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Temporal Change in Nitrous Oxide and Dinitrogen from Denitrification Following Onset of Anaerobiosis†

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Similar temporal patterns were found in three mineral soils for the composition of the gaseous products of denitrification following the onset of anaerobic conditions. During the early period of anaerobiosis (0 up to 1 to 3 h), N_2 was the dominant product of denitrification. The $NO_3^- \rightarrow N_2O$ activity then increased, but was not accompanied by a corresponding increase in N_2O-reducing activity. This resulted in a relatively extended period of time (1 to 3 up to 16 to 33 h) during which N_2O was a major product. Eventually (after 16 to 33 h), an increase in N_2O-reducing activity occurred without a comparable increase in the N_2O-producing activity. The increase in the rate of N_2O reduction did not occur in the presence of chloramphenicol and required the presence of N_2O or NO_3^- during the preceding anaerobic incubation. During the final period (16 to 33, up to 48 h), N_2 was generally the sole product of denitrification, since the rate of N_2O reduction exceeded the rate of N_2O production. A similar sequential pattern was also found for a culture of a denitrifying *Flavobacterium* sp. shifted to anaerobic growth. A staggered synthesis of the enzymes in the denitrification sequence apparently occurred in response to anoxia, which caused first a net production of N_2O followed by consumption of N_2O.

The suggestion that soil-evolved nitrous oxide is an important factor in destruction of the earth's ozone shield has stimulated much interest in the production of N_2O during microbial nitrogen metabolism in soil. Although soil may be an important source of N_2O (4, 6), it may also serve as a sink for atmospheric N_2O (2, 5). It is generally accepted that denitrification of nitrate or nitrite results in N_2O and N_2 as the major gaseous products. This microbial process can thus produce N_2O or consume N_2O through the reduction of N_2O to N_2. The composition of the gaseous products of denitrification in soils varies tremendously with the conditions in soil, and depending on the environment in which it occurs, denitrification can serve either as a source or a sink for N_2O. Some progress has been made in understanding the environmental parameters (e.g., NO_3^-, NO_2^-, O_2) that influence the relative proportion of N_2O and N_2 produced during denitrification (3; M. K. Firestone, M. S. Smith, R. B. Firestone, and J. M. Tiedje, Soil Sci. Soc. Am. J., in press).

In this study, we investigated the changes in the relative production of N_2O and N_2 that occur after a shift to anaerobic conditions. We had previously noticed that the ratio of N_2O/N_2 increased within the first several hours after soils were exposed to anaerobiosis (13, 15). In the studies reported here, we identify the general temporal pattern of change in the ratio of N_2O/N_2 produced in response to anoxia and provide some evidence for the physiological changes causing the pattern. We used both cultures of a denitrifying bacterium and well-mixed soil slurries to observe the behavior of the indigenous denitrifying community.

The acetylene inhibition technique was used in a major portion of the investigation. In the presence of sufficient acetylene and NO_3^-, N_2O is the sole product of denitrification (1, 13, 17). The assumption that the ratio of the quantity of N_2O produced in the absence of acetylene to the quantity of N_2O produced in the presence of acetylene reflects the $N_2O/(N_2O + N_2)$ ratio that would occur in the absence of the inhibitor was confirmed by using ^{13}N methods (13).

MATERIALS AND METHODS

Bacterial culture and incubation. The denitrifying soil isolate *Flavobacterium* sp. strain 175, used in the experiments reported here, has been characterized previously (7). The cells were grown aerobically in 500 ml of 3% tryptic soy broth (Difco) in a 2-liter

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Erlenmeyer flask on a rotary shaker at 300 rpm at 28°C for 12 h. Cells were harvested by centrifugation at 2,000 × g for 15 min at 2°C and suspended in 20 mM phosphate buffer, pH 7.2. After two washings, the cells were resuspended in phosphate buffer containing the appropriate glucose, NO_3^-, and chloramphenicol amendments. The 6-ml final volume contained 10 mg of glucose and, when desired, 60 μmol of KNO_3 or 1.2 mg of chloramphenicol (Sigma Chemical Co., St. Louis, Mo.) or both; all treatments were performed in duplicate. The vials were capped with Hungate septa, sealed with aluminum crimp seals, and evacuated and filled with helium three times. To the vials containing NO_3^-, 1 ml of acetylene (99.6%, Matheson Gas, Joliet, Ill.) was added; to the other vials either no electron acceptor or 0.5 ml of N_2O was added. The vials were then incubated at room temperature on a rotary shaker at 250 rpm.

Soils used and preparation. The soils used in this study were a Brookston loam, a Miami sandy loam, and a Conover loam; their characteristics have been previously reported (13). After collection, the moist soils were passed through 5-mm sieves to remove gravel and debris and either used immediately or stored in sealed plastic bags at 2°C.

For all soil experiments (except those requiring removal of indigenous NO_3^-), 50 g (fresh weight) of soil and 50 ml of water, containing the desired quantity of KNO_3, were added to 125-ml Erlenmeyer flasks, which were sealed with rubber stoppers pierced by a glass tube capped with a serum stopper. The soil slurries were made anaerobic by evacuating and filling with helium three times. This procedure required a total of 10 min: the time at the end of the third gas replacement was recorded as the zero time for start of the anaerobic incubation. Acetylene (0.1 atm) was added to flasks to be used for assay of denitrification rate. The flasks were incubated on a rotary shaker at 250 rpm at room temperature. All treatments were performed in triplicate.

Chloramphenicol (0.25 g) was added where indicated during the incubations. We assumed that a portion of the antibiotic added would be bound to soil surfaces and, by adding an amount twice the solubility, hoped to supply enough excess chloramphenicol to maintain an effective concentration in solution.

When it was necessary to remove indigenous soil NO_3^- and NO_2^- before the beginning of an experiment, 50-g samples of soil were extracted three times with 75 ml of water. The effectiveness of this extraction was confirmed by the absence of detectable NO_3^-/NO_2^- in the supernatant of the third extraction and the observation that the extracted soild did not produce measurable N_2O when incubated anaerobically in the presence of acetylene. The procedure required approximately 1 h. The concentrations of NO_3^- are expressed as micrograms of nitrogen per gram (fresh weight) of soil.

Gas chromatographic analyses. A Perkin-Elmer model 900 gas chromatograph (Norwalk, Conn.) with a Hot Wire detector was used for gas analysis after the first 2 h of anaerobic incubation. A Porapak Q column (3 mm by 1.8 m) at ambient temperature, with a helium carrier gas flow of 15 ml/min, was used to separate N_2O, CO_2, acetylene, and air. For N_2O analysis during the first 2 h of anaerobiosis, a Perkin-Elmer model 910 gas chromatograph with dual ^{63}Ni electron capture detectors (ECD) operated at 300°C was used. The carrier gas was 5% CH_4 in argon with a flow rate of 30 ml/min, and the column was Porapak Q at 50°C. The sensitivities of the two detection systems were complementary in that the analytical range of the ECD extended from below ambient N_2O (approximately 300 nl/liter) well past the lower limit (50 μl/liter) of N_2O quantitation using the Hot Wire detector. Peak areas were determined with a computing integrator, and N_2O standard curves were prepared for every experiment.

Samples of the headspace gas (0.5 ml for soil slurries; 0.2 ml for cultures) were periodically removed with a 1-ml syringe equipped with a Mininert valve (Precision Scientific Co., Baton Rouge, La.). In all experiments, the concentration of N_2O in solution was calculated from the measured headspace concentration, and the quantities of N_2O were corrected accordingly. We had previously verified that the published values of the Bunsen absorption coefficient (0.66) approximated the N_2O solubility in the slurries used (13).

In soil slurries, the rate of denitrification was determined by measuring the rate of N_2O production in the presence of about 0.1 atm of acetylene, and the net rate of N_2O accumulation was determined in the absence of acetylene. The ratio of these two rates of N_2O production (absence of acetylene/presence of acetylene) should represent the proportion of the total gaseous products appearing as N_2O. The difference in these two rates (presence of acetylene minus absence of acetylene) was used to calculate the rate of N_2O reduction. In bacterial culture experiments, the rate of N_2O reduction was determined directly by measuring N_2O disappearance from vials to which N_2O had been added. The soil rates were determined using linear regressions of three to six data points, each point being the mean of three replicates, over a 3- to 5-h assay period.

Nitrate effect. From previous work (3; Firestone et al., in press), we knew that the concentration of NO_3^- or NO_2^- influenced the $N_2O/(N_2O + N_2)$ ratio. Hence, we maintained a relatively high and constant concentration of NO_3^- in the soils and cultures. At the beginning of each soil experiment, 100 μg of NO_3^--N per g of soil was added to the indigenous NO_3^- present. In most of the experiments (both soils and culture), additional NO_3^- was added during the experiments by injecting an NO_3^--containing solution stored under a helium atmosphere.

RESULTS

Soil experiments. The rates of net N_2O production and denitrification for a Brookston soil are shown in Table 1. Both net N_2O production and denitrification reached maximum linear rates during the 7- to 12-h period. The rate of denitrification then decreased until about 26 h, after which it remained relatively constant at about 24 to 25 nmol g^{-1} h^{-1}. After 12 h of anaerobiosis, the net rate of N_2O production continuously declined, with a negative value

TABLE 1. *Rates of net N_2O production and denitrification following the onset of anaerobic conditions in a Brookston soil*

Time period (h)	Rate (nmol of gas g of soil^{-1} h^{-1}) of:			Proportion of total product as N_2O
	Net N_2O production	Denitrification	N_2O reduction	
3–5	15.7 (0.99)[a]	34.1 (0.99)	18.4	0.46
7–12	24.4 (0.99)	51.2 (0.99)	26.8	0.48
18–23	19.7 (0.97)	41.2 (0.98)	21.5	0.48
26–29	11.5 (0.60)	24.2 (1.00)	12.7	0.48
33–37	5.0 (0.89)	25.2 (0.97)	20.2	0.20
48–53	−1.1 (0.21)	24.2 (0.98)	25.3	N_2O consumption

[a] Values in parentheses are coefficients of determination (r^2).

being determined at 48 h. The calculated rate of N_2O reduction at 48 h exceeded the rate of denitrification, indicating that N_2O could be reduced faster than it could be produced. Nitrous oxide comprised a relatively constant proportion of the total denitrification product (about 48%) during the first 29 h, after which the N_2O component sharply declined. This decrease in the proportion of total product as N_2O paralleled an increase in N_2O-reducing activity.

The decreases in rates of denitrification and N_2O reduction that occurred between 12 to 26 h probably resulted from partial depletion of the available carbon. In analogous experiments, the addition of 0.1% glucose at 24 h caused the denitrification rate to immediately increase threefold.

To test for possible inhibitory effects on denitrification rates of long-term exposure to acetylene, parallel sets of flasks incubated in identical manner received their acetylene additions at three intervals staggered over the 2-day incubation period. The validity of this approach was indicated by finding the same denitrification rate (51.2 versus 54.7 nmol g^{-1} h^{-1}) for the 8- to 12-h period from flasks continuously exposed to acetylene and from those which had received acetylene at 8 h.

A very similar temporal pattern was found with the Miami soil. Between 3 and 26 h after the onset of anaerobiosis, N_2O was the dominant product of denitrification, but by 32 h no net N_2O production occurred, and by 47 h N_2O was consumed. Again the sharp decrease in proportion of gaseous product as N_2O reflected a comparable increase in N_2O-reducing activity.

The decline in the proportion of total product as N_2O, or the decline in the net rate of N_2O production after a period of anaerobiosis, appeared to result from an increase in N_2O-reducing activity. To determine whether the increase in N_2O-reducing activity resulted from de novo enzyme synthesis, chloramphenicol (an inhibitor of protein synthesis) was added to soil slurries just before the decline in N_2O appearance (Table 2). In the absence of chloramphenicol, a temporal pattern of gas production similar to that observed in the Brookston and Miami soils was found. A sharp decrease in the proportion of total product as N_2O occurred between 10 and 22 h, reflecting an increase in N_2O-reducing activity. In the soils to which chloramphenicol was added at 22 h, N_2O continued to be the dominant product of denitrification, whereas the N_2O-reducing activity remained low. The overall rate of denitrification was not strongly affected by the presence of chloramphenicol. In both the presence and absence of the inhibitor, the lower denitrification rate probably reflected carbon depletion.

The results of a comparable experiment using chloramphenicol in a Brookston soil are shown in Table 3. There was no significant difference in the rates of denitrification found in the presence and absence of the inhibitor, but in the presence of chloramphenicol a threefold-lower rate of N_2O reduction was determined. A similar effect of chloramphenicol was also observed in the Miami soil (data not shown). In the presence

TABLE 2. *Rates of net N_2O production and denitrification in the presence and absence of chloramphenicol in a Conover soil*

Chloramphenicol	Time period (h)	Rate (nmol of gas g of soil^{-1} h^{-1}) of:			Proportion of total product as N_2O
		Net N_2O production	Denitrification	N_2O reduction	
Absent	3–4	12.2 (1.00)	13.5 (1.00)	1.3	0.91
	5–10	21.2 (0.94)	25.5 (0.99)	4.3	0.83
	22–25	4.5 (0.99)	17.4 (1.00)	12.9	0.26
	27–30	−5.1 (0.21)	7.7 (0.99)	12.8	N_2O consumption
	32–36	0.5 (0.01)	12.8 (0.78)	12.3	0.04
Present[a]	26–30	7.9 (0.99)	8.5 (0.92)	0.6	0.92
	33–36	7.7 (0.88)	9.1 (0.98)	1.4	0.84

[a] Added at 22 h.

of the inhibitor of protein syntesis, N_2O remained the dominant product of denitrification.

To determine whether the presence of NO_3^- or N_2O was required to produce the characteristic increase in N_2O-reducing activity, the Brookston soil, with indigenous NO_3^- and NO_2^- removed, was incubated with added NO_3^-, N_2O, or no added electron acceptor. In the presence of NO_3^-, the decline in the net rate of N_2O production occurred between 12 and 23 h, corresponding to an increase in N_2O-reducing activity (Table 4). A similar pattern was observed in the soils incubated with N_2O for 22 h. However, in the soils incubated anaerobically, in the absence of NO_3^- and N_2O, the net rate of N_2O production remained high (N_2O-reducing activity, low) through 42 h, and N_2O was the dominant product of denitrification from 22 to 42 h. Hence the presence of NO_3^- or N_2O during the first 22 h of incubation was required to produce the observed increase in N_2O-reducing activity.

The data in Table 4 include rates determined shortly after the onset of anaerobic conditions (0.7 to 1.7 h). The proportion of product which occurred as N_2O during this period was low (0.13), since the rate of N_2O reduction was relatively close to the rate of denitrification. The pattern occurring during this early period of anaerobiosis was investigated further by using the more sensitive ^{63}Ni electron capture detector; an example of the data is shown in the inset of Fig. 1. For the first 70 min after the imposition of anaerobic conditions, the net rate of N_2O production was very low relative to the rate of denitrification (a ratio of rates of 0.14). The rate of denitrification then began to increase (at about 60 min), as did the net rate of N_2O production (at about 80 min). However, the rate of N_2O reduction did not increase as rapidly as did the overall rate of denitrification (Table 4; Fig. 1), which resulted in a period of time between 1 to 3 and 16 to 33 h during which N_2O was a significant and often dominant product.

Culture experiments. The pattern of gas evolution after the imposition of anaerobic conditions on the *Flavobacterium* culture is shown in Fig. 2. In this experiment, the rate of denitrification was measured in the presence of acetylene, as before; however, the rate of N_2O reduction was measured directly, since the organisms had been aerobically grown in the absence of NO_3^-. The denitrification activity exhibited an

TABLE 3. *Rates of net N_2O production and denitrification in the presence and absence of chloramphenicol in a Brookston soil*

Chloramphenicol	Time period (h)	Rate (nmol of gas g of soil^{-1} h^{-1}) of: Net N_2O production	Denitrification	N_2O reduction	Proportion of total product as N_2O
Present[a]	30–34	37.1 (0.97)	49.2 (0.96)	12.1	0.75
	44–47	28.2 (0.88)	40.3 (0.81)	12.1	0.70
Absent	30–34	15.6 (0.77)	51.1 (0.99)	35.5	0.30
	44–47	7.0 (0.83)	41.8 (1.00)	34.8	0.17

[a] 0.25 g of chloramphenicol was added to each flask at 21 h after the onset of anaerobic conditions.

TABLE 4. *Rates of net N_2O production and denitrification after anaerobic incubation with NO_3^-, without NO_3^-, and with N_2O in a Brookston soil*

Status of electron acceptors	Time period (h)	Rate (nmol of gas g of soil^{-1} h^{-1}) of: Net N_2O production	Denitrification	N_2O reduction	Proportion of total product as N_2O
100 μg of NO_3^--N/g of soil	0.7–1.7	4.6 (0.99)	34.3 (0.99)	29.7	0.13
	2–4	25.1 (0.98)	71.8 (1.00)	46.7	0.35
	5–12	48.8 (0.99)	87.1 (1.00)	38.3	0.56
	23–28	4.2 (0.37)	54.6 (0.94)	50.4	0.08
	47–51	−22.1 (0.85)	32.6 (0.71)	54.7	N_2O consumption
N_2O added at zero time[a]	23–26	15.6 (0.80)	74.6 (0.96)	59.0	0.21
	47–50	−9.9 (0.96)	60.6 (0.94)	70.5	N_2O consumption
No electron acceptor added at zero time[b]	22–26	52.8 (1.00)	68.5 (0.99)	15.7	0.77
	37–42	32.9 (0.95)	37.2 (0.98)	4.3	0.88

[a] 18 μmol of N_2O was added at zero time. At 22 h, 100 μg of NO_3^--N per g of soil was added for assay.

[b] 100 μg of NO_3^--N per g of soil was added at 22 h for assay.

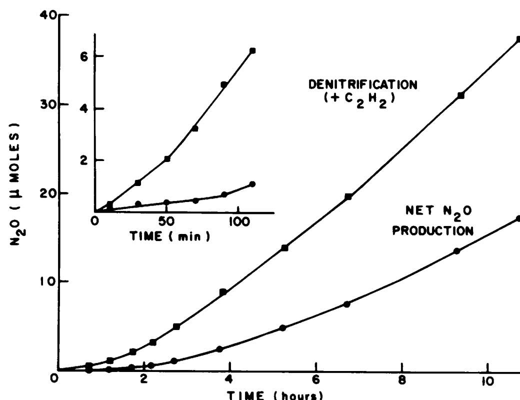

FIG. 1. *Net N_2O production (●) and denitrification (□) following the onset of anaerobic conditions in a Brookston soil. Total gas production was determined on a per-flask basis.*

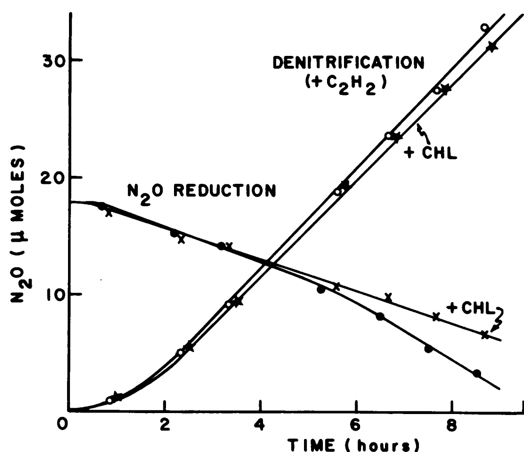

FIG. 2. *N_2O reduction and denitrification following the onset of anaerobic conditions, in the presence and absence of chloramphenicol (CHL), in a Flavobacterium sp. Nitrous oxide reduction and production were determined on a per-flask basis.*

initial lag (1 to 2 h), after which a linear rate was established. In the presence of chloramphenicol, the rate of denitrification was only slightly lower (after 5 h) than in its absence. After a very slight initial lag, the rate of N_2O reduction in the presence of chloramphenicol remained relatively constant throughout the assay. However, in the absence of chloramphenicol, the rate of N_2O reduction began to increase significantly after about 4 h. Hence, de novo synthesis of enzymes involved in N_2O reduction occurred after a period of anaerobiosis in the *Flavobacterium* sp. Resting cells were also incubated anaerobically in the absence of N_2O and NO_3^-. At 29 h, N_2O was added and the rate of its reduction was found to be 17.6 nmol of gas mg of cells^{-1} h^{-1} (29 to 31 h; data not shown). This rate was lower than the rate of N_2O reduction for this period in the presence (32.1 nmol of gas mg of cells^{-1} h^{-1}) and absence (69.4 nmol of gas mg of cells^{-1} h^{-1}) of chloramphenicol (data not shown). Hence, the presence of NO_3^- or N_2O was required to produce the increase in N_2O-reducing activity. This result is similar to that obtained in soil.

It should be noted that similar experiments were attempted with a denitrifying strain of *Pseudomonas fluorescens*. However, for 20 h after the abrupt shift from aerobic to anaerobic conditions, very little N_2O-reducing or denitrifying activity could be detected. Apparently this organism could not adapt to a rapid aerobic to anaerobic shift.

DISCUSSION

In the three soils investigated, a consistent pattern of N_2O versus N_2 production was found, corresponding to the length of time of anaerobiosis. This pattern is summarized in Table 5; the explanations are based on the soil and culture evidence discussed below. The denitrifying activity and product gases found very early (from 0 up to 1 to 3 h) after the onset of anaerobic conditions probably reflect the preexisting enzymatic capacity (14). In the study reported here and in a previously reported study using ^{13}N-labeled NO_3^- to directly quantify product gases during the first 1 to 2 h of anaerobiosis (13, 15), we found N_2 to be the dominant denitrification product during this early period. Since the early activity probably reflects the preexisting physiological state of the soil microflora, the result would be expected to vary with the aeration status of the soil examined. The soils that we studied were well-structured agricultural soils and reasonably well aerated at the time of sampling. We consistently found N_2 to be the dominant early product of denitrification in soil collected 30 min before the start of the experiment as well as in soil that had been stored at 4°C for up to 4 months.

The increase in the denitrification activity that occurred after the short early period of N_2 dominance was not immediately accompanied by a comparable increase in N_2O-reducing activity. As we have shown elsewhere (14), this early acceleration in the denitrification rate in soils was blocked by chloramphenicol, indicating that the increased N_2O production was caused by synthesis of enzymes that reduce NO_3^- to N_2O. This resulted in a relatively long period (of 16 to 32 h duration) during which N_2O production was enhanced relative to N_2 production. After 16 to 33 h of anaerobiosis, the proportion of denitrification product that appeared as N_2O sharply

TABLE 5. *Generalized temporal activity pattern after the onset of anaerobic conditions in soils*

Time period (h)	Characteristic major product	Explanation
0 to 1-3	N_2 dominant (60-80%)[a]	Reflects preexisting conditions of soil.
1-3 to 16-33	N_2O (40-90%)	$NO_3^- \rightarrow N_2O$ activity increased without comparable increase in N_2O-reducing activity
16-33 to 48	N_2 dominant (80-100%) Net N_2O consumption	N_2O-reducing activity increased without a comparable increase in $NO_3^- \rightarrow N_2O$ activity

[a] Percentages reflect the more commonly observed range of nitrogen gas composition for this time period.

decreased. This decrease did not occur in the presence of chloramphenicol, which indicates that an increase in N_2O-reducing activity resulted from de novo protein synthesis. This interpretation is supported by the culture studies which show an increased rate of N_2O reduction occurring after 5 h in the absence of chloramphenicol. The more compressed sequence of events in the pure culture compared with soil is probably due to the greater cell density and the better nutritional and physiological state of the cells in culture.

A limited amount of work has been reported on the sequence of synthesis of denitrifying enzymes in bacterial cultures after the onset of anaerobic conditions (8, 11, 12). Payne and Riley (11), working with *Pseudomonas perfectomarinus*, found that all of the enzymes involved in denitrification were present after 40 min of anaerobiosis. However, these investigators did not report the relative activities of the specific enzymes at 40 min or changes occurring with longer periods of anaerobiosis.

We found that the presence of N_2O or NO_3^- (possibly N_2O from NO_3^- reduction) was required during the anaerobic incubation of the soil and the *Flavobacterium* culture to produce the characteristic increase in N_2O-reducing activity. It is possible that N_2O or NO_3^- induced synthesis of an enzyme involved in N_2O reduction. Enhancement of N_2O-reducing activity by growth on N_2O has been shown in *Pseudomonas denitrificans* (8). However, the low N_2O-reducing activity found after a period of anaerobic incubation in the absence of NO_3^- and N_2O may also reflect the lack of energy-yielding metabolism under these conditions. Aerobically grown *Flavobacterium* sp. was capable of almost immediate reduction of N_2O when shifted to anaerobiosis. This may imply that the "aerobically" grown denitrifier had produced or experienced localized areas of oxygen depletion during growth.

It is generally accepted that carbon availability is a factor controlling denitrification in soils. Several investigators have suggested that the availability of an electron donor influences the composition of the gaseous products of denitrification as well as the quantity of denitrification (10, 16). Thus, the temporal pattern of N_2O and N_2 production reported here may be influenced somewhat by the availability of electron donor as well as by the enzymatic capacities of the soil microflora.

Nitrous oxide accumulation and subsequent reduction is a pattern commonly reported in soils and cultures (2, 3, 6, 9, 10). In most of these reports, quantities of N_2O accumulate until the ionic species are nearly depleted; the amount of N_2O then declines. The activity patterns that we describe here apparently result from staggered synthesis of denitrification enzymes in response to anoxia, not from changing NO_3^- concentration. The occurrence of similar N_2O and N_2 production patterns can be discerned in data from another report in which NO_3^- concentration did not change significantly (3).

Although total anaerobiosis rarely occurs in most natural soils, the responses reported in this work may be typical of the proportion of anaerobic microsites in soils. It is now thought that anaerobic zones grow and decline in volume in response to moisture conditions (K. A. Smith, Abstr. 11th Int. Congr. Soil Sci. **1**:304, 1978). Thus, the changes following onset of anaerobiosis, reported here, would be expected to occur in newly created or growing anaerobic zones in soil. The short-term changes in the composition of the gaseous products of denitrification, however, may reflect the sequence of events that occurs during short periods of O_2 depletion after rainfall or irrigation.

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LITERATURE CITED

1. **Balderston, W. L., B. Sherr, and W. J. Payne.** 1976. Blockage by acetylene of nitrous oxide reduction in *Pseudomonas perfectomarinus*. Appl. Environ. Microbiol. **31**:504-508.
2. **Blackmer, A. M., and J. M. Bremner.** 1976. Potential

of soil as a sink for atmospheric nitrous oxide. Geophys. Res. Lett. **3**:739-742.

3. **Blackmer, A. M., and J. M. Bremner.** 1978. Inhibitory effect of nitrate on reduction of N_2O to N_2 by soil microorganisms. Soil Biol. Biochem. **10**:187-191.
4. **Bremner, J. M.** 1978. Effects of soil on the atmospheric concentration of nitrous oxide, p. 477-491. *In* D. R. Nielsen and J. G. MacDonald (ed.), Nitrogen in the environment: nitrogen behavior in field soil. Academic Press Inc., New York.
5. **Brice, K. A., A. E. J. Eggleton, and S. H. Penkett.** 1977. An important ground surface sink of atmospheric nitrous oxide. Nature (London) **268**:127-129.
6. **Freney, J. R., O. I. Denmead, and J. R. Simpson.** 1978. Soil as a source or sink for atmospheric nitrous oxide. Nature (London) **273**:530-532.
7. **Gamble, T. N., M. R. Betlach, and J. M. Tiedje.** 1977. Numerically dominant denitrifying bacteria from world soils. Appl. Environ. Microbiol. **33**:926-939.
8. **Matsubara, T.** 1971. Studies on denitrification. XIII. Some properties of the N_2O-anaerobically grown cell. J. Biochem. **69**:991-1001.
9. **Matsubara, T., and T. Mori.** 1968. Studies on denitrification. IX. Nitrous oxide, its production and reduction to nitrogen. J. Biochem. **64**:863-871.
10. **Nommik, H.** 1956. Investigations on denitrification in soil. Acta Agric. Scand. **6**:195-228.
11. **Payne, W. J., and P. S. Riley.** 1969. Suppression by nitrate of enzymatic reduction of nitric oxide. Proc. Soc. Exp. Biol. Med. **132**:258-260.
12. **Payne, W. J., P. S. Riley, and C. D. Cox.** 1971. Separate nitrite, nitric oxide, and nitrous oxide reducing fractions from *Pseudomonas perfectomarinus*. J. Bacteriol. **106**: 356-361.
13. **Smith, M. S., M. K. Firestone, and J. M. Tiedje.** 1978. The acetylene inhibition method for short-term measurement of soil denitrification and its evaluation using nitrogen-13. Soil Sci. Soc. Am. J. **42**:611-615.
14. **Smith, M. S., and J. M. Tiedje.** 1979. Phases of denitrification following oxygen depletion in soil. Soil Biol. Biochem. **11**:261-267.
15. **Tiedje, J. M., M. K. Firestone, M. S. Smith, M. R. Betlach, and R. B. Firestone.** 1978. Short-term measurement of denitrification rates in soils using ^{13}N and acetylene inhibition methods, p. 132-137. *In* M. W. Loutit and J. A. R. Miles (ed.), Microbial ecology. Springer-Verlag, Berlin.
16. **Wijler, J., and C. C. Delwiche.** 1954. Investigations on the denitrifying process in soil. Plant Soil **5**:155-169.
17. **Yoshinari, T., R. Hynes, and R. Knowles.** 1977. Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil. Soil Biol. Biochem. **9**:177-183.