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Nitrate limitation of N_2O production and denitrification from tropical pasture and rain forest soils

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Abstract. Nitrous oxide production was measured in intact cores taken from active pasture and old-growth forest Inceptisols in the Atlantic Lowlands of Costa Rica. Following additions of aqueous KNO_3 or glucose, or the two combined amendments, the cores were incubated in the laboratory to determine if N_2O production rates were either N-limited or C-limited in the two land use types. Differences in rates of denitrification (N_2O + N_2 production) among amended forest and pasture soils were determined by addition of 10% C_2H_2.

The forest soils were relatively insensitive to all amendment additions, including the acetylene block. Forest N_2O production rates among the treatments did not differ from the controls, and were consistently lower than those of the pasture soils. With the addition of glucose plus nitrate to the forest soils, production of N_2O was three times greater than the controls, although this increase was not statistically significant. On the other hand, the pasture soils were definitely nitrogen-limited since N_2O production rates were increased substantially beyond controls by all the amendments which contained nitrate, despite the very low N level (5 mg N kg^{-1} soil) relative to typical fertilizer applications. With respect to the nitrate plus glucose plus acetylene treatment, denitrification was high in the pasture soils; N_2O production in the presence of C_2H_2 was 150% of the rate of N_2O production measured in the absence of the acetylene block. The results are discussed in relation to the effects of agricultural land use practices and subsequent impacts of disturbance on N_2O release.

Introduction

Atmospheric concentrations of nitrous oxide have increased about 0.3% per year for the last 20 to 30 years (Rasmussen & Khalil 1986; Prinn et al.

1990; Khalil & Rasmussen 1992). Increasing nitrous oxide emissions have two serious consequences: along with CO_2, CH_4, O_3 and chlorofluorocarbons, N_2O contributes to global warming (Dickinson & Cicerone 1986); and following its oxidation to NO, N_2O catalyzes stratospheric ozone depletion (Cicerone 1987). Biogenic emissions of N_2O from tropical forest soils have been identified as the most important global source of this trace gas (Matson & Vitousek 1990).

Microbial oxidation of NH_4^+ to NO_3^- (nitrification) and anaerobic reduction of NO_3^- and NO_2^- to N_2 (denitrification) both can lead to the production of N_2O (Firestone & Davidson 1989). The partial-pressure of O_2 in the soil atmosphere and the availability of inorganic-N and labile organic carbon are the factors most directly responsible for controlling the production of N_2O (Firestone et al. 1980; Firestone & Davidson 1989). At larger scales, nitrous oxide production is regulated by climate, soil type and disturbance (Groffman et al. 1988; Robertson 1989), as well as land management practices (Aulakh et al. 1991). Patterns of N_2O production have been correlated with soil fertility for humid tropical forests (Matson & Vitousek 1987; Vitousek & Matson 1988; Matson & Vitousek 1990), but less is known about fluxes of gaseous N species from tropical forest soils subject to disturbance or conversion to other land uses (e.g. Keller et al. 1986; Goreau & de Mello 1988; Robertson & Tiedje 1988; Luizão et al. 1989; Steudler et al. 1991).

We wished to determine which proximal variables contribute to differences in nitrous oxide emissions from rain forest and active pastures as has been observed in field studies in the Atlantic Lowlands of Costa Rica. Keller and Reiners (in preparation) found that N_2O emissions from rain forest soils increased exponentially with increasing saturation of the soil pore space. This result and previous findings from fertilization experiments (Keller et al. 1988) strongly link the nitrous oxide emissions to denitrification in clay-rich rain forest soils. No comparable relation between N_2O emissions and water-filled pore space (WFPS) was found for pasture sites that had been actively grazed for over two decades. We assume that denitrification is the major process resulting from N_2O release from these soils. We wanted to test whether any of three selected controls on denitrification (alone or in combination) contribute to the different patterns found at forest and pasture sites. We tested for effects of possible substrate limitation through addition of nitrate or labile carbon (as glucose). We also tested whether differences in the N_2O emission might result from a difference in the ratio of N_2O and N_2 as end products of denitrification by using the acetylene block technique (Yoshinari et al. 1977; Walter et al. 1979).

Site description

Our study was conducted on and adjacent to La Selva Biological Station in the Atlantic Lowlands of Costa Rica (10°26′N 84°0′W). Three sites of primary forest and active pasture were established on Inceptisols of the Matabuey soil series (Reiners et al. in press). The active pastures had been created from primary forest 21—31 years ago. In addition to marked differences in vegetational composition, soil properties differed between the active pasture and primary forest, as shown in Table 1. Detailed descriptions of soil and vegetation properties at these sites are given by Reiners et al. (in press).

Table 1. Physico-chemical properties characterizing the A horizons of old-growth rain forest and active pasture soils near La Selva Biological Station (from Reiners et al. in press). Values represent means (std. dev.) of three sites.

Soil property	Forest	Pasture
Bulk density (Mg m^{-3})	0.674	0.804
	(0.036)	(0.014)
Porosity (m^3 m^{-3})	0.744	0.704
	(0.013)	(0.004)
Total organic carbon (%)	3.02	2.02
	(1.15)	(0.18)
Total Kjeldahl nitrogen (%)	0.63	0.60
	(0.06)	—
Extractable NH_4^+ (mg N kg^{-1} soil)	2.09	3.64
	(0.66)	(0.92)
Extractable NO_3^- (mg N kg^{-1} soil)	4.30	1.76
	(1.70)	(0.74)
7-d mineralization (mg N kg^{-1} soil)	13.00	7.38
	(1.70)	(2.42)
7-d Nitrification (mg N kg^{-1} soil)	13.83	9.84
	(1.84)	(2.58)

Materials and methods

Field sampling procedure

Soils in the six active pasture and primary forest sites were collected during late January and early February, 1992. Sampling alternated between the pasture and forest sites; the dates of sampling and the order of the

replicate land use types were randomly selected. Forty soil cores were taken at regular intervals (pasture, 75 cm; forest, 150 cm) along two randomly-placed transect lines in each site (pasture, 15 m long; forest, 30 m long). Bevel-edged PVC tubes (5 cm i.d. × 10 cm) were inserted into the soil to 10-cm depth. After removal from the soil, both ends of the PVC tubes were capped for transport to the laboratory.

Sample incubation and gas measurement

The soils were analyzed on the same day they were collected. Cores were randomly selected to receive eight possible treatments. The eight possible treatments were: (1) 10 mL deionized water; (2) 10 mL of nitrate solution (506 mg $NaNO_3$ L^{-1}); (3) 10 mL of glucose solution (894 mg glucose L^{-1}); (4) 10 mL of mixed nitrate-glucose solution; (5—8) the above four treatments plus acetylene block (described below). The C and N additions were calculated on the basis of a nominal fresh core mass of 300 g (exclusive of the PVC core tube) and a moisture content of about 0.80 g H_2O g^{-1} dry soil. The addition of 10 mL of deionized water alone was our 'control' treatment because all other amendments were added in this volume of water. Additions of water alone were known from preliminary experiments to enhance N_2O production rates (Fig. 1a), and the 10-mL aliquots were sufficiently large to distribute the amendments throughout the cores.

Nitrate was applied to the soil cores at a level comparable to the mean soil N concentration measured in old-growth forest soils during the wet season ($\sim$ 5 mg NO_3-N kg^{-1} soil, Reiners et al. in press). Glucose was added to the soil assuming a 5:1 molar ratio between organic-C and inorganic-N (29.77 mM C: 5.95 mM N). This C:N ratio was similar to that used by Schuster and Conrad (1992), but greater than a 3:1 to 4:1 ratio suggested as being effective for obtaining denitrifying potential (Payne 1981, cited in Beauchamp et al. 1989).

The samples were prepared for incubation by inverting the cores and injecting the aqueous solutions into the exposed soil surface at quincuntially-distributed injection points. The 18-gauge spinal needle used to inject solution was pushed downward through the soil to within 1-cm of the capped, topside surface. A 2-mL aliquot of solution was injected gradually at each point. The needle was rotated and withdrawn slowly during injection to distribute solution throughout the core. The core bottoms were recapped after injection, the cores were reinverted, and the top ends were uncapped for incubation.

Incubations of the cores were performed in 1-L Mason jars. The cores rested on wire supports contained within the jars, which were then sealed

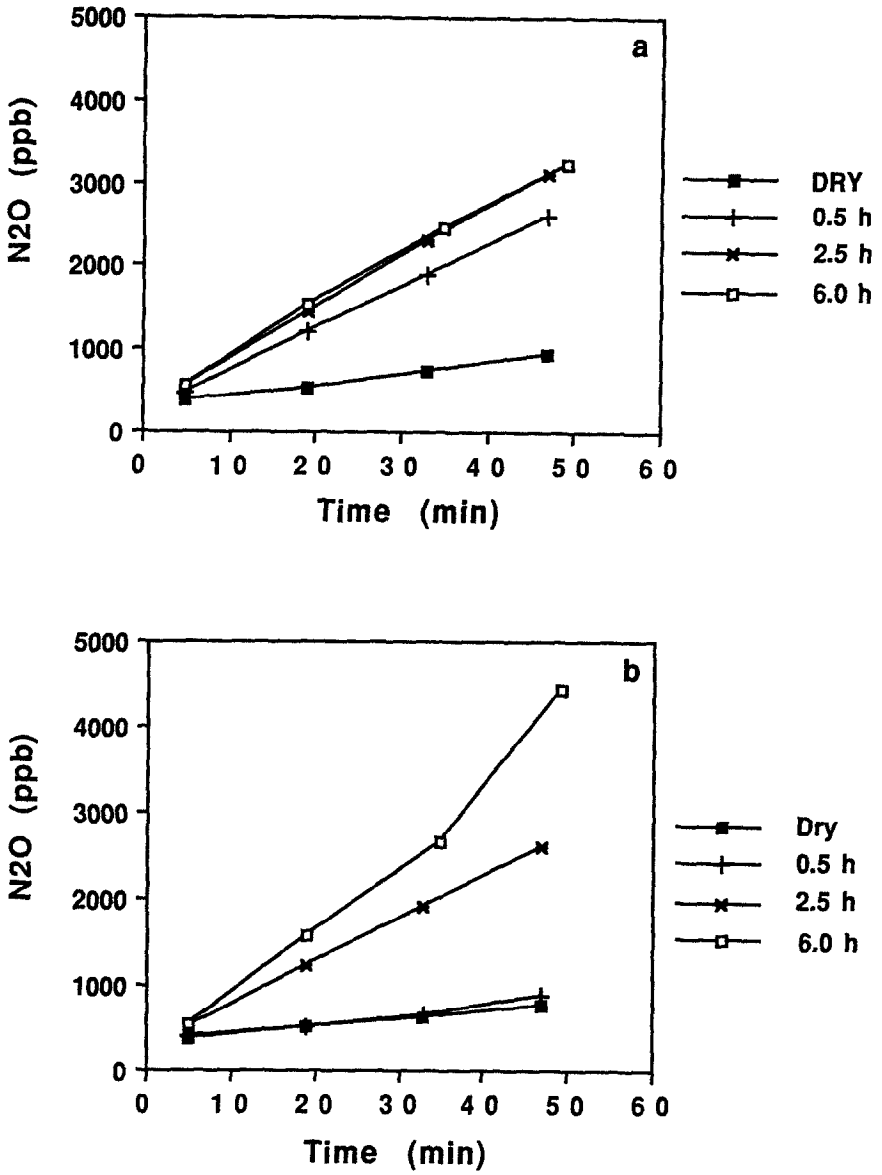

Fig. 1. Forty-two minute time-courses of N_2O production for individual soil cores after incubation for 0.5 h, 2.5 h and 6 h following the addition of 15 mL distilled water (a) or 15 mL distilled water plus KNO_3 (b). The respective 'dry' core measurements refer to N_2O production rates in soil cores taken from primary forest immediately adjacent to the La Selva station and incubated in the laboratory under ambient moisture and available-N conditions (panel a: 13.23 ppbv N_2O min^{-1}, WFPS = 63.9%; panel b: 9.66 ppbv N_2O min^{-1}, WFPS = 73.0%). The nitrate addition raised available soil nitrogen by ~ 10 mg N kg^{-1} soil.

with gas-tight lids and brass o-ring seal ports (Swagelok) fitted with rubber septa. The jars were evacuated 4 times, each for 30 s duration to remove residual traces of N_2O, and slowly returned to normal atmospheric pressure. Following the final evacuation, the jars were re-equilibrated with ambient air, or air was added following addition of 90 cm^3 of CaC_2-generated acetylene gas to the partially-evacuated jars. Acetylene was applied to five cores in each of amendment groups (5) to (8) to limit the activity of N_2O reductase (Yoshinari et al. 1977). The head space C_2H_2 partial-pressure was about 10 kPa, sufficient to block conversion of N_2O to N_2.

During preliminary observations, we had observed maximum activity between 2.5 and 6 h following amendments (Fig. 1). In the current experiment, the jars were incubated for four hours on the laboratory bench under ambient conditions (~ 26 °C). Since it was not possible to incubate all forty cores simultaneously, four of the eight treatments were assigned randomly to each 4-hour-long incubation. The preparation of a given block of incubation jars was separated, in turn, by 1 h from the preceding and following groups. Within temporal blocks, the injection and incubation of each core was staggered, since the process of evacuation and re-equilibration took about 4 min to complete for each jar. Prior to incubating each jar (and immediately prior to the removal of head space samples), the head space gases were mixed by withdrawing and reintroducing three successive 25-cm^3 volumes of the air through the ports.

After 4 h, the jars were re-evacuated and re-equilibrated with the atmosphere, repeating the aforementioned procedure. Freshly-generated acetylene was added back to the appropriate jars. Nitrous oxide concentrations in the head space were analyzed at 0, 16 and 32 min after resealing the jars. Samples and standards were held in gas-tight nylon syringes prior to injection into the GC. Standard gas mixtures (343.8, 543.7 and 808.7 ppbv N_2O), run every 24 samples, were supplied by Scott Speciality Gases and calibrated by the Climate Modeling and Diagnostic Laboratory (NOAA, Boulder, CO).

Head space samples were analyzed using a gas chromatograph equipped with a ^{63}Ni electron capture detector (Shimadzu GC-8A ECD). Gas samples were separated on two 1.83 m stainless-steel columns linked in series and packed with 50/80 mesh Poropak Q (Waters Associates). The flow rate of carrier gas (5% CH_4—95% Ar) to the detector was ~ 25 mL min^{-1}. Column and detector temperatures were 63 °C and 330 °C, respectively. Samples were injected into the first column through a 12-port valve (designed by James Elkins, NOAA/Climate Modeling and Diagnostic Laboratory, Boulder, CO, and manufactured by Valco Instru-

ments). The first column was backflushed automatically after 1.76 min to remove C_2H_2 and higher molecular-weight constituents which otherwise would contaminate the ECD. The retention time of N_2O was 2.65 min. The N_2O peak areas were measured by using a Hewlett Packard HP3396A integrator.

Intact cores were weighed following the collection of the last gas sample in each block of incubation jars. The individual cores then were bulked, and gravimetric moisture content was calculated from 3 replicate subsamples of the composite (100 g fw; 100—110 °C for 48 h). We calculated the core bulk density, % WFPS, and the head space volume for each incubation jar.

Nitrous oxide concentration measurements made at 0, 16 and 32 min were used to calculate instantaneous N_2O production for each soil core (ppbv N_2O min^{-1}). In preliminary tests, we observed linear responses to the additions of water (Fig. 1a) and nitrate (Fig. 1b) during 30—45 min assays conducted with or without C_2H_2 (Fig. 2). Assuming this linear response, we simply calculated the overall N_2O production rate from the change in N_2O concentration over the entire 32-min assay period, for the head space volume with a core in place (pasture mean = 818 mL; forest mean = 845 mL). Production rates were expressed on a soil-dry mass basis as ng N_2O g^{-1} soil h^{-1}.

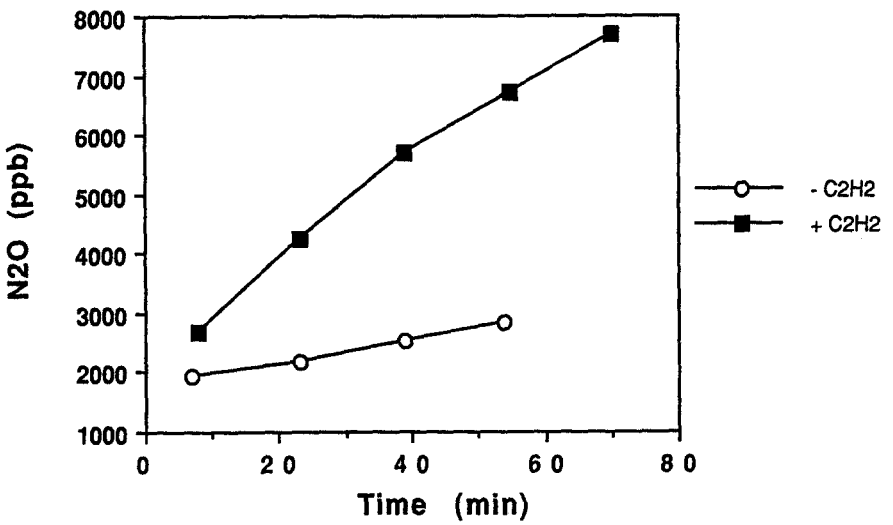

Fig. 2. Laboratory time courses of N_2O production in individual forest soil cores incubated in the presence (closed squares) or absence (open circles) of an acetylene block (10% v/v). The soils were incubated under ambient moisture conditions.

Statistical analysis

In addition to land use (pasture vs forest), there are two levels for each of three, completely-crossed factors: with and without C_2H_2; with and without NO_3^-; with and without glucose. There were two levels of replication in the experiment. The three sites for each land use type were the true replicates, and the 40 cores sampled within each site (5 per assigned treatment) represented a random variance component outside the fixed-effects of the original model.

The treatment structure of incubation experiment was a four-way factorial analysis of covariance (ANCOVA) design. Rather than average over the 5 replicates in each treatment group, the WFPS of each core was used as the covariate in the analysis to correct the N_2O production data for variability in soil moisture attributable to land use, individual sites and sampling date. However, during the evacuation and re-equilibration procedure, ~ 6% of the cores lost solution; the N_2O production data for these samples were not used in the subsequent statistical analysis. Prior to the analysis of covariance within SAS (SAS Institute Inc. 1985), the N_2O production rates were transformed ($\log_{10}[X + 1]$) to linearize the relationship with WFPS and to satisfy assumptions of normality and homogeneity of variance.

Results

The glucose and nitrate amendments individually exerted no appreciable effect on the rate of N_2O production from forest soil cores beyond that produced by deionized water (Table 2a). The mean rate of N_2O production for the forest soils amended with nitrate plus glucose was about three times the controls, although there was no significant difference between the two treatments (20.97 vs 6.01 ng N_2O g^{-1} soil h^{-1}). Forest soil cores amended with both glucose and nitrate produced almost 50% less N_2O in the presence of C_2H_2 than in its absence (9.24 vs 20.97 ng N_2O g^{-1} soil h^{-1}); again, there was no significant difference. Otherwise, forest cores incubated under C_2H_2 with the individual amendments emitted, on average, 1.1—1.8 times as much N_2O as those cores incubated without C_2H_2, but differences among the means were not significant (Table 2a).

The addition of glucose to the pasture soils, like the forest sites, had no effect on rates of N_2O production in the absence of supplemental nitrate (Table 2b). In the absence of NO_3^-, rates of N_2O production were comparable between the two land use types. However, nitrate-amended pasture soils produced about eleven times as much N_2O as the deionized

Table 2. Nitrous oxide production rates (ng N_2O g^{-1} soil h^{-1}) in incubated, intact soil cores taken from a) old-growth forest and b) active pasture sites. Each value has been estimated by ANCOVA on log-transformed data, and is the average of 13–15 measurements from three sites. Subsequent pairwise comparisons of the geometric means were performed using Bonferroni-adjusted 95% confidence intervals. The back-transformed values followed by the same bold letter do not differ significantly at $P = 0.05$.

a) Forest:		−Nitrate	+Nitrate
$-C_2H_2$	−Glucose	6.01**a**	7.49**a**
	+Glucose	4.89**a**	20.97**ab**
$+C_2H_2$	−Glucose	9.56**a**	8.56**a**
	+Glucose	7.92**a**	9.24**a**
b) Pasture:		−Nitrate	+Nitrate
$-C_2H_2$	−Glucose	3.88**a**	41.58**b**
	+Glucose	3.48**a**	137.93**c**
$+C_2H_2$	−Glucose	11.69**a**	108.45**c**
	+Glucose	7.44**a**	203.10**d**

water controls; likewise, N_2O production in pasture soils amended with nitrate in the presence of acetylene was about nine times greater than control rates. Moreover, the pasture soils responded very differently from the forest soils to the addition of nitrate plus glucose (Table 2b). There was a synergistic effect of adding glucose combined with nitrate to pasture cores, regardless of whether C_2H_2 was added or not. In the absence of acetylene, N_2O production rates were increased significantly (36-fold) beyond the controls by the nitrate plus glucose treatment (3.88 vs 137.93 ng N_2O g^{-1} soil h^{-1}). In the presence of the C_2H_2 block, mean N_2O production rate observed for the nitrate plus glucose treatment was ~ 17 times control rates (11.69 vs 203.48 ng N_2O g^{-1} soil h^{-1}).

Log-transformation of the N_2O production rates satisfied the assumption of homoscedasticity among treatments. Moreover, cumulative probability plots (Finney 1952) indicated that the N_2O production data were log-normally distributed (although not shown here), consistent with earlier reports (Parkin 1987; Parkin et al. 1988). The overall coefficient of variation for the log-N_2O production data was 61%.

According to ANCOVA (Table 3), log-N_2O production was significantly different between the two levels of acetylene ($F_{1,32} = 5.82$, $P < 0.05$) and highly significantly different between the levels of nitrate ($F_{1,32} = 86.63$, $P < 0.001$). The two-way interaction between land use type and

Table 3. Significance tests on log-transformed N_2O production rates, as estimated by four-way ANCOVA, in lab incubations of soil cores from active pasture and primary forest sites under glucose and nitrate amendments, with and without addition of C_2H_2.

Source	MS	df	F	*P*
Land (L)	0.025	1	0.09	>0.50
Acetylene (A)	1.576	1	5.82	<0.05
Nitrate (N)	23.476	1	86.63	<0.001
Glucose (G)	0.908	1	3.35	<0.10
L × A	1.794	1	6.62	<0.05
L × N	15.784	1	58.24	<0.001
L × G	0.042	1	0.15	>0.50
A × N	0.362	1	1.34	>0.10
A × G	0.404	1	1.49	>0.10
N × G	1.676	1	6.18	<0.05
L × A × N	0.156	1	0.57	>0.10
L × A × G	2.665E-5	1	9.815E-5	>0.50
L × N × G	0.058	1	0.21	>0.50
A × N × G	0.064	1	0.24	>0.50
L × A × N × G	0.041	1	0.15	>0.50
Error (Sites)	0.271	32	0.74	>0.50
WFPS	6.903	1	18.96	<0.001
Error (Cores)	0.364	176		

nitrate was very strong ($F_{1,32} = 58.24$, $P < 0.001$). Moderately strong interactions were found, respectively, between land use type and C_2H_2 ($F_{1,32} = 6.62$, $P < 0.05$), and between nitrate and glucose ($F_{1,32} = 6.18$, $P < 0.05$), but the remaining interaction terms were not significant (Table 3). Likewise, differences between levels of the main effects of land use and glucose were not significant (Table 3), although these factors contributed to the differences observed among the means, especially in the pasture soils (Table 2b).

Nitrous oxide production rates exhibited a significant, positive exponential relationship with the water-filled pore space percentage (Fig. 3), but only for the forest soils ($r = 0.570$; $F_{1,110} = 52.82$, $P < 0.001$). In ANCOVA, log-transformed N_2O was significantly correlated with WFPS (Table 3: $F_{1,176} = 18.96$, $P < 0.001$), consistent with the results of simple linear regression. However, much of the core-to-core variation in log-N_2O production rates was explained, not by WFPS ($r^2 = 0.05$), but rather by the significant main effects and interactions within the ANCOVA ($r^2 = 0.35$). Inclusion of the covariate in the analysis eliminated inter-site differences, and thus, potential day-to-day variation, which could have con-

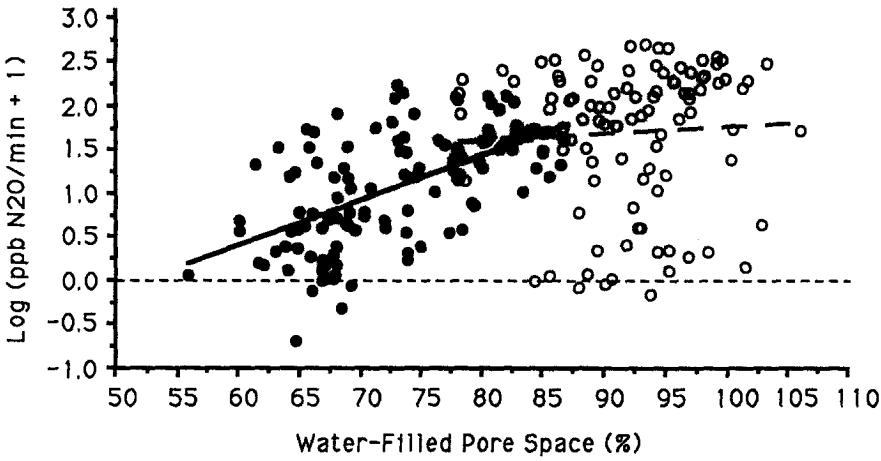

Fig. 3. Log_{10}-transformed nitrous oxide production as a function of the water-filled pore space (% WFPS) of individual primary forest (closed circles) and active pasture (open circles) soil cores. The relationship between log-N_2O and WFPS (indicated by the dashed bold line) for soil cores (n = 113) from the active pasture soils is not significant. For the forest soils, the solid bold line is described by the following least-squares regression equation (n = 112; r^2 = 0.324): log_{10}(ppbv N_2O/min + 1) = −2.681 (± 0.517) + (WFPS) * 0.051 (± 0.007).

founded the interpretation of the treatment effects (F-test of Sites: $F_{32,176} = 0.74, P > 0.50$).

Discussion

Overall, the strong relation between N_2O production and WFPS suggests that denitrification was the principle source of N_2O production in our soil cores. Increased water content causes reduced air diffusion into the soil, increasing the likelihood that anaerobic microsites will develop (Linn & Doran 1984; Sexstone et al. 1985; Davidson et al. 1986; Aulakh et al. 1991; Davidson 1991; Rudaz et al. 1991; Davidson 1992).

Individually, or in combination, the effect of nitrate, glucose, or acetylene additions had no significant effect on N_2O production by the forest soil cores. The levels of substrate additions used in this study are substantially lower than those employed by other investigators studying denitrification and nitrous oxide production. Nitrate applications, taken on an areal basis, would translate to 5 kg-N/ha, far below nitrogen additions typical of field fertilization trails and very low relative to other incubation studies employing combinations of C and N (e.g. deCatanzaro & Beauchamp 1985; Davidson et al. 1986, deCatanzaro et al. 1987; Lalisse-

Grundman et al. 1988; Robertson & Tiedje 1988; Ambus & Lowrance 1991; Peterjohn & Schlesinger 1991; Lescure et al. 1992). The lack of a significant response to substrate additions may reflect the low level of substrate addition relative to high background mineralization rates.

The lack of response to addition of 10 kPa C_2H_2 is more difficult to interpret. This level of C_2H_2 addition will not only limit reduction of N_2O to N_2, it will also effectively inhibit nitrification (Davidson et al. 1986). Acetylene addition did not significantly reduce N_2O production by the cores; thus, it seems unlikely that nitrification alone is the major pathway for N_2O production in this system. On the other hand, the failure of the C_2H_2 block to increase the core N_2O production does not unambiguously indicate that N_2O is the principle end-product of denitrification in this system. It is plausible that the lack of a significant effect results from a combination of a reduced nitrification contribution to N_2O production with an increased denitrification contribution under the C_2H_2 block condition.

In contrast to the forest cores, N_2O production from the pasture cores was clearly limited by the availability of nitrate. Nitrous oxide production rates for nitrate and nitrate plus glucose amended cores were consistently higher in the pasture soils than in forest soils, which agrees with the findings of Groffman et al. (1991) for grass covered and forested vegetation strips in Rhode Island. Glucose alone had no significant effect on the nitrous oxide production from pasture cores. This suggests that as in forest soils, carbon availability alone did not limit nitrous oxide production in pasture soils. However, we found a significant effect when nitrate and glucose were added together, especially under the acetylene block. This synergistic effect indicates that when nitrate supply is adequate, denitrification activity is increased by the addition of available carbon. The observed glucose effect may result from the direct availability of glucose to the denitrifiers or through indirect effects. Two possible indirect effects are increased availability of fermentation products to the denitrifiers and expanded anaerobic soil microenvironments resulting from increased soil metabolism (deCatanzaro & Beauchamp 1985; Parkin 1987; Beauchamp et al. 1989; Aulakh et al. 1991).

Conversion of tropical forest to pastures is potentially an important contributor to observed increases of atmospheric N_2O concentrations (Luizão et al. 1989). Our results suggest that nitrous oxide production from some pasture soils may be nitrogen limited. Nitrogen fertilization of tropical pastures under intensive production systems may substantially increase emissions of N_2O (Vitousek et al. 1989). Understanding the nitrogen cycle in tropical forest and pasture soils appears to be critical to understanding the controls on nitrous oxide emissions from these systems.

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References

Ambus P & Lowrance R (1991) Comparison of denitrification in two riparian soils. Soil Sci Soc Amer J 55: 994–997

Aulakh MS, Doran JW & Mosier AR (1991) Soil denitrification: significance, measurement, and effects on management. Adv. Soil Sci. 18: 1–57

Beauchamp EG, Trevors JT & Paul JW (1989) Carbon sources for bacterial denitrification. Adv. Soil Sci. 10: 113–142

Cicerone RJ (1987) Changes in stratospheric ozone. Science 337: 35–42

Davidson EA (1991) Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. In: Rodgers JE & Whitman WB (Eds) Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes (pp 219–235). American Society of Microbiology, Washington, DC

Davidson EA (1992) Sources of nitric oxide and nitrous oxide following wetting of dry soil. Soil Sci. Soc. Amer. J. 56: 95–102

Davidson EA, Swank WT & Perry TO (1986) Distinguishing between nitrification and denitrification as sources of gaseous nitrogen production in soil. Appl. Environ. Microbiol. 52: 1280–1286

deCatanzaro JB & Beauchamp EG (1985) The effects of some carbon substrates on denitrification rates and carbon utilization in soil. Biol. Fertil. Soils 1: 183–187

deCatanzaro JB, Beauchamp EG & Drury CF (1987) Denitrification vs dissimilatory nitrate reduction in soils with alfalfa, straw, glucose and sulfide treatments. Soil Biol. Biochem. 19: 583–587

Dickinson RE & Cicerone RJ (1986) Future global warming from atmospheric trace gases. Nature 319: 109–115

Finney DJ (1952) Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve (2nd ed). Cambridge University Press, Cambridge, UK

Firestone MK, Firestone RB & Tiedje JM (1980) Nitrous oxide from soil denitrification: factors controlling its biological production. Science 208: 749–751

Firestone M & Davidson EA (1989) Microbial basis of NO and N_2O production and consumption. In: Andreae MO & Schimel DS (Eds) Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere (pp 7–21). Dahlem Konferenzen, Wiley, Chichester

Goreau TJ & de Mello WZ (1988) Tropical deforestation: some effects on atmospheric chemistry. Ambio 17: 275–281

Groffman PM, Axelrod EA, Lemunyon JL & Sullivan WM (1991) Denitrification in grass and forest vegetated filter strips. J. Environ. Qual. 20: 671–674

Groffman PM, Tiedje JM, Robertson GP & Christensen S (1988) Denitrification at different temporal and geographical scales: proximal and distal controls. In: Wilson JR (Ed) Proceedings of Symposium on Advances in Nitrogen Cycling in Agricultural Ecosystems (pp 174–192). Commonwealth Agricultural Bureau, Perth

Keller M, Kaplan WA & Wofsy SC (1986) Emissions of N_2O, CH_4, and CO_2 from tropical soils. J. Geophys. Res. 91: 791–802

Keller M, Kaplan WA, Wofsy SC & da Costa JM (1988) Emissions of N_2O from tropical forest soils: response to fertilization with NH_4^+, NO_3^-, and PO_4^{3-}. J. Geophys. Res. 93: 1600–1604

Khalil MAK & Rasmussen RA (1992) The global sources of nitrous oxide. J. Geophys. Res. 97: 14,651–14,660

Lalisse-Grundman G, Brunel B & Chalament A (1988) Denitrification in a cultivated soil: optimal glucose and nitrate concentrations. Soil Biol. Biochem. 20: 839–844

Lescure C, Menendez L, Lensi R, Chalamet A & Pidello A (1992) Effect of addition of various carbon substrates on denitrification in a vertic Mollisol. Biol. Fertil. Soils 13: 125–129

Linn DM & Doran JW (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and non-tilled soils. Soil Sci. Soc. Amer. J. 48: 1267–1272

Luizão F, Matson P, Livingston G, Luizão R & Vitousek P (1989) Nitrous oxide flux following tropical land clearing. Global Biogeochem. Cycles 3: 281–285

Matson PA & Vitousek PM (1987) Cross-system comparisons of soil nitrogen transformations and nitrous oxide flux in tropical forest ecosystems. Global Biogeochem. Cycles 1: 163–170

Matson PA & Vitousek PM (1990) Ecosystem approach to a global nitrous oxide budget. BioScience 40: 667–671

Parkin TB (1987) Soil microsites as a source of denitrification variability. Soil Sci. Soc. Amer. J. 51: 1194–1199

Parkin TB, Meisinger JJ, Chester ST, Starr JL & Robinson JA (1988) Evaluation of statistical estimation methods for lognormally distributed variables. Soil Sci. Amer. J. 52: 323–329

Peterjohn WT & Schlesinger WH (1991) Factors controlling denitrification in a Chihuahuan desert ecosystem. Soil Sci. Soc. Amer. J. 55: 1694–1701

Prinn R, Cunnold D, Rasmussen R, Simmonds P, Alyea F, Crawford A, Fraser P & Rosen R (1990) Atmospheric emissions and trends of nitrous oxide deduced from 10 years of ALE-GAGE data. J. Geophys. Res. 95: 18,369–18,385

Rasmussen RA & Khalil MAK (1986) Atmospheric trace gases: trends and distributions over the last decade. Science 232: 1623–1624

Reiners WA, Bouwman AF, Parsons WFJ & Keller M (1994) Tropical rain forest conversion to pasture: changes in vegetation and soil properties. Ecological Applications, in press

Robertson GP (1989) Nitrification and denitrification in humid tropical ecosystems: potential controls on nitrogen retention. In: Proctor J (Ed) Mineral Nutrients in Tropical Forest and Savanna Ecosystems (pp 55–69). Blackwell Sci Publ, Oxford

Robertson GP & Tiedje JM (1988) Deforestation alters denitrification in a lowland tropical rain forest. Nature 336: 756–759

Rudaz A, Davidson EA & Firestone MK (1991) Sources of nitrous oxide production following wetting of dry soil. FEMS Microbial. Ecol. 85: 117–124

SAS Institute Inc (1985) SAS User's Guide: Statistics, Version 5. SAS Institute Inc, Cary, NC 956 pp

Schuster M & Conrad R (1992) Metabolism of nitric oxide and nitrous oxide during nitrification and denitrification in soil at different incubation conditions. FEMS Microbiol. Ecol. 101: 133–143

Sexstone AJ, Parkin TB & Tiedje JM (1985) Temporal response of soil denitrification rates to rainfall and irrigation. Soil Sci. Soc. Amer. J. 49: 99–103

Steudler PA, Melillo JM, Bowden RD, Castro MS & Lugo AE (1991) The effects of natural and human disturbances and trace gas fluxes in a Puerto Rican wet forest. Biotropica 23: 356–363

Vitousek PM & Matson PA (1988) Nitrogen transformations in a range of tropical forest soils. Soil Biol. Biochem. 20: 361–367

Vitousek PM, Matson PA, Volkmann C, Maass JM & Garcia-Mendez G (1989) Nitrous oxide flux from seasonally dry tropical forests: a survey. Global Biogeochem. Cycles 3: 375–382

Walter HM, Keeney DR & Fillery IR (1979) Inhibition of nitrification of acetylene. Soil Sci. Soc. Amer. J. 43: 195–219

Yoshinari T, Hynes R & Knowles R (1977) Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil. Soil Biol. Biochem. 9: 177–183