Soil Biol. Biochem. Vol. 19, No. 2, pp. 187–193, 1987
Printed in Great Britain.

0038-0717/87 $3.00 + 0.00

NITROUS OXIDE SOURCES IN AEROBIC SOILS: NITRIFICATION, DENITRIFICATION AND OTHER BIOLOGICAL PROCESSES

G. P. ROBERTSON* and J. M. TIEDJE
Departments of Crop and Soil Sciences and of Microbiology and Public Health, Michigan State University, East Lansing, MI 48824-1114, U.S.A.

(*Accepted* 12 *August* 1986)

Summary—Nitrous oxide (N_2O) production in aerobic, intact soil cores collected from two forest sites was partitioned into different sources of N_2O by subjecting soils to specific inhibitors during short-term incubations. We used acetylene (10 Pa) to selectively inhibit N_2O production by nitrifiers, 100 kPa (100% v/v) O_2 to inhibit N_2O production by denitrifiers, and sterilized soil to evaluate chemical sources of N_2O. Individual soil cores were incubated in a recirculating atmosphere system under air, 100 kPa O_2, and air + 10 Pa C_2H_2 atmospheres for 3–4 h under each condition. Rates of N_2O production in air ranged from 2.8 to 8.5 ng $N\,g^{-1}\,h^{-1}$ in soils from the Rose Lake site and from 0.1 to 0.94 ng $N\,g^{-1}\,h^{-1}$ in soils from Warren Woods. The importance of specific N_2O sources in these soils varied among soil cores within each site. In the recently disturbed, more fertile Rose Lake site nitrifiers and denitrifiers were both important N_2O sources, but in many cores a significant portion of the N_2O flux was inhibited by neither acetylene nor O_2, implying an alternate N_2O source. Sterilized soil produced little N_2O, which suggests that the alternate source is biological. In the old-growth Warren Woods site nitrifiers were significant but relatively unimportant sources of N_2O. O_2 consistently stimulated rather than inhibited N_2O production in cores from this site, suggesting (a) that denitrifiers in this site are a sink for N_2O produced by other sources, and (b) that most of the N_2O produced in this site is from sources other than nitrification and denitrification. Biological sources of N_2O other than nitrifiers and denitrifiers were not further identified, although low soil pH values suggest that fungi may be important.

INTRODUCTION

The importance of nitrous oxide to atmospheric photochemistry has become increasingly well-defined in recent years (Crutzen, 1983; Whitten *et al.*, 1984), and recently-documented increases in atmospheric N_2O concentrations (Weiss, 1981; Rasmussen and Kahlil, 1986) have stimulated substantial interest in identifying and measuring rates of N_2O flux from terrestrial sources to the atmosphere. Biological sources of N_2O appear to dominate present-day global fluxes (Soderlund and Svensson, 1976; Banin *et al.*, 1984), but there is little agreement about which of the several soil biological processes that are capable of producing N_2O are in fact responsible for the N_2O fluxes observed *in situ*.

In vitro experiments using soil slurries and repacked soil cores have demonstrated significant N_2O production by both denitrifiers (Firestone *et al.*, 1980) and nitrifiers (Blackmer *et al.*, 1980), although N_2O from nitrifiers may in fact be from nitrifier denitrification (Poth and Focht, 1985). Pure culture studies suggest other soil biological processes may also be important N_2O sources. These latter processes include dissimilatory nitrite and nitrate reduction to ammonium (Bleakley and Tiedje, 1982; Smith, 1982; Smith and Zimmerman, 1981), nitrate assimilation (Satoh *et al.*, 1981), and yet-to-be identified processes carried out under aerobic conditions by fungi, yeasts, nitrate-assimilating bacteria and green algae (Bollag and Tung, 1972; Bleakley and Tiedje, 1982; Burth and Ottow, 1983; Weathers, 1984).

Studies of agricultural soils that produce additional N_2O following NH_4^+ but not following NO_3^- additions, and in which little N_2O is produced after additions of nitrate and nitrifier inhibitors (Bremner and Blackmer, 1979; Freney *et al.*, 1979; Aulakh *et al.*, 1984) suggest that nitrifiers are the principal agents of N_2O production in many agricultural soils. In soils from forested or other noncultivated sites, however, this generalization may not apply. Such soils are not heavily fertilized and pool sizes of NH_4^+ and NO_3^- fluctuate very differently from pool sizes in cultivated systems. Because fluctuations of inorganic N pools can have important consequences for the strengths of both sources and sinks of N_2O in soils (Blackmer and Bremner, 1978; 1979; Firestone *et al.*, 1979; Terry and Tate, 1980), and because the microbial community in forested vs cultivated sites is very different, N_2O dynamics in nonagricultural soils may differ substantially from those in cultivated systems. In particular, sources of N_2O in noncultivated systems may be more diverse.

Partitioning this diversity into meaningful process-level compartments has not yet been achieved. Few soils appear to contain only two or three classes of potential N_2O producers, and selective inhibitors that do not affect existing electron donor–acceptor distributions are rare. Nevertheless, two inhibitors that may only minimally affect these distributions are available: acetylene and oxygen. Acetylene severely inhibits nitrification at concentrations far below those

*Present address: W.K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI 49060-9516, U.S.A.

Table 1. Outline of experimental treatments to partition N_2O sources in intact soil cores. Symbols indicate whether N_2O production is potentially present (+) or inhibited (−)

N_2O source	Air	O_2 (100 kPa)	C_2H_2 (10 Pa)	Sterilization
Nitrifiers				
Non-denitrifying	+	+	−	−
Denitrifying	+	−	−	−
Denitrifiers	+	−	+	−
Dissimilatory nitrate reduction to ammonium	+	−	+	−
Other organisms	+	+[1]	+	−
Chemical	+	+	+	+

[1] O_2 has little or no effect on N_2O production by many of these organisms but this has not been extensively examined.

that affect N_2O reductase in soil (Berg *et al.*, 1982), and oxygen in sufficient quantity inhibits respiratory denitrification (Knowles, 1982). If one can experimentally verify the effectiveness of these inhibitors in a given soil, it becomes possible to partition N_2O sources in otherwise undisturbed soil cores into at least four classes (Table 1): N_2O from nitrifiers is inhibited by 10 Pa acetylene; N_2O from denitrifiers, including denitrifying nitrifiers, is inhibited by elevated O_2; N_2O from chemical sources is produced in sterilized soil; and N_2O from other biological sources can be deduced by difference.

Our objective in the present study was to determine the relative importance of these four potential sources of N_2O in two temperate forest soils.

MATERIALS AND METHODS

Site description and sampling strategy

Intact soil cores (4.7 cm dia × 10 cm depth) were removed from an early successional forest at the Rose Lake Wildlife Research Area near East Lansing, Michigan, and from an old growth beech–maple forest at Warren Woods in southwest Michigan. Soils from both sites had earlier produced N_2O at significant rates (Robertson and Tiedje, 1984). Selected soil characteristics appear in Table 2; more detailed site and soil descriptions can be found in the description of our earlier study.

N_2O production in most sites studied to date demonstrates extreme spatial variability (Mosier *et al.*, 1981; Goodroad and Keeney, 1984; Folorunso and Rolston, 1984). In order to better understand the diversity of N_2O sources throughout our sites, we partitioned N_2O sources in individual soil cores rather than in composited soil samples. This strategy reduced our ability to generalize results over the entire sites, but we believe it offers greater resolution at the process level than would a strategy that included a large number of replicated samples from a single site composite. The four cores per site that constitute the bulk of the results reported in this paper were typical of the >16 per site actually analyzed. We limit our report to these four for clarity.

N_2O assays

Short-term N_2O production in individual cores with and without specific inhibitors was monitored using the soil atmosphere recirculation system described by Parkin *et al.* (1984). In this system, four sealed, intact cores stored at 4°C since sampling 3–4 days earlier were vented, brought up to 22°C, and incubated in line with individual diaphragm pumps that continuously circulated core + headspace atmosphere from the top of each core to its base via external 0.32 mm dia stainless steel tubing. Periodically, a valve along each recirculation stream delivered 0.5 ml of core atmosphere to an injection port of a Perkin–Elmer 910 GC fitted with dual ^{63}Ni electron capture detectors. Gas flows were optimized for separation of N_2O and CO_2, and detection of both peaks was completely independent of sample O_2 concentration from 0.1 to 100 kPa O_2 (Siever *et al.*, 1979). Total airspace volume inside each core was measured with a pressure transducer (Parkin *et al.*, 1984); total core plus recirculating system air space ranged from 80 to 150 ml.

We incubated soil cores on the recirculating-atmosphere system under the treatments noted in Table 1 in the sequence: (1) air (21 kPa or 21% v/v O_2) to establish a baseline rate of N_2O production, (2) 100 kPa (100% v/v) O_2, to inhibit denitrification, (3) air to re-establish a baseline N_2O flux, and (4) 10 Pa (0.01% v/v) C_2H_2 to selectively inhibit nitrification. Autoclaved soil from different, otherwise untreated cores at each site was also assayed for N_2O production under each treatment to evaluate chemical N_2O production. Cores were incubated under each experimental condition for 3–4 h, with 30–45 min venting periods (100 ml min^{-1} O_2 or air)

Table 2. Selected soil characteristics (mean ± SE) of the two research sites (from Robertson and Tiedje (1984) except pH)

Site	Bulk density ($g\,cm^{-3}$)	Texture	pH	Organic C (%)	C/N	Mineralization potentials (NO_3^- ($mg\,N\,m^{-2}\,day^{-1}$))	Mineralization potentials (N_{min} ($mg\,N\,m^{-2}\,day^{-1}$))
Rose Lake	0.88 (0.07)	Loamy sand	5.2 (0.4)	2.49 (0.17)	12.3 (1.5)	60.3 (15.7)	211.0 (68.1)
Warren Woods	1.14 (0.05)	Loamy sand	3.8 (0.09)	1.62 (0.36)	20.9 (6.4)	142.0 (28.0)	181.0 (32.0)

preceding each treatment to flush the previous atmosphere. Both CO_2 production and O_2 consumption were monitored along with N_2O production during these assays; rates of N_2O production were calculated from periods under each treatment when all three rates were linear. This usually occurred within the first 15 min of each treatment period. Acetylene was generated from CaC_2. Previous experiments with soil cores collected on the same date as our principal cores showed that rates of N_2O production in both the presence and absence of 10 kPa C_2H_2 were constant for at least 20 h.

To evaluate whether 10 Pa C_2H_2 effectively blocked nitrification in our soils we monitored NO_3^- production in the presence and absence of 10 Pa C_2H_2 in soils from both sites. Twelve 10 g replicates of soil from each site were placed in individual 160 ml serum vials sealed and treated with air plus 10 Pa C_2H_2 or with air. Vials were held at 25°C and periodically were replenished with original atmosphere (air or air plus 10 Pa C_2H_2). On days 1, 3 and 7, three replicate vials from each site were extracted with 100 ml of 2 M KCl, and the extracts analyzed for NO_2^- and NO_3^- by colorimetric techniques (Technicon, 1973).

In a second experiment, to evaluate the short-term sensitivity of nitrifiers to C_2H_2, we used the chlorate inhibition assay (Belser and Mays, 1980) to examine 24 h ammonium oxidation in Rose Lake soils amended with different concentrations of C_2H_2. Warren Woods soils were also assayed but no NO_2^- accumulated under control conditions. Soils (25 g) were incubated in replicate 160 ml serum vials containing 100 ml media plus $NaClO_3$; different amounts of C_2H_2 injected into the sealed headspace of each vial brought solution concentrations of C_2H_2 to 0, 0.009, 0.09 and 0.9 Pa C_2H_2. Headspace concentrations ranged to 7.8 Pa C_2H_2. Vials were held on a shaker for 24 h and each vial subsampled periodically for NO_2^- determinations.

Potential effects of 10 Pa C_2H_2 on denitrification were evaluated by monitoring N_2O production in soil from the Rose Lake site incubated under anaerobic conditions and treated sequentially with 0, 0.01 and 10 kPa C_2H_2. Replicate serum vials (160 ml) containing 25 g soil were incubated on the recirculation system with an Ar atmosphere under each condition for ≤ 3 h.

To evaluate chemical sources of N_2O in these soils, 25 g soil samples were autoclaved in sealed serum vials three times during a 5-day period, vented to dissipate N_2O formed during autoclaving, and then incubated on the recirculating atmosphere system described above. N_2O production was monitored under air, 100 kPa O_2 and 10 Pa C_2H_2 atmospheres for 3 h each.

All soils asssayed for N_2O production were subsequently analyzed for soil moisture content, pH and nitrate. Soil from cores was mixed and sieved (<4 mm). Two 10 g samples were analyzed for pH in a 2:1 H_2O:soil slurry.

RESULTS AND DISCUSSION

Inhibitor effectiveness and selectivity

Initial experiments were designed to test the effectiveness with which 10 Pa C_2H_2 in these soils inhibited nitrification without affecting denitrification. Figure 1 shows that nitrate production in both soils was severely curtailed by 10 Pa C_2H_2 during a 7 day incubation; that this curtailment was rapid is suggested by the results of the chlorate inhibition experiment, wherein only 0.9 Pa C_2H_2 in solution markedly depressed 24 h nitrite production in chlorate-treated Rose Lake soils (Fig. 2). The 10 Pa C_2H_2 treatment had little effect on denitrification (Fig. 3): under anaerobic conditions

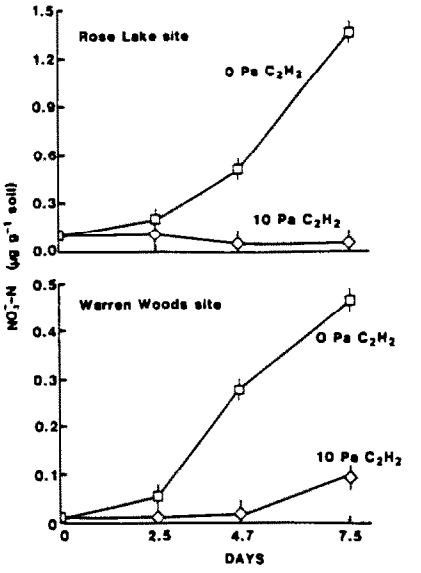

Fig. 1. Effect of 10 Pa (0.01% v/v) acetylene on nitrate accumulation in laboratory-incubated soils from Rose Lake and Warren Woods research sites. Bars represent standard errors ($n = 3$).

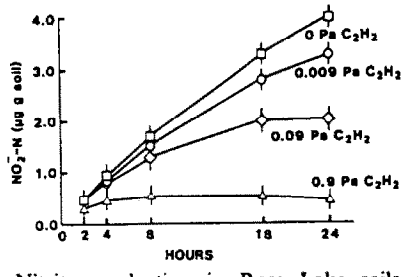

Fig. 2. Nitrite production in Rose Lake soils during chlorate-inhibition assay for short-term nitrification activity. Solution phase C_2H_2 concentrations noted; gas phase concentrations were 0, 0.078, 0.78, and 7.8 Pa C_2H_2. Bars represent standard errors ($n = 2$).

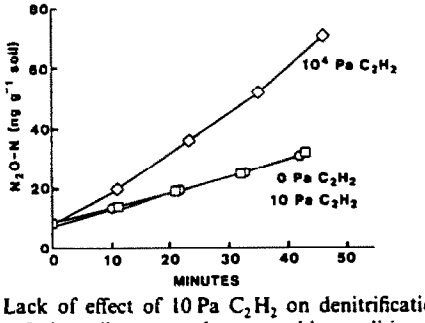

Fig. 3. Lack of effect of 10 Pa C_2H_2 on denitrification in Rose Lake soil cores under anaerobic conditions.

favorable to denitrifiers, N_2O production in Rose Lake soils was not significantly affected by 10 Pa C_2H_2 (34.2 vs 32.4 ng N g^{-1} h^{-1}). That a 10 kPa C_2H_2 atmosphere resulted in significantly higher N_2O production (85.2 mg N g^{-1} h^{-1}) shows that denitrifiers were present and active under the 0 and 10 Pa C_2H_2 treatments.

We judge the effectiveness of 100 kPa O_2 as a selective inhibitor of denitrification on our understandings of O_2 effects on denitrifiers and of O_2 dynamics in soil microsites. First, O_2 is the most effective and selective inhibitor of denitrifiers (Allison *et al.*, 1960; Knowles, 1982); Parkin and Tiedje (1984), for example, found that in intact soil cores only 2% of the soil's capacity to denitrify was expressed unless O_2 concentrations declined to <3 kPa O_2. Second, O_2 microelectrode studies have shown that soil denitrification goes on only in aerobic microsites (Sexstone *et al.*, 1985), with the extent of the anaerobic zone around a microsite defined by macropore O_2 concentration, the gaseous diffusion coefficient and O_2 consumption in the microsite. In our recirculating system soil macropores were brought rapidly to 100 kPa O_2, thereby steepening the O_2 gradient around soil microsites and presumably reducing zones of anaerobiosis. The rapid reduction of the zone of anaerobiosis should be especially marked in poorly structured soils such as the loamy sand soils used in this study. We cannot conclude that we have totally inhibited denitrifying sources of N_2O in our soils because we could not directly measure denitrification in our aerobic acetylene-free atmospheres. Nevertheless, our conclusions do not depend on complete inhibition of denitrification and it seems likely that denitrification was severely inhibited by our 100% (v/v) O_2 atmospheres.

Enhanced soil aeration should also affect N_2O production by nitrifiers (Goreau *et al.*, 1980), especially where nitrifier-derived N_2O is the result of denitrification (Poth and Focht, 1985). In our experiment N_2O from nitrifier denitrification is classified as both nitrification and denitrification because this source should be inhibited by both 100 kPa O_2 and 10 Pa C_2H_2 (see Table 1).

N_2O partitioning

In three of four soil cores from the Rose Lake site, 10 Pa C_2H_2 inhibited N_2O production 39–64% (Table 3). This implies that except in core RL4, in which acetylene did not significantly affect N_2O production, nitrifiers were directly responsible for a major portion of the N_2O flux from soils of this site. In core RL3, for example, acetylene reduced N_2O production from 8.5 to 3.1 ng N g^{-1} h^{-1}.

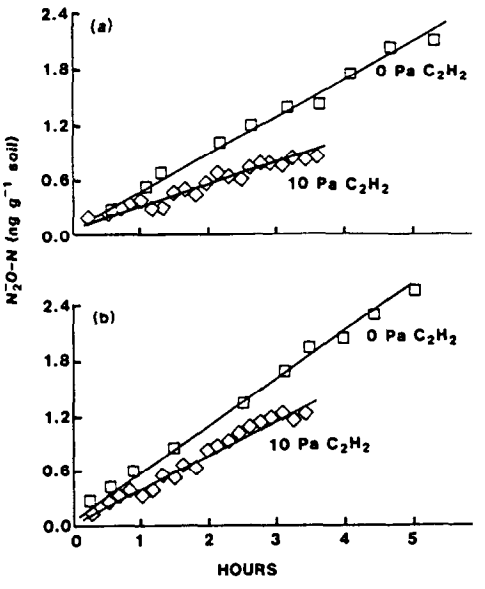

Fig. 4. Effects of 10 Pa C_2H_2 added to a 100 kPa (100% v/v) O_2 atmosphere on N_2O production in two Rose Lake soil cores.

Denitrification also appears to be a component of the Rose Lake site's N_2O flux. A 100% oxygen atmosphere in these cores inhibited N_2O production (Table 3) by 16% in core RL4 to 46% in core RL2. Some of this lost N_2O may have been from denitrifying nitrifiers, but probably only a relatively small proportion: in companion cores amended with 100 kPa O_2 to inhibit denitrification, N_2O production was further inhibited by 10 Pa C_2H_2 (Fig. 4). If denitrifying nitrifiers were important in these cores acetylene would have had little effect since denitrification was already inhibited by the O_2. Instead, acetylene depressed the N_2O flux under both air and oxygen to the same degree (Fig. 4, Table 3), suggesting that under both conditions denitrifying nitrifiers were unimportant relative to non-denitrifying nitrifiers.

In Warren Woods soils, 10 Pa acetylene in air also inhibited N_2O production, but to a lesser degree than in Rose Lake soils. N_2O fluxes from Warren Woods cores were about an order of magnitude lower than those from Rose Lake cores, and nitrification appeared to account for negligible portions of the flux in two cores (WW3 and WW4; Table 4) and only 11–23% in the remaining two described here. This implies that non-nitrifier denitrification and other sources of N_2O must account for most of the N_2O flux from these soils.

Surprisingly, however, results from the O_2-inhibition experiments suggest that denitrifiers are a net

Table 3. Effect of 10 Pa C_2H_2 and 100% O_2 on rates of N_2O produced by intact soil cores from Rose Lake Research Area. Values in parentheses represent standard errors of the slopes; n = 10–12 sample points per 2 h incubation period

Treatment	Core RL1	RL2	RL3	RL4
	(ng N_2O-N g^{-1} h^{-1})			
Air	8.06 (0.57)	2.77 (0.11)	8.45 (0.27)	4.12 (0.15)
Air + 10 Pa C_2H_2	4.91 (0.15)	1.51 (0.05)	3.07 (0.55)	4.00 (0.12)
100 kPa O_2	6.32 (1.48)	1.55 (0.03)	4.78 (0.29)	3.47 (0.04)

Table 4. Effect of C_2H_2 and O_2 on N_2O flux in intact cores from Warren Woods. Values in parentheses represent standard errors of the slopes; n = 10–12 sample points per 2 h incubation period

Treatment	Core WW1	WW2	WW3	WW4
	($ng\ N_2O\text{-}N\ g^{-1}\ h^{-1}$)			
Air	0.216 (0.012)	0.109 (0.011)	0.330 (0.013)	0.935 (0.063)
Air + 10 Pa C_2H_2	0.167 (0.002)	0.097 (0.002)	0.324 (0.004)	1.022 (0.017)
100 kPa O_2	0.871 (0.008)	0.447 (0.016)	0.880 (0.023)	2.574 (0.057)

Table 5. N_2O production (±SE) in air, 100 kPa O_2, and 10 Pa C_2H_2 atmospheres after autoclaving soils. Values below are means and standard errors

Site	Air	100 kPa O_2	10 Pa C_2H_2
		($ng\ N_2O\text{-}N\ g^{-1}h^{-1}$)	
Rose Lake	0.21 (0.01)	0.19 (0.01)	0.17 (0.02)
Warren Woods	0.012 (0.001)	0.015 (0.002)	0.019 (0.002)

sink rather than a net source of N_2O in these soils. In all four of our Warren Woods soils a 100% O_2 atmosphere resulted in substantially enhanced rates of N_2O production (Table 4). This increase was not due to chemical reactions at high pO_2 (Table 5), but rather appears to represent the net consumption of N_2O by denitrifiers under a normal soil atmosphere. Under the O_2 atmosphere denitrifiers were inhibited so that N_2O produced by other sources accumulated rather than was further reduced to N_2 by denitrifiers. We thus surmise that total N_2O production in these soils is actually 3–4 times greater than that observed under an ambient air atmosphere, and that consumption by denitrifiers accounts for the difference between total N_2O produced and total N_2O emitted.

Chemical production of N_2O as judged by autoclaved cores is present but it is less than 10% of the total N_2O flux (Table 5).

Figure 5 summarizes our understanding of N_2O production in these soils. Nitrification appears to account for 3–40% of the net N_2O flux from Rose Lake soils and for 0–23% of the net N_2O flux from Warren Woods soils. Some of this N_2O may be from denitrification by nitrifiers. Denitrification from all sources may account for as much as 46% of the net N_2O flux in Rose Lake soils. Denitrification in Warren Woods soils appears to be a sink rather than a source of N_2O.

In soils from both sites, organisms other than nitrifiers or denitrifiers appear to be major contributors to the net N_2O flux. Such sources appear to account for up to 80% of the N_2O flux from our Rose Lake cores and for 77–100% of the N_2O emitted by Warren Woods cores. We have not attempted to identify these sources more precisely. Bollag and Tung (1972), Bleakley and Tiedje (1982) and Burth

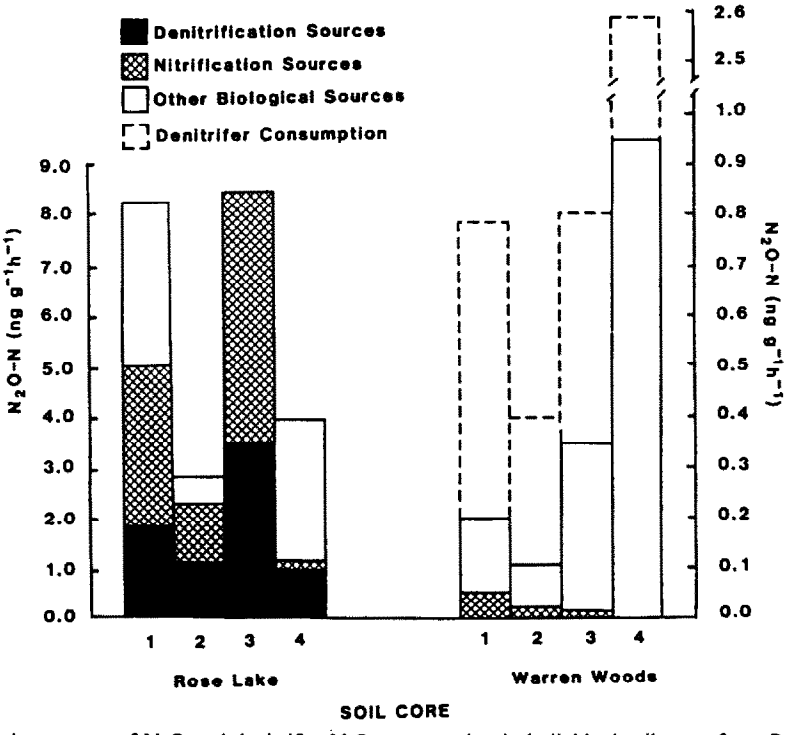

Fig. 5. Major sources of N_2O and denitrifier N_2O consumption in individual soil cores from Rose Lake and Warren Woods study sites. Note that y-axis scales differ by site.

and Ottow (1983) reported N_2O production by fungi in pure culture. That both our Rose Lake Site and especially our Warren Woods site have low pH soils suggests that fungi may be important sources.

Overall, our results suggest that N_2O production in noncultivated soils is from a suite of organisms that encompass a greater diversity than simply nitrifiers and denitrifiers. A basic understanding of the factors underlying the global N_2O balance requires understanding this diversity and identifying the factors that regulate process-level interactions *in situ*.

Acknowledgements—We thank U. Rönner and P. Groffman for helpful discussions during the preparation of this paper and for comments on an early draft. J. Golphin provided very capable laboratory assistance. A. Gillespie drafted the figures. This work was performed under a grant from the U.S. National Science Foundation's Division of Biotic Systems and Resources to Michigan State University. Contribution No. 12230 of the Michigan State Agriculture Experiment Station.

REFERENCES

Allison F. E., Cartner J. N. and Sterling L. D. (1960) The effect of partial pressures of oxygen on denitrification. *Soil Science Society of America Proceedings* **24**, 283–285.

Aulakh M. S., Rennie D. A. and Paul E. A. (1984) Gaseous N losses from soils under zero-till as compared with conventional-till management systems. *Journal of Environmental Quality* **13**, 130–136.

Banin A., Lawless J. C. and Whitten R. C. (1984) Global N_2O cycles—terrestrial emissions, atmospheric accumulation and biospheric effects. In *Advances in Space Research, Proceedings of COSPAR XXV*, pp. 141–153. Pergamon Press, New York.

Belser L. W. and Mays E. L. (1980) The specific inhibition of nitrite oxidation by chlorate and its use in assessing nitrification in soils and sediments. *Applied and Environmental Microbiology* **39**, 505–510.

Berg P., Klemedtsson L. and Rosswall T. (1982) Inhibitory effect of low partial pressures of acetylene on nitrification. *Soil Biology & Biochemistry* **14**, 301–303.

Blackmer A. M. and J. M Bremner (1978) Inhibitory effect of nitrate on reduction of nitrous oxide to dinitrogen gas by soil microorganisms. *Soil Biology & Biochemistry* **10**, 187–191.

Blackmer A. M. and J. M. Bremner (1979) Stimulatory effect of nitrate on reduction of nitrous oxide to dinitrogen by soil microorganisms. *Soil Biology & Biochemistry* **11**, 313–315.

Blackmer A. M., Bremner J. M. and Schmidt E. L. (1980) Production of N_2O by ammonia-oxidizing chemoautotrophic microorganisms in soil. *Applied and Environmental Microbiology* **40**, 1060–1066.

Bleakley B. H. and Tiedje J. M. (1982) Nitrous oxide production by organisms other than nitrifiers or denitrifiers. *Applied and Environmental Microbiology* **44**, 1342–1348.

Bollag J. M. and Tung G. (1972) Nitrous oxide release by soil fungi. *Soil Biology & Biochemistry* **4**, 271–276.

Bremner J. M. and Blackmer A. M. (1979) Effects of acetylene and soil water content on emission of nitrous oxide from soils. *Nature* **280**, 380–381.

Burth I. and Ottow J. C. G. (1983) Influence of pH on the production of N_2O and N_2 by different denitrifying bacteria and *Fusarium solani*. In *Environmental Biogeochemistry* (R. Hallberg. Ed.) *Ecological Bulletins (Stockholm)* **35**, 207–215.

Crutzen P. J. (1983) Atmospheric interactions—homogeneous gas reactions of C, N, and S containing compounds. In *The Major Biogeochemical Cycles and their Interactions*, (B. Bolin and R. B. Cook, Eds), pp. 67–112. Wiley, New York.

Firestone M. K., Firestone R. B. and Tiedje J. M. (1979) The influence of nitrate, nitrite, and oxygen on the composition of the gaseous products of denitrification in soil. *Soil Science Society of America Journal* **43**, 1140–1144.

Firestone M. K., Firestone R. B. and Tiedje J. M. (1980) Nitrous oxide from soil denitrification: factors controlling its biological production. *Science* **208**, 749–751.

Folorunso O. A. and Rolston D. E. (1984) Spatial variability of field-measured denitrification gas fluxes, *Soil Science Society of America Journal* **48**, 1214–1219.

Freney J. R., Denmead O. T. and Simpson J. R. (1979) Nitrous oxide emission from soils at low moisture contents. *Soil Biology & Biochemistry* **11**, 167–173.

Goodroad L. L. and Keeney D. R. (1984) Nitrous oxide emission from forest, marsh and prairie ecosystems. *Journal of Environmental Quality* **13**, 448–452.

Goreau T. J., Kaplan W. A., Wofsy S. C., McElroy M. B., Valois F. W. and Watson S. W. (1980) Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. *Applied and Environmental Microbiology* **40**, 526–532.

Knowles R. (1982) Denitrification. *Microbiological Reviews* **46**, 43–70.

Mosier A. R., Stillwell M., Parton N. J. and Woodmansee R. G. (1981) Nitrous oxide emissions from a native shortgrass prairie. *Soil Science Society of America Journal* **45**, 617–619.

Parkin T. B. and Tiedje J. M. (1984) Application of a soil core method to investigate the effect of oxygen concentration on denitrification. *Soil Biology & Biochemistry* **16**, 331–334.

Parkin T. B., Kaspar H. F., Sexstone A. J. and Tiedje J. M. (1984) A gas-flow soil core method to measure field denitrification rates. *Soil Biology & Biochemistry* **16**, 323–330.

Poth M. and Focht D. D. (1985) ^{15}N kinetic analysis of N_2O production by *Nitrosomonas europaea*: an examination of nitrifier denitrification. *Applied and Environmental Microbiology* **49**, 1134–1141.

Rasmussen R. A. and Kahlil M. A. K. (1986) Atmospheric trace gases: trends and distributions over the last decade. *Science* **232**, 1623–1624.

Robertson G. P. and Tiedje J. M. (1984) Denitrification and nitrous oxide production in successional and old-growth Michigan forests. *Soil Science Society of America Journal* **48**, 383–389.

Satoh T. H., Hom S. S. M. and Shanmugam K. T. (1981) Production of nitrous oxide as a product of nitrite metabolism by enteric bacteria. In *Genetic Engineering of Symbiotic Nitrogen Fixation and Conservation of Fixed Nitrogen* (J. M. Lyons, R. C. Valentine, D. A. Phillips, D. W. Rains and R. C. Huffaker, Eds), pp. 481–497. Plenum Press, New York.

Sexstone A. J., Revsbech N. P., Parkin T. B. and Tiedje J. M. (1985) Direct measurement of oxygen profiles and denitrification rates in soil aggregates. *Soil Science Society of America Journal* **49**, 645–650.

Siever R. E., Phillips M. P., Barkley R. M., Wizner M. A., Bollinger M. J. Hutte R. S. and Fehsenfeld F. C. (1979) Selective electron-capture sensitization. *Journal of Chromatography* **186**, 3–14.

Smith M. S. (1982) Dissimilatory reduction of nitrite to ammonium and nitrous oxide by a soil *Citrobacter* sp. *Applied and Environmental Microbiology* **43**, 865–871.

Smith M. S. and Zimmerman K. (1981) Nitrous oxide production by nondenitrifying soil nitrate reducers. *Soil Science Society of America Journal* **45**, 865–871.

Söderlund R. and B. H. Svensson (1976) The global nitrogen cycle. In *Nitrogen, Phosphorus and Sulfur—Global Cycles. Ecological Bulletin (Stockholm)* **22**, 23–73.

Technicon Instruments (1973) Nitrite and nitrate in water and wastewater. Industrial Method No. 100-70W, Technicon Instruments, Tarrytown, New York.

Terry R. E. and Tate R. L. (1980) The effect of nitrate on nitrous oxide reduction in organic soils and sediments. *Soil Science Society of America Journal* **44**, 744–746.

Weathers P. J. (1984) N_2O evaluation by green algae. *Applied and Environmental Microbiology* **48**, 1251–1253.

Weiss R. J. (1981) The temporal and spatial distribution of tropospheric nitrous oxide. *Journal of Geophysical Research* **86**, 7185–7195.

Whitten R. C., Banin A. and Lawless J. G. (1984) Terrestrial N_2O cycles and atmospheric effects. In *Proceedings of the AIAA 22nd Aerospace Sciences Meeting*, pp. 1–9. American Institute of Aeronautics and Astronautics, New York.