reduced forms of carbon and O_2 (Knowles, 1982). Whereas nitrification is predominantly regulated by ammonium (NH_4^+) availability (Firestone and Davidson, 1989). The amount of N_2O produced by nitrification relative to NO_3^- is thought to increase as O_2 partial pressure (Goreau *et al.*, 1980; Poth and Focht, 1985) or pH (Martikainen, 1985) decrease. Substrate availability for each of these microbial processes is determined by the relative rates of N-mineralization and N-assimilation by plants and microbes and by diffusional constraints.

The semi-arid shrub-steppe is the largest grassland-type region in North America, totaling over 64,500,000 ha (Rogers and Rickard, 1988). Despite the potential importance of N_2O losses from the shrub-steppe to global atmospheric chemistry little information is available on how processes resulting in N_2O production from shrub-steppe ecosystems are regulated.

No attempts have been made to identify sources of N_2O from shrub-steppe ecosystems, however, Parton *et al.* (1988) found that nitrifiers accounted for 60–80% of the total N_2O flux from a semiarid shortgrass steppe ecosystem in Colorado where soil

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water content is usually too low to favor denitrification. In an early successional forest ecosystem soil core studies showed that 50% of the N_2O produced was from nitrification (Robertson and Tiedje, 1987). In addition, Davidson *et al.* (1993) reported that nitrification was the dominant source of N_2O in soil from a seasonally-dry tropical forest.

Matson *et al.* (1991) examined the temporal and spatial variation in N_2O flux rates from a Wyoming shrub-steppe ecosystem and its relationship to soil N characteristics. They found a positive relationship between N_2O flux and soil NO_3^- concentration. However, the variation in N_2O flux rates could not be explained on the basis of variation in soil N pools alone.

Soils in the shrub-steppe undergo wetting periods followed by long periods of desiccation, resulting in microbial semi-dormancy (Rickard, 1988). Birch (1958), Van Schreven (1967), Sorensen (1974), Marumoto *et al.* (1982) and Kieft *et al.* (1987) have shown that air dried soils have generally larger C and N mineralization rates upon wetting than soils which remain wet. Wetting of air dry soils is also known to result in pulses of N_2O flux from denitrification (Patten *et al.*, 1980; Groffman and Tiejde, 1988; Rudaz *et al.*, 1991) and nitrification (Rudaz *et al.*, 1991; Davidson *et al.*, 1993). Therefore soil water potential dynamics is a critical controlling factor for nutrient cycling processes in semi-arid ecosystems.

Knowledge of the sources of N_2O and the factors limiting N_2O flux rates in semi-arid ecosystems are important for understanding (i) spatial and temporal variability, thus facilitating quantification (ii) the effects of ecosystem disturbance, and (iii) how flux rates may change in response to climatic changes. Our objectives were to determine the importance of nitrification, denitrification and abiotic sources to N_2O flux and to investigate the roles played by soil moisture content, nutrient availability and soil wetting events in regulating N_2O flux rates from an undisturbed shrub-steppe ecosystem.

MATERIALS AND METHODS

Study site and soil

The study site is located on the Arid Lands Ecology reserve (ALE) within the U.S. Department of Energy's Hanford Site in south central Washington State. The ALE site has been unaltered by human disturbance since the early 1940s. The dominant plant-species are *Artemisia tridentata* (big sagebrush) intermixed with the perennial grasses *Elytrigia spicatum* (bluebunch wheatgrass) and *Poa secunda* (Sandberg bluegrass). A soil crust consisting of cryptograms, lichens, moss and algae is found in all undisturbed interplant areas. The soil at the study site is classified as coarse-silty, mixed, mesic, Xerollic Camborthid. The site has a semi-arid climate, receiving two-thirds of the 220 mm annual precipitation in the winter (Rickard, 1988).

Soil sampling methods

Soil-cores were obtained using PVC pipe sections (5 cm dia × 10 cm) which were beveled at one end to facilitate insertion into the soil while minimizing compaction. The pipes were driven into the soil until the tops were flush with the soil surface. Tight clusters of six soil-cores were taken from points arranged in a 2.4 m square grid centered around the base of an *A. tridentata* shrub. The bottom of each soil-core was covered with plastic and sealed with masking tape prior to transport to the laboratory. Several smaller soil-cores (10 mm dia × 10 cm depth) were sampled at the same time immediately adjacent to each six soil-core cluster for analysis of initial soil NO_3^--N, NH_4^+-N, and moisture contents. The inorganic-N concentrations of the 10 mm soil-cores were considered to be similar to that of the associated six soil-core cluster. Soil-cores were collected on 27 April 1992 and transferred to cold storage (4°C) within 24 h.

Sources of N_2O

The N_2O produced by abiotic sources was determined using autoclaved soil-cores. Field dry soil-cores were autoclaved in loosely sealed canning jars (121°C, 40 min) and allowed to equilibrate to room temperature for 24 h. Soil-cores were then moistened to 65% water holding capacity (WHC) with sterile distilled water and kept for 24 h. Jar headspace N_2O content was determined at 4 and 24 h by gas chromatography (g.c.). For our purposes water holding capacity (WHC) was the water content (w/w) at saturation or a matric potential of 0 kPa. The % water holding capacity of this soil corresponding to a water potential of −33 kPa is 77%.

The contribution of nitrification and denitrification to total N_2O flux was determined using sieved (2 mm), field dry soil (0.07% WHC) from a composite of nine randomly-selected soil-cores (5 cm dia × 10 cm depth). 10 g samples were kept in 40 ml serum bottles at four soil moisture contents (0.07, 36, 72, and 100% WHC). These water contents span the range of water contents thought to be optimal for N_2O production by nitrification and denitrification (Davidson, 1991). Four replicates at each moisture content were sealed in serum bottles using gas sampling valves for screw-top bottles and used to quantify N_2O produced by both nitrification and denitrification. A second set of replicate soil samples were treated with the same moisture contents and kept at 10 Pa C_2H_2 immediately after sealing the serum bottles to inhibit N_2O production from nitrification (Davidson *et al.*, 1986).

To determine if N availability affects the partitioning of N_2O flux between nitrification and denitrification additional samples with or without C_2H_2 were moistened to 36 and 72% WHC and amended with 100 μg NO_3^--N g^{-1} soil plus 100 μg NH_4^+-N g^{-1} soil.

Gas samples from all bottles were removed at 4 and 24 h for the analysis of N_2O.

A partial pressure of 10 Pa C_2H_2 is known to irreversibly inhibit the ammonia monooxygenase enzyme of nitrifying microorganisms while having little effect upon the nitrous oxide reductase enzyme of denitrification (Davidson *et al.*, 1986; Klemedtsson *et al.*, 1988). Thus the total N_2O flux is the water-only treatment, N_2O flux from denitrification is the water plus 10 Pa C_2H_2 treatment, and N_2O flux from nitrification is the difference between the water-only treatment and the water-plus 10 Pa C_2H_2 treatment.

Factors limiting N_2O production

To determine the factors affecting N_2O production from soil in this ecosystem, a laboratory experiment was made using intact soil-cores. Three groups of cores were collected to (i) test moisture effects with no N additions, (ii) test nutrient effects at one moisture content, and (iii) evaluate the role of immobilization as a controller of N_2O flux.

The effects of moisture on N_2O production were examined by moistening a group of soil cores to four different water contents (Table 1). Field dry soil cores served as controls for this experiment.

A second set of soil cores were used to evaluate nutrient limitations on N_2O production. These soil cores were amended with C or N in the moistening solution (Table 1). All nutrient treatments were delivered in distilled H_2O to bring the soil to 65% WHC. This water content is conducive to N_2O production by both nitrification and denitrification as suggested by the results of the N_2O source experiments (explained previously). A control soil-core was collected in the field immediately adjacent to each soil-core used for nutrient treatment. This allowed each soil-core used in the nutrient limitation experiment to have a control soil-core from close to the same field location. Control soil-cores received only water.

A third set of soil-cores were used to verify *in situ* activity of denitrification enzymes and the role of N assimilation during N_2O production. These soil-cores were subjected to identical treatments (Table 1), with the addition of 300 mg chloramphenicol l^{-1} in the added solution. The nutrient treated soil-cores not receiving chloramphenicol served as controls for this experiment.

Chloramphenicol is a broad-spectrum bacteriostatic agent that inhibits new protein synthesis by binding to ribosomes (Brooks *et al.*, 1992), therefore halting *de novo* synthesis of enzymes including those of the pathways resulting in N_2O production and N assimilation by both prokaryotic and eukaryotic organisms. Brooks *et al.* (1992) found that chloramphenicol completely inhibited N_2O production when added at the beginning of C_2H_2-block incubations.

For each of the three experiments four soil-cores were subjected to each of the treatments listed in Table 1 and incubated in 475 ml glass jars equipped with gas sampling ports. Water and nutrient solutions were added in equal amounts to the top and bottom of the soil-cores to ensure even water distribution. Headspace gas samples from each soil-core were taken at 4 and 24 h following wetting and immediately analyzed for N_2O and CO_2 content by g.c.

Wet–dry cycle

A laboratory procedure was used to investigate the dynamics of N_2O production and inorganic soil N transformations following rapid changes in water potential. Nine field dry soil-cores (0.07% WHC) were pooled and repeatedly sieved (2 mm) to ensure soil mixing. Dry soil (10 g) was placed in tared serum bottles and moistened to field capacity (*ca* 77% WHC) with distilled H_2O. Immediately following, the serum bottles were weighed to determine their initial water content. Serum bottles were not sealed and were kept at laboratory temperature. Periodically four serum bottles were sealed for 2 h, using valves for screw-top bottles, to allow N_2O to accumulate in the headspace. Gas samples were then taken for analysis of N_2O concentration followed by the determination of gravimetric soil water content and soil NH_4^+-N and NO_3^--N concentrations.

Analytical methods

N_2O and CO_2 were determined using a g.c. equipped with a ^{63}Ni electron capture detector. Gas samples were transferred directly from incubation vessels to the g.c. using syringes with airtight stopcocks. Concentrations were corrected for dissolved N_2O in the liquid fraction (Tiedje, 1982). Soil NO_3^--N and NH_4^+-N extracts were prepared by shaking soil with 2.5 M KCl for 1 h and filtering the mixture through washed, medium-fast filters. The NH_4^+-N and NO_3^--N concentrations in the extracts were determined using a colorimetric continuous-flow analyzer.

Table 1. Water and nutrient treatments used to elucidate the limiting factors for N_2O production

Water treatments		Nutrient treatments at 65% WHC* (g^{-1} soil)
−kPa	WHC (%)	
−92	40	100 μg glucose-C
−74	50	20 μg NO_3^--N
−51	65	100 μg glucose-C + 20 μg NO_3^--N
−26	81	20 μg NH_4^+-N
		100 μg glucose-C + 20 μg NH_4^+-N

*Percent of water holding capacity.

RESULTS

Sources of N_2O

No detectable N_2O was evolved from field dry soil (0.07% WHC) or from autoclaved soil cores during the 24 h incubation, suggesting that abiotic N_2O production from soil in this ecosystem is negligible.

For soil treatments not receiving supplemental N, nitrifiers were responsible for 97.8 and 93.7% of the total N_2O produced at 36 and 72% WHC respectively

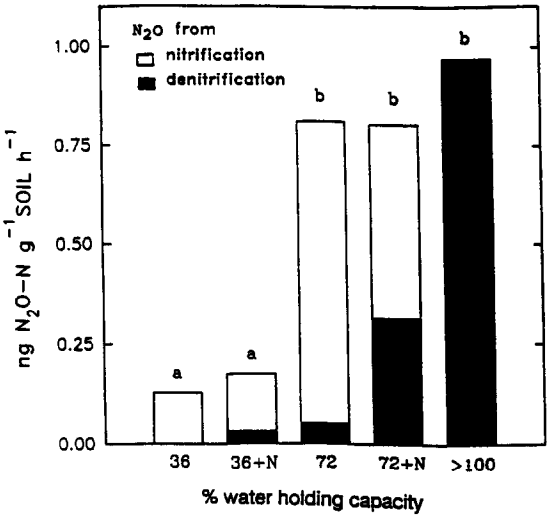

Fig. 1. N_2O produced by nitrification and denitrification in water and water plus nutrient treatments. Bars with different letters indicate significant differences at $P < 0.05$. N_2O concentration was analyzed at 4 and 24 h.

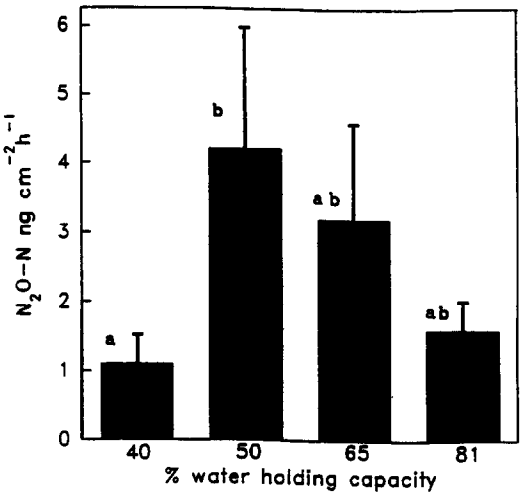

Fig. 2. Effect of water additions on N_2O production from intact soil cores. Bars with different letters indicate significant differences at $P < 0.05$. N_2O flux rates were calculated from the change in N_2O headspace concentration between 4 and 24 h after moistening.

(Fig. 1). For treatments receiving supplemental N, nitrifiers were responsible for 82.5 and 61% of the total N_2O in the 36 and 72% WHC treatments respectively (Fig. 1). Nitrification is the prominent N_2O source in this soil when water contents are below saturation. However, under saturated conditions (100% WHC) the difference in N_2O production for the C_2H_2 treatment and treatment with water only were negligible, suggesting that denitrification is the dominant N_2O-producing process. Total N_2O produced and denitrifier N_2O production increased with increased soil water content (Fig. 1). Nitrate-N and NH_4^+-N amendments did not significantly increase the total N_2O production in the 36 or 72% WHC treatments, however, the additions resulted in a greater proportion of N_2O from denitrification (Fig. 1). The increased N_2O from denitrification was only statistically significant ($P < 0.01$) for the 72% WHC plus N treatment.

Factors limiting N_2O flux rates

Nitrous oxide flux rates from intact soil cores were greatest in the 50% WHC treatment and decreased in the wetter and drier treatments (Fig. 2). The only significant difference ($P < 0.05$) in N_2O flux was found between the 50 and 40% WHC treatments. The 50% WHC treatment cores also had significantly more NH_4^+-N and NO_3^--N after 24 h (data not shown) and higher CO_2 flux rates (data not shown) than the other water-only treatments. No significant differences in inorganic-N concentrations were noted among the 40, 65, and 81% WHC treatments. When values for all soil samples receiving a water only treatment were combined, N_2O flux rates were found to be more strongly correlated with soil NH_4^+-N content after incubation ($r^2 = 0.77$) than NO_3^--N

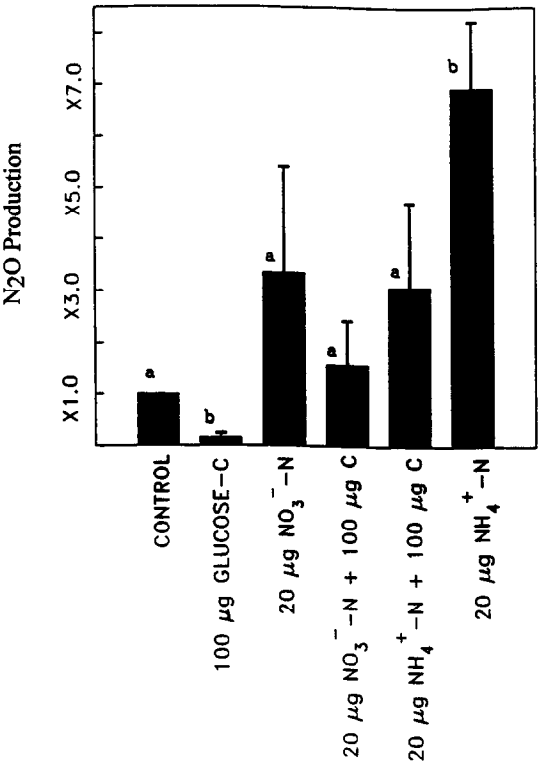

Fig. 3. Relative change in intact soil cores receiving supplemental C and N at 65% WHC over water only control soil cores. Bars with different letters indicate significant differences at $P < 0.05$. N_2O flux rates were calculated from the change in headspace N_2O concentration between 4 and 24 h after moistening.

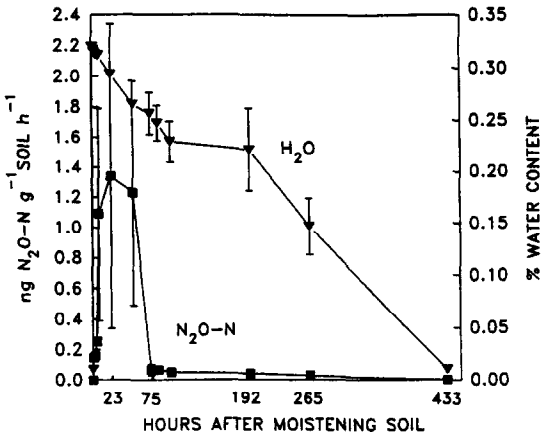

Fig. 4. Changes in N_2O flux rate and soil H_2O after moistening air-dry soil.

content after incubation ($r^2 = 0.48$) or CO_2 flux rate ($r^2 = 0.63$).

Figure 3 shows the effect of C and N additions on N_2O production, each set of treated cores is compared to its own untreated control core. Soil-cores receiving supplemental glucose-C without supplemental N had significantly ($P < 0.05$) lower N_2O flux rates than control soil-cores receiving only water (Fig. 3). Soil-cores receiving supplemental NO_3^--N or NH_4^+-N, with or without supplemental C, exhibited greater N_2O flux rates than control soil-cores (Fig. 3) with only the NH_4^+-N treatment being significantly greater than the control ($P < 0.05$). Ammonium amended soil-cores yielded the greatest N_2O flux rates and soil-cores amended with 100 μg C g^{-1} soil plus NO_3^--N or NH_4^+-N yielded lower N_2O flux rates than treatments receiving only NO_3^--N or NH_4^+-N (Fig. 3).

Treatment of C-only amended soil cores with chloramphenicol, preventing cell protein synthesis, resulted in a 3–5 fold increase in N_2O production in contrast to soil-cores receiving supplemental N and chloramphenicol which showed, on average, a 50% decrease in N_2O production over 24 h (data not shown). This suggests that *de novo* enzyme synthesis for N_2O production and N assimilation is an important factor in short-term N_2O flux rate studies.

Wet–dry cycle

Moistening of air-dried soil to field capacity resulted in a sharp pulse of N_2O flux during the subsequent 60 h (Fig. 4). Although N_2O production was detected within 2 h, a lag was observed in which N_2O flux increased slowly, followed by a sharp increase in rate after 5 h. Increased soil NH_4^+-N was observed within 2 h of moistening the dry soil (Fig. 5). Soil NH_4^+-N peaked at 5 h and fell to below initial concentrations at the end of the incubation (433 h). The N_2O flux rate was significantly correlated with soil NH_4^+-N concentration ($r^2 = 0.67$, $P < 0.05$), while there was no significant correlation between N_2O flux and soil moisture or NO_3^--N content. Soil NO_3^--N concentration increased sharply within 2 h of moistening, indicating that nitrifier activity resumes rapidly following rewetting. Soil NO_3^--N began to decrease when soil NH_4^+-N concentration decreased to *ca* 5 μg N g^{-1} soil at 192 h, suggesting that NO_3^--N assimilation was greater than production at this time.

DISCUSSION

Nitrification is the dominant N_2O-producing process at all but saturated soil conditions when it is then surpassed by denitrification (Fig. 1). In a 2 yr study, Wildung *et al.* (1975) reported maximum soil moisture contents in this ecosystem were less than field capacity. The low soil moisture content commonly found in this ecosystem allows surface soils to remain relatively well aerated, thus retarding the overall contribution of denitrification to total N_2O production. Therefore, on a yearly basis nitrification is likely to be the dominant source of N_2O in this ecosystem.

The relative importance of nitrification to N_2O flux from most terrestrial ecosystems is poorly defined. However, nitrification is known to make substantial contributions to the total N_2O produced by some fertilized agricultural soils (Bremner and Blackmer, 1978; Goodroad and Keeney, 1984), some forest soils (Martikainen, 1985; Robertson and Tiedje, 1987) and following wetting of a seasonally dry tropical forest soil (Davidson *et al.*, 1993). Parton *et al.* (1988) found that nitrification accounted for 60–80% of the total N_2O flux from a semi-arid shortgrass steppe ecosystem in Colorado. The results of Parton *et al.* (1988) and our own study suggest that nitrification may be the dominant N_2O source from semi-arid grassland-type ecosystems.

Determining the factors limiting N_2O flux from shrub-steppe soil revealed several environmental and chemical variables regulating N_2O flux. First, field

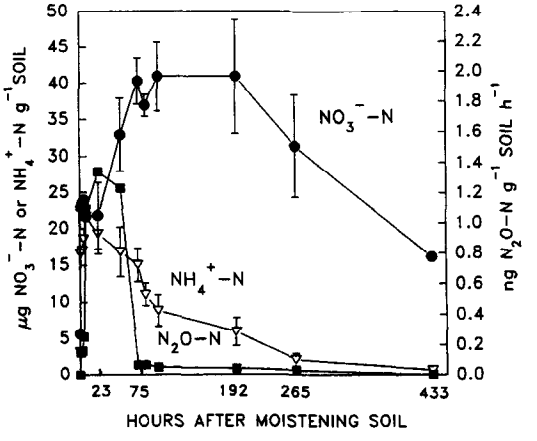

Fig. 5. Changes in N_2O flux rate and soil NH_4^+-N and NO_3^--N content after moistening air-dry soil.

dry soil produced no detectable N_2O, while N_2O production was detected from all soil receiving water amendments (Fig. 2). This indicates that N_2O production is controlled by soil moisture content and that little N_2O production can be expected during the dry months of June to September (Rickard, 1988). Second, soil cores receiving supplemental N produced more N_2O than control soil cores receiving only water (Fig. 3), indicating that N_2O flux rates are also limited by N availability. Due to high variance and small sample size only the NH_4^+-N treatment produced significantly ($P < 0.05$) greater N_2O than the control. However, N_2O flux rates were stimulated by either NO_3^--N or NH_4^+-N (Fig. 3) suggesting that both nitrification and denitrification contribute to N_2O flux at 65% WHC. Third, additions of C reduced N_2O flux rates (Fig. 3), suggesting that N immobilization is important in regulating N_2O production. If the immobilization process is inhibited more N should be available for nitrification or denitrification. Treatment of C-amended cores with chloramphenicol, which presumably inhibits immobilization of N, resulted in greater N_2O production, confirming that N immobilization is a highly competitive process with respect to nitrification and denitrification. In the N-amended soil-cores *de novo* enzyme synthesis was inhibited with chloramphenicol resulting in less N_2O production than control soil-cores. This suggests that the induction and rapid production of the enzymes of N_2O producing pathways is important to the overall magnitude of N_2O production.

The results of the wet–dry cycle experiment clearly show that relatively large pulses of N_2O and N-mineralization occur in this soil after wetting (Figs 4 and 5) and that N_2O production is positively correlated with soil NH_4^+-N content ($r^2 = 0.67$, $P < 0.05$). Increased N-mineralization following wetting of dry soil is generally thought to be due to the release of readily-decomposable organic matter into the soil environment from non-living organic matter and from the death of the microbial population after stress from desiccation and rapid changes in water potential. Christenson *et al.* (1990) found that addition of dead bacterial cells to an anaerobic soil slurry doubled denitrification activity within 2 h. Following a wetting event, carbon and nitrogen availability is often high (Smith *et al.*, 1985). Biomass-C released into the soil environment upon rapid change in water potential in two California grassland soils was shown to range from between 17 to 70% of the total biomass-C initially present (Kieft *et al.*, 1987).

Ammonium availability initially increased then declined after reaching a peak at 8 h (Fig. 5). The decline in NH_4^+-N availability is probably due to a combination of immobilization, nitrification and gaseous losses. Ammonium is generally considered to be the preferred N-source for microorganisms (Rice and Tiedje, 1989; Jackson *et al.*, 1989) and nitrifying bacteria are generally considered to be poor competitors for NH_4^+-N relative to heterotrophic microorganisms (Verhagen and Laanbroek, 1991). The inorganic N turnover estimated from the magnitude of N change from Fig. 5 suggests that inorganic N was turned over at least once during the incubation period.

Nitrate losses, presumably due to immobilization reactions and gaseous loss, surpassed production when soil NH_4^+-N content decreased to *ca* 5 $\mu g\, g^{-1}$ soil (Fig. 5) even though NH_4^+-N concentrations of 0.1 $\mu g\, g^{-1}$ soil are known to effectively inhibit NO_3^--N assimilation by soil microbes (Jackson *et al.*, 1989; Rice *et al.*, 1989). The presence of NH_4^+-depleted microsites may allow for assimilation of soil NO_3^--N, which is more mobile in the soil environment than NH_4^+. Soil N_2O flux dropped to low rates while NO_3^--N was still being produced (Fig. 5) suggesting that when substrate concentrations were at an optimum for denitrification soil aeration status was unfavorable for the process. Nitrate immobilization occurring after 200 h (Fig. 5) may limit substrate availability for denitrification during subsequent wetting events when other conditions are favorable.

Our results indicate that N_2O flux from this shrub-steppe ecosystem is regulated by interactions between soil water content, and N-mineralization and N-immobilization processes. Low soil moisture content and intense competition among microorganisms and plants for available N probably result in low N_2O flux rates for much of the year. However, even though moisture and inorganic N are generally low for this shrub-steppe ecosystem, after wetting available substrate and conditions for N_2O loss increase considerably. In addition, soil N and C pools, N-mineralization rates and microbial biomass are known to be associated spatially with vegetation in this ecosystem (Bolton *et al.*, 1990, 1993) and therefore areas under plant canopies would be expected to produce more N_2O than interplant areas following precipitation events. Thus on a ecosystem basis conditions may only be favorable for appreciable N_2O production after precipitation events and mostly in soil associated with plant cover.

Nitrification accounts for over 60% of the N_2O from this ecosystem which is consistent with estimates from shortgrass steppe (60–80%) (Parton *et al.*, 1988), humid forest (50%) (Robertson and Tiedje, 1987) and dry tropical forest ecosystems (Davidson *et al.*, 1993). From the core experiments and periodic field measurements (data not presented) we estimated the annual N_2O flux from this ecosystem to be 0.15 kg N_2O-N $ha^{-1}yr^{-1}$. This estimate is less than a 0.21 kg N_2O-N $ha^{-1}yr^{-1}$ estimate for a Wyoming shrub-steppe ecosystem (Matson *et al.*, 1991) and greater than a 0.10 kg N_2O-N $ha^{-1}\,yr^{-1}$ estimate for a shortgrass steppe (Parton *et al.*, 1988) and a 0.10 kg N_2O-N $ha^{-1}yr^{-1}$ estimate for Wisconsin prairies (Goodroad and Keeney, 1984). Further research is needed to quantify the spatial relationship between

vegetation and N_2O flux in different ecosystems and to determine the importance of nitrification in the total annual N_2O flux from all undisturbed terrestrial ecosystems.

Acknowledgements—We gratefully acknowledge the skilled technical assistance of Debbie Bikfasy. This research was supported in part by the Ecological Research Division, Office of Health and Environmental Research, U.S. Department of Energy. Pacific Northwest Laboratory is operated for the DOE by Battelle Memorial Institute.

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