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Relative Rates of Nitric Oxide and Nitrous Oxide Production by Nitrifiers, Denitrifiers, and Nitrate Respirers

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Biogenic emissions of nitric and nitrous oxides have important impacts on the photochemistry and chemistry of the atmosphere. Although biogenic production appears to be the overwhelming source of N_2O, the magnitude of the biogenic emission of NO is very uncertain. In soils, possible sources of NO and N_2O include nitrification by autotrophic and heterotrophic nitrifiers, denitrification by nitrifiers and denitrifiers, nitrate respiration by fermenters, and chemodenitrification. The availability of oxygen determines to a large extent the relative activities of these various groups of organisms. To better understand this influence, we investigated the effect of the partial pressure of oxygen (pO_2) on the production of NO and N_2O by a wide variety of common soil nitrifying, denitrifying, and nitrate-respiring bacteria under laboratory conditions. The production of NO per cell was highest by autotrophic nitrifiers and was independent of pO_2 in the range tested (0.5 to 10%), whereas N_2O production was inversely proportional to pO_2. Nitrous oxide production was highest in the denitrifier *Pseudomonas fluorescens*, but only under anaerobic conditions. The molar ratio of NO/N_2O produced was usually greater than unity for nitrifiers and much less than unity for denitrifiers. Chemodenitrification was the major source of both the NO and N_2O produced by the nitrate respirer *Serratia marcescens*. Chemodenitrification was also a possible source of NO and N_2O in nitrifier cultures but only when high concentrations of nitrite had accumulated or were added to the medium. Although most of the denitrifiers produced NO and N_2O only under anaerobic conditions, chemostat cultures of *Alcaligenes faecalis* continued to emit these gases even when the cultures were sparged with air. Based upon these results, we predict that aerobic soils are primary sources of NO and that N_2O is produced only when there is sufficient soil moisture to provide the anaerobic microsites necessary for denitrification by either denitrifiers or nitrifiers.

Nitric and nitrous oxides are key trace gas species affecting the photochemistry and chemistry of the atmosphere (26, 27). In the troposphere, NO is an important species controlling the concentration of ozone and is converted via nitrogen dioxide to nitrous and nitric acids. Nitric acid is the fastest growing component of acid precipitation (18). Because N_2O is chemically inert in the troposphere, it readily diffuses up to the stratosphere where it initiates chemical reactions that lead to the chemical destruction of O_3. In addition, N_2O absorbs surface-emitted infrared radiation and, therefore, impacts the climate of the earth via the greenhouse effect.

Photochemical models have been developed to help predict possible anthropogenic-induced perturbations in the composition and chemistry of the atmosphere. Such models require accurate information on the global annual emissions of gases such as NO and N_2O. The biosphere is the overwhelming source of N_2O, although the exact amount of N_2O produced by the biosphere remains uncertain. Considerably less is known about the relative importance of the various sources of NO, including biogenic activity. For example, Baulch et al. (5) have recently summarized information on the global sources of NO, along with estimates of source strengths in units of 10^{12} g of N per year as follows: biomass burning, 10 to 40; industry, 8.2 to 18.5; lightning, 3 to 4; soil, 0 to 15; oxidation of N_2O in the stratosphere, 0.5 to 1.5; and jet aircraft, 0.25. Other estimates give a similar range of uncertainty (14, 30, 41). In addition, oxidation of ammonia is another source of NO ($<8 \times 10^{12}$ g of N per year) (11). The most uncertain of the source strengths listed above is that given for soil. Attempts to assess the amount of NO contributed by biogenic emissions have been limited until recently by the lack of a sufficiently sensitive and inexpensive analytical tool. The development of the chemiluminescence detector in the late 1970s (17) has permitted detection of NO in parts per billion (nanoliters per liter), and with modification, the sensitivity can be extended to parts per trillion (picoliters per liter) (12).

Autotrophic nitrifiers can produce nitric oxide either by oxidation of hydroxylamine during nitrification or by reduction of nitrite (denitrification) (22, 23, 34). Nitric oxide has been observed in pure cultures of the nitrifier *Nitrosomonas europaea* (28, 29). Denitrifying bacteria also produce NO by denitrification, although it is not yet clear whether NO is a direct intermediate or is in rapid equilibrium with a bound intermediate (2, 15, 20). Nitric oxide has been observed in cultures, resting cell suspensions, and partially purified extracts of a variety of denitrifiers (3, 4, 6, 28, 31, 40).

Formation of N_2O has been observed in cultures of both autotrophic and heterotrophic nitrifiers (9, 21, 28, 29) and in cultures of denitrifiers (6, 9, 32, 33, 40) and nitrate-respiring organisms (7, 38, 39). The production of N_2O by autotrophic nitrifiers appears to be primarily a result of nitrite reduction under anaerobic conditions (23, 34, 36). In denitrifiers N_2O has been identified as a free obligatory intermediate of denitrification (16, 25, 33). Another possible source of NO and N_2O is chemodenitrification, the nonenzymatic decomposition of nitrite. Chalk and Smith (10) have suggested that chemodenitrification is the major source of the NO emitted from soils.

There is currently available a much larger data base on N_2O emissions from both bacterial cultures and soils (13, 24)

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than there is for NO. We know of only a few reports (6, 16, 28, 29, 35, 40) which describe the simultaneous measurement of NO and N_2O in bacterial cultures. Such information might allow use of the N_2O data base in predicting NO emissions to the atmosphere. Before attempting field measurements of biogenic NO and N_2O emissions from soils, a laboratory investigation was performed to determine how the partial pressure of oxygen (pO_2) affects emissions of NO and N_2O from nitrifying, denitrifying, and nitrate-respiring bacteria. The organisms which were chosen for the study are commonly found in soils (19).

MATERIALS AND METHODS

Cultures. *N. europaea* ATCC 19718, *Alcaligenes faecalis* ATCC 8750, *Serratia marcescens* ATCC 13880, and *Citrobacter freundii* ATCC 8090 were purchased from the American Type Culture Collection, Rockville, Md. *Nitrosomonas* strain ATCC 25981 (from a marine source) was provided by F. Valois, Woods Hole Oceanographic Institution, Woods Hole, Mass. *Pseudomonas fluorescens* was provided by M. Rhodes, Virginia Institute of Marine Sciences, Gloucester Point, Va. *Rhizobium japonicum* 143 was provided by J. Neal, Virginia Polytechnic Institute and State University, Blacksburg, Va.

Media. *N. europaea* was grown in a medium containing 3.0 g of $(NH_4)_2SO_4$, 0.5 g of K_2HPO_4, 0.05 g of $MgSO_4$, 0.004 g of $CaCl_2$, 0.1 mg of chelated iron (Sequestrene 138Fe; CIBA-GEIGY Corp., Greensboro, N.C.), and 0.05 mg of cresol red in 1 liter of distilled water. The medium was adjusted to pH 8.2.

Nitrosomonas strain ATCC 25981 was grown in a seawater medium containing 1.32 g of $(NH_4)_2SO_4$, 200 mg of $MgSO_4 \cdot H_2O$, 20 mg of $CaCl_2 \cdot 2H_2O$, 1 mg of chelated iron (Sequestrene 138Fe), 100 μg of $Na_2MoO_4 \cdot 2H_2O$, 200 μg of $MnCl_2 \cdot 4H_2O$, 2 μg of $CoCl_2 \cdot 6H_2O$, 100 μg of $ZnSO_4 \cdot 7H_2O$, 8.7 mg of K_2HPO_4, 2.38 g of HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (Sigma Chemical Co., St. Louis, Mo.), and 0.05 mg of cresol red in 1 liter of diluted aged seawater (diluted twofold with distilled water). The medium was adjusted to pH 7.5.

A. faecalis, *P. fluorescens*, *S. marcescens*, and *C. freundii* were grown in the same denitrification medium but with different energy sources. The medium was a modification of that described by Smith (38). $Ca(NO_3)_2$ was omitted; 0.2 g of $MgSO_4 \cdot 7H_2O$ and 0.1 g of KNO_3 were added per liter of medium. The pH was adjusted to 7.5. Sodium citrate at 11.8 g/liter served as the energy source for *A. faecalis* and *C. freundii*, and glucose at 2.0 g/liter was the energy source for *P. fluorescens* and *S. marcescens*. The KNO_3 concentrations were varied in the experiments with *S. marcescens*, as described below.

R. japonicum was grown in a *Rhizobium* medium containing 10 g of mannitol, 0.4 g of yeast extract, 1 g of KNO_3, 0.1 g of NaCl, 0.5 g of K_2HPO_4, and 0.2 g of $MgSO_4 \cdot 7H_2O$ per liter. The pH was adjusted to 6.8.

Batch cultures. Pure cultures of bacteria were grown in a batch culture apparatus (Fig. 1). The medium was continuously sparged with gas containing a pO_2 varying from 0 to 20%, with the balance of the gas made up of nitrogen. Gases were either purchased premixed from Scott Environmental Technology, Inc., Plumsteadville, Pa., or were mixed in our laboratory. The concentration of oxygen in the final gas mixture was determined by injecting samples into a Carle 8500 gas chromatograph (Fig. 1) equipped with a thermal conductivity detector and molecular sieve column held at ambient temperature. The flow rate through the batch culture device was maintained at 150 ml/min by using a flow controller and was monitored with a mass flowmeter. The flushing time was 3.7 min. The gas supplied to nitrifiers also contained 300 ppm (microliters per liter) of carbon dioxide. Organisms were grown to the early stationary phase and diluted 5- to 100-fold into fresh, pregassed medium at the start of each experiment. All cultures were grown at 28 to 30°C unless otherwise specified.

Chemostat cultures. *A. faecalis* was maintained in a chemostat at a constant growth rate to study the effect of dissolved oxygen (DO) partial pressure on denitrification. DO partial pressure was determined with a Dissolved Oxygen Analyzer (DO-50; New Brunswick Scientific Co., Inc., Edison, N.J.) equipped with a Series 900 DO electrode. The denitrification medium was the same as that described above, except that sodium citrate was supplied at a concentration of 2.94 mg/liter to limit growth. An early-stationary-phase culture of *A. faecalis* (50 ml) was injected with a syringe into 350 ml of pregassed medium in the chemostat vessel. Gas supply and sampling ports on the chemostat vessel were similar to those shown in Fig. 1 for the batch culture apparatus. The temperature was maintained at 30°C in a water bath. The medium was supplied by a peristaltic pump at a flow rate of 46 ml/h.

Bacterial enumeration. Nitrifiers were counted directly either in a Petroff-Hausser counter or on filters with an epifluorescence microscope after staining with proflavine (1). *A. faecalis*, *P. fluorescens*, *C. freundii*, and *S. marcescens* were enumerated by serial dilution and plating on trypticase soy agar. *R. japonicum* was enumerated on *Rhizobium* medium with added agar.

Cell transfer studies. For cell transfer studies, exponentially growing cultures of *N. europaea* were collected on cellulose filters (Type GS, 0.22 μm; Millipore Corp., Bedford, Mass.), washed with 3 volumes of medium 221 without ammonium, and resuspended in fresh medium with ammonium.

Biological inactivation studies. To stop biological activity in growing bacterial cultures, saturated mercuric chloride was added at a final concentration of 0.6% (vol/vol).

Analytical methods. Nitric oxide was analyzed by pumping effluent gas through a mass flowmeter into a chemiluminescence detector (CSI-1600; Columbia Scientific Industries Corp., Austin, Tex.) with a detection limit of 2 ppb. Nitrogen was mixed with the sample gas to provide a flow rate of 520 ml/min into the instrument. The instrument was zeroed with the same nitrogen source and calibrated with a standard containing 113 ppb of NO in nitrogen (Scott Environmental

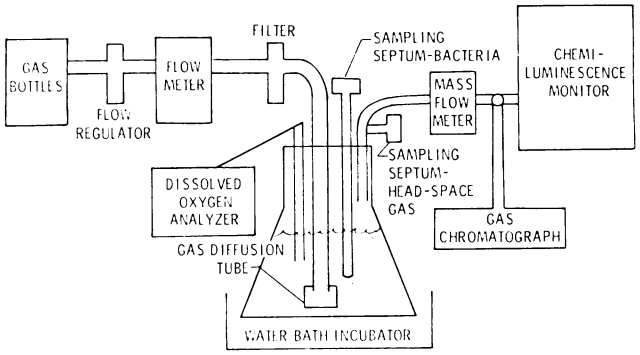

FIG. 1. Batch culture apparatus.

Technology). The NO standard was periodically checked against a National Bureau of Standards source (SRM 2628).

Samples of headspace gas for N_2O determination were taken by syringe through the sampling septum (Fig. 1). These samples were injected through a 2-ml loop into an F and M 810 gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif.) equipped with a Poropak Q column (9 ft by 1/8 in [ca. 274 by 0.3 cm]) at ambient temperature and an Ni^{63} electron capture detector held at 350°C. The precision of this instrument was 6% (standard deviation/mean) in ambient air. The carrier gas (5% CH_4–95% argon) was supplied at a flow rate of 18 ml/min. Peak area was computed by an integrator (model 3390A; Hewlett-Packard). Carbon dioxide was scrubbed from the samples by injecting them through an ascarite column.

Nitrite analysis was performed by Environmental Protection Agency method 353.2 with a Technicon Autoanalyzer II (Technicon Instruments Corp., Tarrytown, N.Y.).

Calculations. In Fig. 2 through 5, the *y* axes represent concentrations (in microliters per liter) of NO or N_2O in the effluent gas stripped from the batch cultures. The concentrations of NO were corrected for dilution by nitrogen gas. These concentrations can be converted to production rates (microliters per minute) by multiplying them by 0.15 liters/min (the flow rate of the gas used to strip the cultures). Increases in the concentrations of NO or N_2O were results of increasing cell numbers during logarithmic growth of the organisms. Production rates normalized for cell number were calculated whenever cell enumerations were performed. These data are shown in Table 1.

TABLE 1. Production rates of NO and N_2O in nitrifiers, denitrifiers, and a nitrate respirer

Organism	pO_2 (%)	Production rate (10^{12} mmol/cell per day) of[a]:		Molar ratio of NO/N_2O[a,b]
		Nitric oxide	Nitrous oxide	
Nitrifiers				
N. europaea	0.5	5.35 (2.31)	7.41 (4.47)	0.9 (0.21)
	5.0	3.92 (2.59)	0.45 (0.50)	8.5 (15.9)
	10.0	4.83 (2.31)	0.85 (0.12)	3.7 (1.21)
Nitrosomonas strain ATCC 25981	0.5	6.86 (2.49)	2.62 (0.78)	5.6 (2.28)
Denitrifiers				
P. fluorescens	0.5	0.09 (0.03)	10.09 (4.41)	0.01 (0.0)
A. faecalis	0.5	0.45 (0.07)	0.19 (0.09)	3.0 (1.36)
	1.0	0.05 (0.01)	0.07 (0.03)	0.87 (0.27)
	5.0	0.02 (0.01)	0.13 (0.01)	0.18 (0.09)
	10.0	0.02 (0.01)	0.11 (0.02)	0.24 (0.08)
	20.0	0.05 (0.01)	0.21 (0.03)	0.21 (0.05)
R. japonicum	0	0.19 (0.05)	0.82 (0.70)	0.30 (0.06)
	0.5	0.01 (0.0)	0.16 (0.07)	0.10 (0.03)
	5.0	0	0	—
Nitrate respirer				
S. marcescens	0.5	0.01 (0.0)	0.05 (0.03)	0.08 (0.04)

[a] Values are means (95% confidence limits).

[b] Molar ratios were determined from concentrations of NO and N_2O in the effluent gas. The concentrations were not normalized for cell number as production rates were.

RESULTS

Nitrifiers (*N. europaea* and *Nitrosomonas* strain ATCC 25981) produced the highest concentrations of NO (Table 1). Production of NO (millimoles per cell per day) was independent of pO_2 in the range tested (0.5 to 10%) (Table 1). Below a pO_2 of 0.5% the NO production rate declined (data not shown). The yield of NO was constant relative to the amount of nitrite produced (data not shown). The concentration of accumulated nitrite as each culture reached the stationary phase ranged from 1.7 to 2.5 mM. N_2O production by nitrifiers was inversely proportional to pO_2 (Table 1), and the yield of N_2O relative to that of nitrite declined with increasing pO_2. The molar ratio of NO/N_2O produced was in most cases greater than unity.

To test the effect of NO_2^- concentration on the production of NO and N_2O, filtered and washed *N. europaea* cultures were suspended in medium containing nitrite concentrations of 0 to 2.2 mM. NO and N_2O emissions increased with increasing nitrite concentration (Fig. 2A and B). To determine the source of the NO and N_2O produced (chemodenitrification versus biological production), cultures were poisoned with saturated $HgCl_2$ with or without added nitrite (Fig. 3A and B). $HgCl_2$ was added to an early-stationary-phase culture of *N. europaea* which contained approximately 2.2 mM NO_2^- (Fig. 3A). The rate of production of NO decreased rapidly, although some residual activity remained through the 20-h test period. The addition of $HgCl_2$ and additional 2.2 mM NO_2^- to a parallel culture (final concentration 4.4 mM NO_2^-) (Fig. 3A and B) resulted in initial increases in both NO and N_2O production. N_2O production then gradually decreased over the 20-h test period, but at a rate slower than that expected from the stripping rate (approximately 0.18 μl/liter per min). Nitric oxide production remained at a high level (Fig. 3A) throughout the test period. After 20 h, both cultures were filtered. The filtrates continued to emit NO at a constant rate over a 3.5-h period.

The three denitrifiers studied (*A. faecalis*, *P. fluorescens*, and *R. japonicum*) produced nitric oxide. Of these organisms, *A. faecalis* produced the highest concentration of NO, but only under near anaerobic or anaerobic conditions. Although NO formation was observed even in *A. faecalis* cultures sparged with air, the rate of production was inversely proportional to pO_2 (Table 1). The final concentration of accumulated nitrite in the medium as each *A. faecalis* culture reached the stationary phase ranged from 0.002 to 0.07 mM and increased with increasing pO_2. Nitric oxide emission was also observed in exponentially growing cultures of *P. fluorescens* (Fig. 4A and Table 1) and *R. japonicum* (Fig. 5 and Table 1). Emissions of both NO and N_2O from cultures of *P. fluorescens* (Fig. 4A and B) and *R. japonicum* (Fig. 5) increased dramatically when nitrogen replaced oxygen as the sparging gas. Nitrite concentrations in early-stationary-phase cultures of *P. fluorescens* and *R. japonicum* averaged 0.2 and 0.5 mM, respectively. Addition of $HgCl_2$ stopped production of both NO and N_2O, suggesting that these gases were of biological origin.

P. fluorescens produced more N_2O per cell than did any other organism studied (Table 1). The relative rates of production of N_2O in cultures of *P. fluorescens*, *N. europaea*, and *A. faecalis*, grown under anaerobic or near anaerobic conditions, were approximately 50:10:1, respectively. *A. faecalis* appeared to be capable of producing NO

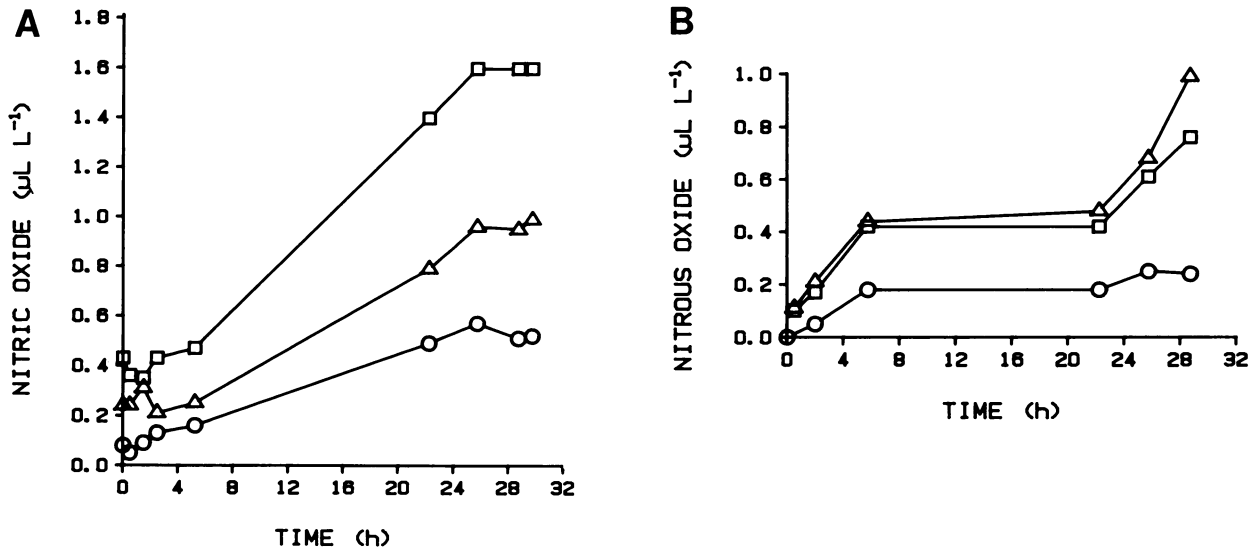

FIG. 2. Nitric oxide (A) and nitrous oxide (B) production by cultures of *N. europaea* grown in medium containing various amounts of nitrite. Exponentially growing cultures were filtered, washed, and suspended in medium containing 0, 1.1, or 2.2 mM nitrite. The cultures were sparged with 0.5% oxygen. Symbols: ○, 0 mM NO_2^-; △, 1.1 mM NO_2^-; □, 2.2 mM NO_2^-.

and N_2O under aerobic conditions, unlike the other denitrifiers studied (Tables 1 and 2). The rates of production (normalized for cell number) of these gases declined with increasing pO_2 (Table 1). To better control pO_2 and thus determine its effect on NO and N_2O production by *A. faecalis*, the organism was grown at a low cell concentration in a chemostat equipped with a DO probe. The carbon-limited culture contained a mean cell concentration of 6.6×10^7 cells per ml. The culture was sparged with gas mixtures containing various concentrations of oxygen. NO and N_2O production was observed at all pO_2s (Table 2). The high rate of NO production observed in batch cultures of *A. faecalis* sparged with 0.5% oxygen (Table 1) was not observed in the chemostat. Because of the higher cell density, conditions in the batch culture were likely to have been closer to anaerobic than conditions in the chemostat; this would explain the higher NO production rate. The DO probe used was not capable of measuring small differences in DO partial pressure at low pO_2s.

Production of NO and N_2O by the fermentative organism *S. marcescens* followed a pattern distinctly different from that observed for the denitrifiers. Nitric and nitrous oxide emissions occurred in denitrifier cultures supplied with 1 mM nitrate, whereas these gases were only observed in *S. marcescens* cultures supplied with 50 to 100 mM nitrate and only after a high concentration of nitrite (53 mM) had accumulated in the medium (Table 1). Nitric oxide emissions from the medium continued at a constant rate for up to 4 h after removal of cells by filtration. Residual N_2O production also occurred at a rate higher than that predicted based upon loss due to stripping. It therefore appears that chemodenitrification was the major source of the NO and N_2O

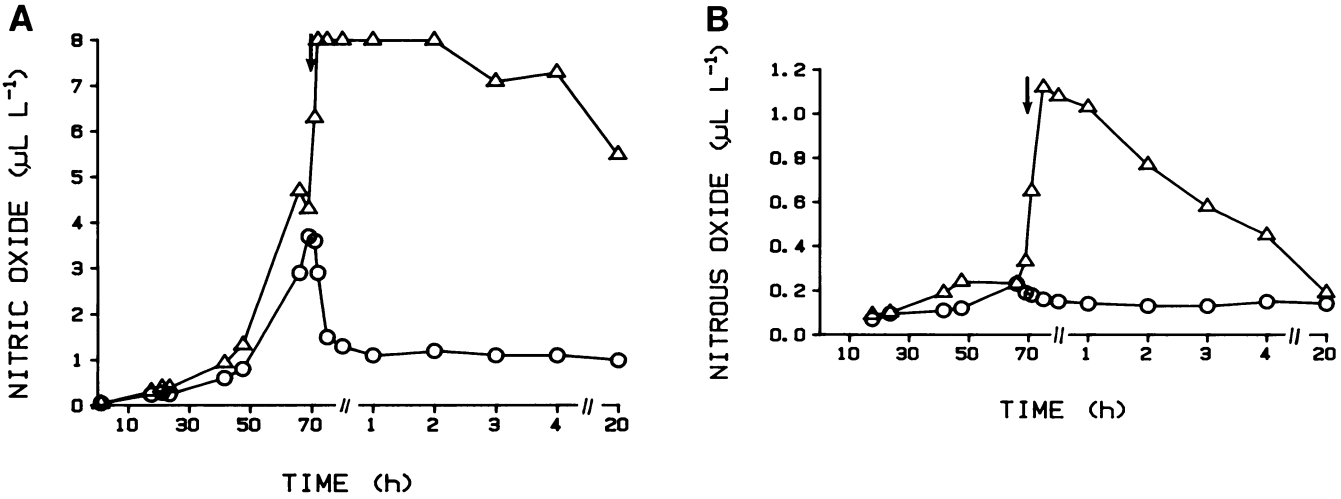

FIG. 3. Chemical versus biological production of nitric oxide (A) and nitrous oxide (B) by *N. europaea*. At the times indicated by the arrows, saturated mercuric chloride with and without 2.2 mM nitrite was added to exponentially growing cultures sparged with 0.5% oxygen. Symbols: ○, no additional nitrite (medium contained 2.2 mM NO_2^-); △, 2.2 mM NO_2^- added (total NO_2^- concentration was 4.4 mM).

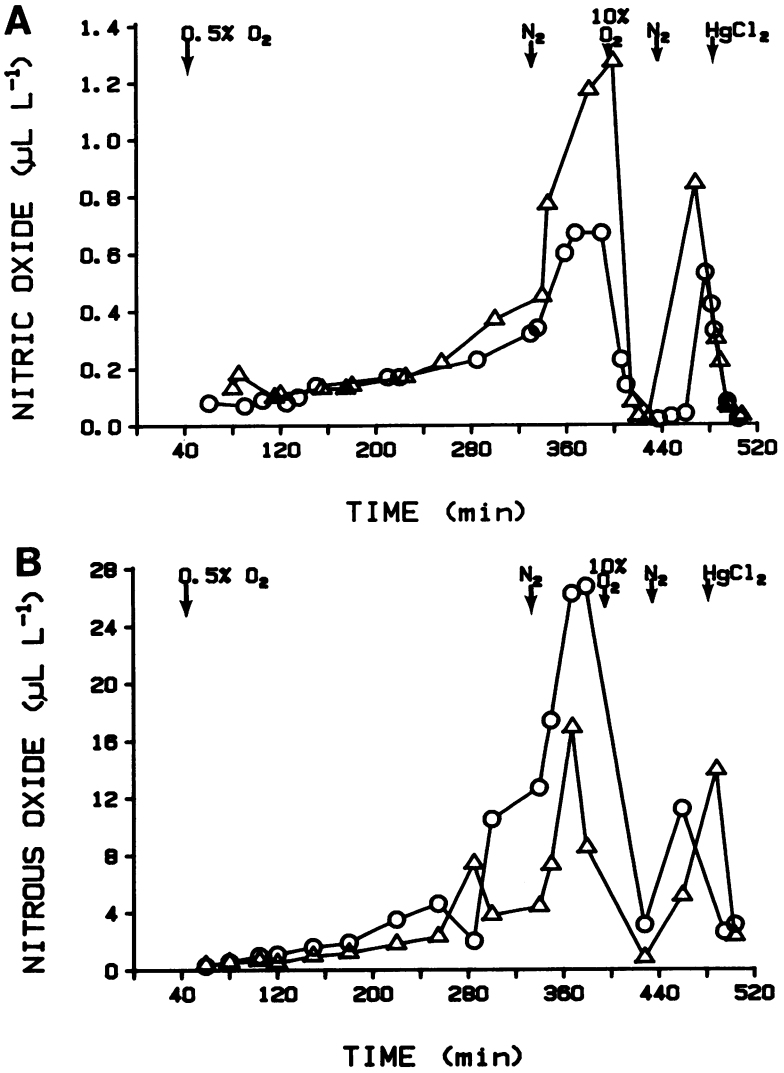

FIG. 4. Production of nitric oxide (A) and nitrous oxide (B) by *P. fluorescens* cultures. Exponentially growing cultures of *P. fluorescens* were diluted 50-fold into fresh denitrification medium at zero time. At the times indicated by the arrows, various gases were used to sparge the cultures. After 480 min of incubation saturated $HgCl_2$ was added. Symbols: ○ and △, duplicate treatments.

observed in cultures of *S. marcescens*. The only other nitrate respirer included in this study was *C. freundii*. Cultures of this organism, sparged with 0.5% oxygen, produced traces of N_2O but no measurable NO.

DISCUSSION

Although it is difficult to extrapolate from laboratory to field conditions, our laboratory results allow us to hypothesize that autotrophic nitrification is likely to be an important source of NO in soil. NO emission from soil should take place over a wide range of soil moisture conditions, provided that soil nitrifiers are not stressed by lack of water and the soil is not water saturated. Hooper and Terry (23) have proposed that, in nitrifiers, production of NO occurs primarily by the aerobic oxidation of hydroxylamine. Under anaerobic conditions, nitrite can replace oxygen as an electron acceptor. Poth and Focht (34) observed that N_2O was a product of this denitrification by nitrifying bacteria. Hooper and Terry (23) noted that both NO and N_2O were emitted as a result of nitrifier denitrification, although with NH_2OH as the electron donor, N_2O predominated. Upon addition of artificial electron acceptors to crude extracts of *N. europaea* cultures held under anaerobic conditions, Ritchie and Nicholas (36) observed that NO was the main end product of denitrification.

The results of our experiments with *N. europaea* were similar in most respects to those reported by Lipschultz et al. (29). One major difference, however, was that they observed an inverse relationship between pO_2 and production of both NO and N_2O, whereas we observed this with N_2O but not with NO. If NO is produced primarily by aerobic nitrification, we would have expected production to be independent of pO_2, provided that the oxygen concentration in our cultures was sufficient to saturate the oxidase system. Goreau et al. (21) have reported that the K_m for oxygen in *N. europaea* is approximately 0.15 mg/liter, which is approximately the concentration expected in a medium sparged with gas containing 0.5% oxygen. Nitric oxide

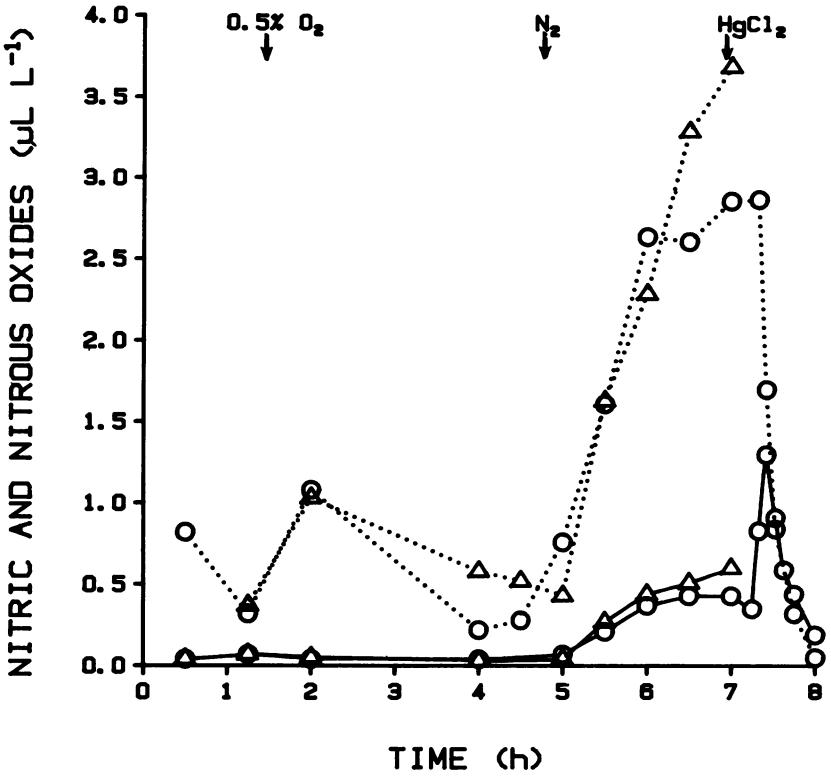

FIG. 5. Production of nitric and nitrous oxides by *R. japonicum* cultures sparged with 0.5% oxygen or with nitrogen. At zero time exponentially growing cultures were diluted 100-fold into fresh *Rhizobium* medium sparged with 0.5% oxygen. At the time indicated by the arrow, nitrogen replaced 0.5% oxygen as the sparging gas. After 7 h of incubation, saturated $HgCl_2$ was added to the cultures. -----, N_2O production; ——, NO production. Symbols: O and △, duplicate treatments.

production should, therefore, have been independent of pO_2 for most of the conditions of growth maintained in our experiments. N_2O emissions, on the other hand, depend upon competition between nitrite and oxygen for electrons removed from ammonium during nitrification. The more oxygen available, the less N_2O should be released, as was observed by Goreau et al. (21), Lipschultz et al. (29), and in our study.

In our experiments, we observed that under near anaerobic conditions, emissions of both NO and N_2O were directly proportional to the nitrite concentration in the growth medium. The predominant gaseous product observed was NO. Therefore, in cultures of *N. europaea* sparged with 0.5% oxygen, nitrite apparently behaved as an electron acceptor for reactions which produced both NO and N_2O.

TABLE 2. Effect of DO partial pressure on NO and N_2O production by *A. faecalis* in chemostat culture

pO_2 of sparging gas (%)	DO (%) in medium	Production rate (10^{12} mmol/cell per day) of[a]:		Molar ratio of NO/N_2O[a,b]
		Nitric oxide	Nitrous oxide	
0.5	0	0.01 (0.002)	0.04 (0.01)	0.20
5.0	3.6	0.03 (0.02)	0.13 (0.09)	0.17
10.0	8.8	0.03 (0.02)	0.10 (0.02)	0.17

[a] Values are means (95% confidence limits).

[b] Molar ratios were determined from concentrations of NO and N_2O in the effluent gas. Concentrations were not normalized for cell number as production rates were.

Under most conditions, addition of $HgCl_2$ to *N. europaea* cultures caused a rapid decline in emissions of both NO and N_2O, suggesting that these gases were of biological origin. However, when high concentrations of nitrite had accumulated or were added to the medium, chemodenitrification was responsible for production of much of the NO and some of the N_2O observed (Fig. 3A and B).

Our results suggest that N_2O emission from soil takes place in a more restricted range of soil moisture conditions than does emission of NO. It is difficult to predict whether nitrifiers or denitrifiers are the more important source of N_2O under these soil moisture conditions. It is clear that N_2O emissions from both nitrifiers and denitrifiers increase with decreasing availability of oxygen. The denitrifier *P. fluorescens* was the most productive organism that we tested with respect to N_2O emission. In order of importance, *P. fluorescens* and *Alcaligenes* spp. have been reported (19) to be the numerically dominant denitrifiers in world soils. We would, therefore, expect that under strictly anaerobic conditions *P. fluorescens* or similar species would be important sources of N_2O in soils. With increasing availability of oxygen, organisms such as *A. faecalis* or nitrifiers are more likely to be sources of N_2O. Based upon results of experiments using acetylene blockage techniques, Bremner and Blackmer (8) have proposed that much of the N_2O emitted from ammonium-treated soils is generated by nitrifying bacteria. They observed that N_2O emissions from soils were directly proportional to percent soil moisture; this supports the hypothesis that N_2O production by nitrifiers results from NO_2^- reduction.

Most denitrifiers emit NO and N_2O only under anaerobic

conditions. In our batch culture studies *A. faecalis* continued to emit these gases in air, although production rates declined. Robertson and Kuenen (37) have reported that cultures of *Thiosphaera pantotropha* are capable of simultaneous reduction of nitrate and oxygen with production of N_2O, a process they call aerobic denitrification; as was the case in our experiment, DO was carefully monitored. Another possible mechanism for the production of N_2O and perhaps NO by *A. faecalis* grown under aerobic conditions is heterotrophic nitrification. Castignetti and Hollocher (9) observed the formation of ammonium ions and N_2O at almost equal rates by *Alcaligenes* sp. grown with NH_2OH. The conditions used in our chemostat experiments did not allow us to distinguish between these two possible mechanisms for NO and N_2O production.

Based upon these laboratory results, we predict that NO emissions in soils result primarily from autotrophic nitrification and occur over a wide range of soil moisture conditions. We predict that N_2O on the other hand, is produced mainly by denitrification, performed either by nitrifiers or denitrifiers, and is emitted primarily under oxygen-limiting conditions. Field measurements of NO and N_2O fluxes are required to test these predictions.

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