FEMS Microbiology Ecology 85 (1991) 117–124

Published by Elsevier
ADONIS 016864969100064N

FEMSEC 00312

Sources of nitrous oxide production following wetting of dry soil

Ariane O. Rudaz [1], Eric A. Davidson [2] and Mary K. Firestone [3]

[1] *Swiss Federal Research Station for Agricultural Chemistry and Hygiene of the Environment, Liebefeld-Berne, Switzerland,* [2] *NASA Ames Research Center, Moffett Field, CA, U.S.A., and* [3] *Department of Soil Science, University of California, Berkeley, CA, U.S.A.*

Received 4 May 1990
Revision received 8 October 1990
Accepted 10 October 1990

Key words: Denitrification; Nitrification; N_2O; Nitrogen cycle

1. SUMMARY

Production of N_2O was detected within 30 min of adding water to very dry soil (matric water potential < -9 MPa) sampled at the end of the dry season from an annual grassland of California, U.S.A. Using C_2H_2 to inhibit nitrification, we demonstrate that nitrification was a modest source of N_2O in sieved soil wetted to a water content below field capacity, but that denitrification was the major source of N_2O in sieved soils wetted to a water content above field capacity and in intact cores wetted either below or above field capacity. Significant abiological sources of N_2O were not detected. De novo enzyme synthesis began within 4–8 h of wetting, and denitrifying enzyme activity doubled within 26 h, indicating that denitrifying bacteria can quickly transform their metabolic state from adaptation to severe drought stress to rapid exploitation of changing resources.

2. INTRODUCTION

Pulses of N_2O flux have been observed in field studies following irrigation and precipitation events [1–5]. Persistent and rapid denitrifying activity has been observed following wetting of air-dried soils in laboratory incubations ([6]; and references therein). However, nitrification and abiological sources [7] might also contribute to observed pulses of N_2O in field studies. Indeed, observations of N_2O pulses that occur within minutes of wetting very dry soil [8] raise the question of how quickly soil microorganisms can respond to increases in soil moisture. The first objective of the present study was to characterize the time-course of N_2O production following wetting of field-dry soil. Secondly, we wished to identify the sources—denitrification, nitrification, abiological—of observed N_2O production. A third objective was to determine how quickly de novo

Correspondence to: A. Rudaz, Swiss Federal Research Station, 3097 Liebefeld-Berne, Switzerland.

synthesis of denitrifying enzymes can occur following wetting.

3. MATERIALS AND METHODS

3.1. Soils and site description

Soil samples were collected from the University of California Sierra Foothills Range Station in the central valley of California. Soils are Argonaut silt loams (Mollic Haploxeralfs) with 25% clay content. Soil pH (in H_2O) ranges from 5.5–5.9. Vegetation and site characteristics of this oak woodland/annual grassland are more completely described elsewhere [9]. Because of distinct differences in N cycling processes between soils under oak canopies and soils in open grassy areas [10], sampling was stratified, with three study plots under oak canopies (hereafter 'canopy') and three in open grassy areas (hereafter 'open'). Twelve plastic cores (4 cm diam. ×9 cm deep) were driven into the soil of each plot near the end of the rainy season in April, 1988. Almost no precipitation falls during the hot summer of this Mediterranean-type climate and the soils become very dry (3% gravimetric moisture) and hard. The soil water potential at the end of the dry season was too low to be measured in a dew point psychrometer designed for readings as low as −9 MPa. The soil cores were retrieved in early September, 1988, just before the first fall rains. At the same time, bulk soil samples of the top 9 cm were excavated with a chisel at each site. The bulk samples were ground to break up large chunks and were sieved (4 mm). Intact cores and bulk soil samples were stored in the laboratory at field-dry moisture at room temperature.

3.2. Sources of N_2O—bulk soil

Sieved soil samples from each plot were combined to form a single bulk sample for the open plots and another for the canopy plots. Subsamples of 20 g were placed in 200 ml canning jars with a septum in each lid. The following treatments were imposed: (1) 16 jars were left at field-dry moisture (3%), 16 jars received 6 ml deionized H_2O (33% moisture) and 16 jars received 9 ml deionized H_2O (48% moisture); (2) at each moisture content, 4 jars had been previously autoclaved twice at 120°C for 20 minutes (sterilized H_2O was used to wet sterilized soil), 4 jars received C_2H_2 to bring headspace concentration to 10 kPa, 4 received C_2H_2 to bring headspace concentration to 10 Pa, and 4 received no C_2H_2. Acetylene was added by syringe through the septa after all jars had been closed. Samples were incubated at room temperature (21°C). Headspace gas of each jar was sampled by syringe 6 or 7 times during the first 10 h after wetting and once again at 24 or 26 h. Gas samples were analyzed for N_2O using a gas chromatograph with an electron capture detector.

3.3. Sources of N_2O production—intact cores

The same experimental design was followed for intact cores except that only 2 treatments of C_2H_2 were used (0 and 10 kPa) and autoclaving was omitted. A uniformly low concentration of C_2H_2 could not be achieved with certainty in intact cores. Two replicate cores from each of the study plots received the same treatment combinations, thus providing 6 replicates for each treatment combination for both canopy and open sites. Either 0, 30, or 45 ml deionized water was added to the top of each core to approximate 3%, 33% and 48% soil moisture treatments, respectively; the approximate weight of each core was 100 g, but the exact amount of soil (particles < 4 mm) in each intact core varied. Following incubation, the cores were broken up, weighed, dried, and sieved to determine dry weight and moisture content. To ensure that C_2H_2 penetrated wetted cores, cores that were to receive both H_2O and C_2H_2 were wetted with acetylated H_2O, and C_2H_2 was also added to headspace by syringe.

3.4. Calculation of rates

Patterns of N_2O accumulation during the first 10 h following wetting were generally nonlinear (Fig. 1). To appropriately describe N_2O accumulation in headspace gas with time, data for the first 10 h were fitted to the non-linear model:

$$y = be^{ax} \quad (1)$$

where y is N_2O-N produced per gram dry soil and x is time in hours. Regressions using eqn. 1 were

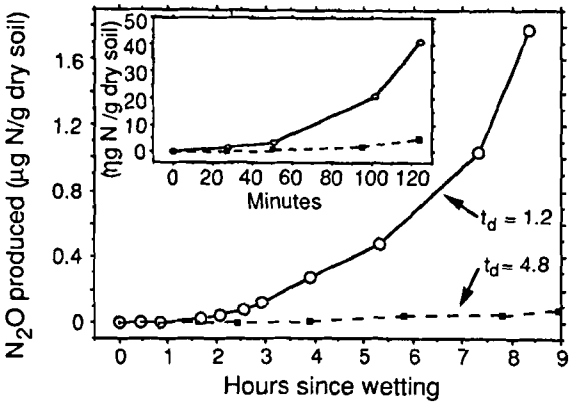

Fig. 1. Examples of time course of N_2O production for samples of bulk mixed soil from an open-grass area. The solid line is for a sample wetted to 48% moisture and treated with 10 Pa C_2H_2 (denitrification N_2O only). The broken line is for a sample wetted to 33% moisture with no C_2H_2 (primarily nitrification N_2O). Data for the first two hours after wetting are given in the inset on a nanogram scale. Doubling times (t_d) of N_2O are expressed in hours.

compared with linear regressions of the same data ($y = ax + b$) for 12 intact cores at approximately 48% moisture. The mean R^2 values were 0.941 and 0.819 for the non-linear (eqn. 1) and linear regressions, respectively. Because R^2 values are percentages of variance accounted for by the regression, they were arcsin transformed [11] and a one-tailed paired t-test of the transformed data revealed that the R^2 values from the non-linear model of eqn. 1 were significantly higher at $\alpha = 0.01$.

Treatment effects were assessed by comparing N_2O doubling times calculated from the following equation:

$$t_d = \ln 2/a \quad (2)$$

where t_d is the doubling time of N_2O in headspace gas and 'a' is calculated from eqn. 1. While doubling time terminology is commonly used in description of a binary microbial growth pattern, this approach is also appropriate to describe any exponential response function. Its use is not meant to imply that growth of microorganisms necessarily occurred.

Effects of sampling site, soil moisture, and C_2H_2, were determined by 3-way analysis of variance of t_d values (all statistical analyses were performed with Statview software for MacIntosh Computers, Abicus Concepts, Berkeley, CA). Because sterilized soil and soil left at field-dry moisture exhibited no N_2O production, these data were not included in the analysis of variance.

Sources of N_2O production at 8 h following wetting were calculated by subtraction of means of C_2H_2 treatments [12]. At 10 kPa C_2H_2, N_2 production by denitrifying bacteria is inhibited, and at 10 Pa C_2H_2 or greater, N_2O production by nitrifying bacteria is inhibited. Denitrification N_2O was estimated directly from 10 Pa C_2H_2 treatment means. Denitrification N_2 was estimated by subtracting the mean N_2O production of the 10 Pa C_2H_2 treatment from the mean N_2O production of the 10 kPa C_2H_2 treatment. Nitrification N_2O was estimated by subtracting the mean N_2O production of the 10 Pa C_2H_2 treatment from the mean N_2O production under no C_2H_2. Because sampling times did not always fall precisely on the hour for each replicate, the cumulative N_2O produced at 8 h following wetting was calculated for each replicate from the regression model of eqn. 1 and these values were used to compute treatment means at 8 h.

3.5. Denitrifying enzyme activity (DEA)

In order to determine if the microbiological capacity to denitrify was changing with time after wetting of the soil, a DEA assay was performed at time increments after wetting. Changes in DEA were measured for the composited sample of bulk soil from canopy sites after wetting to 48% moisture. Subsamples of 10 g field-dry soil were placed in each of 20 serum bottles (70 ml). The bottles were stoppered and 4.5 ml deionized H_2O was added to each. At times 0, 4, 8, and 26 h after wetting, five of the bottles were opened and the DEA assay [6] was begun. Briefly, 25 ml of a solution containing 10 mM glucose, 5 mM KNO_3, and 100 μg chloramphenicol ml^{-1} was added to each bottle; each bottle was flushed three times with N_2; C_2H_2 was added to bring the headspace to 10 kPa; bottles were incubated on an orbital shaker at room temperature; N_2O concentration was analysed after 15, 30, and 60 min; and the DEA was calculated by linear regression of N_2O concentration vs. time.

3.6. Variation in NO_3^-, carbon and N_2O production

Twenty subsamples of 10 g field-dry soil from the canopy site were extracted in 50 ml 2 M KCl and were filtered through Whatman No. 1 filters previously rinsed with KCl. Extracts were analyzed for NO_3^- colorimetrically using a Lachat flow injection autoanalyser [13]. Twenty additional subsamples of 10 g field-dry soil were placed in serum bottles, wetted to 48% moisture, and the bottles then stoppered. Concentrations of N_2O and CO_2 in headspace gas were analyzed at 0, 4, 8, and 26 h after wetting. A gas chromatograph with a thermal conductivity detector was used for CO_2 analysis.

4. RESULTS

4.1. Autoclaved soil incubations

No N_2O production was observed during incubation of autoclaved soil, indicating that abiological reactions were not responsible for N_2O production upon wetting of dry soil. However, it should be noted that some abiological processes, such as reactions of NO_2^- with phenolics [7], could also be affected by autoclaving. Hence, the importance of N_2O production by abiological processes following wetting of field-dry soil cannot be entirely discounted.

4.2. Bulk soil incubations

No production of N_2O was observed during incubations of soils maintained at field-dry condition. The rate of N_2O production increased during the first 8 h of incubation of bulk soil samples at 48% moisture (Fig. 1). This nearly exponential increase was not maintained to 24 h, presumably due to NO_3^- and/or carbon limitation. Production of N_2O was detectable within 30 min of wetting. Analysis of variance indicates that the effects of soil moisture, C_2H_2 treatment, and sampling site were significant at $\alpha = 0.05$ (Table 1). All interactions of the main effects were also significant, except the soil moisture-by-sampling site interaction was not significant. Doubling times were shorter at 48% moisture than at 33% moisture and generally were shorter in samples from open-grass areas than from under oak canopies. The 10 kPa C_2H_2 treatment had no consistent effect, but C_2H_2 at either low or high levels increased t_d values at 33% moisture. Acetylene inhibition of nitrification in the under-canopy samples at 33% moisture may have been incomplete for the 10 Pa treatment, because the 10 kPa treatment caused further inhibition (larger N_2O doubling time; Table 1). Subtraction of C_2H_2 treatment means revealed that most of the observed N_2O at 48% moisture was produced by denitrifying bacteria (Table 2).

Table 1

Doubling times (t_d, in hours) of N_2O concentrations during incubations of mixed bulk soil samples and intact cores

C_2H_2 Treatment	Sample site			
	Open grass		Under oak canopy	
	Soil moisture 33%	Soil moisture 48%	Soil moisture 33%	Soil moisture 48%
Mixed bulk soil samples				
10 kPa	26.6 (3.5) [a]	1.4 (0.1)	60.7 (8.7)	2.5 (0.3)
10 Pa	30.9 (10.4)	1.4 (0.1)	24.6 (3.6)	3.7 (0.5)
None	11.0 (2.0)	1.5 (0.1)	10.7 (0.5)	4.1 (0.6)
Intact cores:				
10 kPa	2.4 (0.4)	1.2 (0.1)	2.9 (0.7)	1.4 (0.3)
None	2.4 (0.2)	1.2 (0.1)	2.6 (0.3)	1.4 (0.1)

[a] Means and (standard errors); $n = 4$ for mixed bulk soil samples and $n = 6$ for intact cores. Least significant difference ($\alpha = 0.05$) = 13.2 for mixed bulk soil and 1.2 for intact cores.

Table 2

Identification of N_2O sources (in ng N_2O-N g^{-1} dry soil) during the first 8 h after wetting mixed soil

Origin of N_2O	Sample site			
	Open grass		Under oak canopy	
	Soil moisture 33%	Soil moisture 48%	Soil moisture 33%	Soil moisture 48%
Abiotic N_2O	0	0	0	0
Nitrification N_2O	4	n.d. [a]	2	n.d. [a]
Denitrification N_2O	2 (2) [b]	490 (316)	<1 (<1)	10 (1)
Denitrification N_2	0	31	0	23

[a] Not detectable because effect of C_2H_2 was not significant ($\alpha = 0.05$) by ANOVA.

[b] Means and (standard errors) of 10 Pa C_2H_2 treatment; variance cannot be estimated for other source estimates because they are calculated by subtraction of C_2H_2 treatment means (see text).

4.3. Intact core incubations

Production of N_2O from intact cores followed the same pattern of increasing rate as shown for bulk soil (Fig. 1), except that t_d values for cores at approximately 33% moisture were considerably shorter (Table 1). Analysis of variance revealed than only soil moisture effects were significant at $\alpha = 0.05$. Mean t_d values appear longer for soils from under canopy than from the open areas, as was observed for bulk soils, but large variation among cores within treatments and sites rendered the difference statistically non-significant.

Table 3

Variation of N_2O accumulation and of potentially limiting factors in mixed soil of the canopy site

Parameter	Hours after wetting			
	0	4	8	26
N_2O produced:				
Mean (ng N_2O-N g^{-1} soil)	ND	8	60	269
C.V. (%)	ND	97	124	71
No. of replicates	ND	19	19	19
Denitrifying enzyme activity:				
Mean (ng N_2O-N g^{-1} min^{-1})	0.27	0.27	0.45	0.56
C.V. (%)	20	23	28	14
No. of replicates	5	5	5	5
Available C (respiration):				
Mean (μg CO_2 – C g^{-1} soil)	ND	36	61	118
C.V. (%)	ND	10	16	16
No. of replicates	ND	19	19	19
NO_3^-				
Mean (μg NO_3^--N g^{-1} soil)	2.7	ND	ND	ND
C.V. (%)	10	ND	ND	ND
No. of replicates	20	ND	ND	ND

ND = no data; C.V. = coefficient of variation.

4.4. Denitrifying enzyme synthesis

A statistically significant ($\alpha = 0.01$; one-way ANOVA) increase in DEA of bulk soil from the canopy sites was observed between 4 and 8 h after wetting (Table 3). The DEA at 26 h after wetting was double the DEA of field dry soil.

5. DISCUSSION

5.1. Effects of C_2H_2 and criteria for identifying N_2O sources

Acetylene is a potent inhibitor of the ammonia monooxygenase of chemoautotrophic nitrifying bacteria [14]. Inhibition of N_2O production at 10 Pa C_2H_2 is strong evidence for autotrophic nitrification as the N_2O source. Higher concentrations of C_2H_2 inhibit N_2O reductase of denitrifying bacteria [15]. Increased N_2O production in the presence of 10 pKa C_2H_2 is evidence for N_2 production via denitrification. When no effect of C_2H_2 is observed, denitrification is the probable source of N_2O. Heterotrophic nitrification can also be a source of N_2O, and current evidence indicates that this process is not affected by C_2H_2 [16,17]. However, evidence for N_2O production via heterotrophic nitrification is limited to aerobic incubations in pure culture [17,18]. While a contribution from heterotrophic nitrification cannot be entirely

ruled out in soils incubated with C_2H_2, the significance of these sources has never been demonstrated in field studies or in studies of intact soil cores. To our knowledge, the only evidence from a soil study for a source of N_2O other than autotrophic nitrification or denitrification occurred when soil samples were incubated in an atmosphere of 100% O_2 [19]. In contrast, denitrifying activity is strongly correlated with soil moisture and degree of anerobiosis [2,4,12,20]. We interpret a lack of C_2H_2 inhibition coupled with a strong positive response to increasing soil moisture as indication of denitrification as a dominant source of N_2O.

5.2. Importance of denitrification

The 33% soil moisture treatment was chosen because this value represents approximate field capacity—i.e., a moist soil with most of the macropores air-filled. The 48% moisture content approaches saturation, when most of the pore spaces are water-filled. Smaller t_d values and lack of an inhibitor effect of C_2H_2 at 48% moisture (Table 1) indicate that denitrification was the most important source of N_2O following near saturation of dry soil from this oak woodland/annual grassland. An inhibitory effect of low C_2H_2 concentrations at 33% moisture in mixed bulk soil (Table 1) shows that the nitrifiers were also producing detectable amounts of N_2O, but the magnitude of their production is dwarfed by production by denitrifiers at 48% moisture (Table 2). These results are consistent with findings of denitrification as the dominant source of N_2O production in incubations of marine sediments [20].

Use of mixed bulk soil permitted application of a low C_2H_2 treatment, thus enabling quantification of modest N_2O production rates via nitrification in mixed soil. Incubations of intact cores provide more realistic conditions for assessing production rates. Lack of inhibitory effects of 10 kPa C_2H_2 on N_2O production in intact cores confirms that denitrification was the main source of N_2O in minimally disturbed soil. However, considerably smaller t_d values for intact cores than for bulk soil at 33% moisture (Table 1), suggest that this moisture content may be sufficient to impede O_2 diffusion and enhance denitrification in intact cores, or that the moisture was not evenly distributed throughout the core, causing 'hot spots' of high denitrifying activity.

The soil is very likely to be wetted above field capacity during major storms and to remain near field capacity for several hours following the storm. Our data for sieved soil indicate that detectable amounts of N_2O are produced by nitrifying bacteria at soil moisture near field capacity, but our data for intact cores show that denitrification is the dominant source of N_2O at both moisture contents when soil structure is minimally disturbed. The DEA data show that denitrifying bacteria can utilize existing denitrifying enzymes within minutes of wetting and begin de novo synthesis within 4–8 h. Hence, short-term fluxes of N_2O via denitrification following wetting events are very plausible. The lack of a significant stimulatory effect of 10 kPa C_2H_2 (Table 1) indicates that N_2 production was not significant at these moisture contents during this time period.

5.3. Increasing rate of N_2O production

Exponential increases of products of microbial activity (Fig. 1) are often interpreted as indications of microbial population growth, but this interpretation may not be entirely appropriate here. We have demonstrated that de novo synthesis of denitrifying enzymes did occur between 4 and 8 h after wetting, but new enzymes may not be the only factor contributing to nearly exponential accumulation of headspace N_2O. Microbial respiration consumes O_2 following wetting of the soil. Response of existing denitrifying enzymes to decreasing O_2 partial pressures may be non-linear. Hence, as O_2 is consumed, the activity of existing denitrifying enzymes may increase, causing the rate of N_2O production to increase. Furthermore, rapid production of N_2O in the liquid phase of the soil could result in transient disequilibrium between N_2O in liquid and gas phases, causing a period of time when N_2O accumulation in headspace gas was non-linear.

5.4. Sources of variation

Denitrification is renowned for its high spatial variability [21,22]. Although incubations of bulk soil in the present study used subsamples of sieved

and mixed composite samples, we observed coefficients of variation (C.V.'s) of N_2O accumulation up to 124% during incubations of soil from canopy sites at 48% moisture (Table 3). This large variation of N_2O produced is probably due to a multiplicative effect of smaller variations in the potentially limiting factors of denitrifying enzymes, available-C, and NO_3^-, which had C.V.'s ranging from 10% to 28% (Table 3). Parkin and Robinson [23] have shown that regression analysis and deterministic models relating these limiting factors to N_2O production perform poorly, but that a stochastic approach based on the variation of each limiting factor within a population of soil samples accounts for the large C.V. commonly observed for N_2O production. It is interesting to note that the largest C.V. for N_2O was observed at 8 h after wetting, when the C.V. for denitrifying enzymes also peaked (Table 3).

5.5. Ecological significance

Persistence of denitrifying enzymes in air-dried mixed soil samples and rapid initiation of denitrifying activity and microbial growth following wetting of dried soils has been previously demonstrated [6,24]. In the present study, the soils became extremely dry in the field. Tropical savannas, seasonally dry tropical forests and Mediterranean climates of temperature zones experience long hot dry seasons. Nitrate can accumulate in dry soil [10] and readily available carbon is released upon wetting dry soil [25,26]. Hence, soil moisture, NO_3^-, and available carbon may be favorable for denitrification during the early rainy season of these climates. Survival of significant populations of denitrifying bacteria and enzymes at very low matric potentials characteristic of field conditions (< -9 MPa in the present study) was uncertain. Our data show that not only do the organisms and enzymes persist in very dry soil, but that resumption of denitrifying activity occurs within minutes of alleviating the severe drought stress.

A rapid response of soil microorganisms to changing resources with the onset of a rainy season has important implications for N cycling processes. Plants that must germinate from seed or grow new vegetative tissue respond to changing soil moisture over days and weeks, not minutes and hours. In addition to N trace gas production, microbial consumption of inorganic-N following early wet season rains could affect N availability to plants and N leaching losses. The focus of the present study demonstrates the importance of this rapid microbial response to changing soil moisture on N_2O production.

ACKNOWLEDGEMENTS

Ariane Rudaz thanks the Swiss Federal Research Station and Director J. von Ah for support while working in the U.S.A. Eric Davidson was supported by the National Research Council Associateship program while preparing this manuscript. This work was supported by NSF grant BSR-88-08187 and performed under the California Agricultural Experiment Station Project 5123-H.

REFERENCES

[1] Anderson, I.C., Levine, J.S., Poth, M.A. and Riggan, P.J. (1988) Enhanced biogenic emissions of nitric oxide and nitrous oxide following surface biomass burning. J. Geophys. Res. 93, 3893–3898.

[2] Davidson, E.A. and Swank, W.T. (1986) Environmental parameters regulating gaseous-N losses from two forested ecosystems via nitrification and denitrification. Appl. Environ. Microbiol. 52, 1287–1292.

[3] Goodroad, L.L. and Keeney, D.R. (1984) Nitrous oxide emissions from forest, marsh, and prairie ecosystems. J. Environ. Qual. 13, 448–452.

[4] Ryden, J.C. (1983) Denitrification loss from a grassland soil in the field receiving different rates of nitrogen as ammonium nitrate. J. Soil. Sci. 34, 355–365.

[5] Hao, W.M., Scharffe, D., Crutzen, P.J. and Sanhueza, E. (1988) Production of N_2O, CH_4, and CO_2 from soils in the tropical savanna during the dry season. J. Atmos. Chem. 7, 93–105.

[6] Smith, M.S. and Parsons, L.L. (1985) Persistence of denitrifying enzyme activity in dried soils. Appl. Environ. Microbiol. 49, 316–320.

[7] Nelson, D.W. (1982) Gaseous losses of nitrogen other than through denitrification, in Nitrogen in Agricultural Soils (Stevenson, F.J., Ed.) pp. 327–364. American Society of Agronomy, Madison, WI., U.S.A.

[8] Vitousek, P., Matson, P., Volkman, C., Maass, J.M. and Garcia, G. (1989) Nitrous oxide flux from dry tropical forest. Global Biogeochem. Cycles 3, 375–382.

[9] Jackson, L.E., Strauss, R.B., Firestone, M.K. and Bartolome, J. (1988) Plant and soil nitrogen dynamics in a California annual grassland. Plant Soil 110, 9–17.
[10] Davidson, E.A., Stark, J.M. and Firestone, M.K. (1990) Microbial production and consumption of nitrate in an annual grassland. Ecology 71, 1968–1975.
[11] Steel, R.G.D. and Torrie, J.H. (1980) Principles and Procedures of Statistics. McGraw-Hill, New York.
[12] Davidson, E.A., Swank, W.T. and Perry, T.O. (1986) Distinguishing between nitrification and denitrification as sources of gaseous nitrogen production in soil. Appl. Environ. Microbiol. 52, 1287–1292.
[13] QuikChem Systems (1986) QuikChem method no. 12-107-06-1-A. QuickChem Systems, Division of Lachat Chemicals, Inc. Mequon, Wiconsin, U.S.A.
[14] Wood, P.M. (1986) Nitrification as a bacterial energy source, in Nitrification (Prosser, J.I., Ed.) pp. 39–62. IRL Press, Oxford.
[15] Yoshinari, T. and Knowles, R. (1976) Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochem. Biophys. Res. Commun. 69, 705–710.
[16] Killham, K. (1986) Heterotrophic nitrification, in Nitrification (Prosser, J.I., Ed.) pp. 117–126. IRL Press, Oxford.
[17] Hynes, R.K. and Knowles, R. (1982) Effect of acetylene on autotrophic and heterotrophic nitrification. Can. J. Microbiol. 28, 334–340.
[18] Papen, H.R., von Berg, R., Hinkel, I., Thoene, B. and Rennenberg, H. (1989) Heterotrophic nitrification by Alcaligenes faecalis: NO_2^-, NO_3^-, N_2O, and NO production in exponentially growing cultures. Appl. Environ. Microbiol. 55, 2068–2072.
[19] Robertson, G.P. and Tiedje, J.M. (1987) Nitrous oxide sources in aerobic soils: nitrification, denitrification and other biological processes. Soil. Biol. Biochem. 19, 187–193.
[20] Jorgensen, K.S., Jensen, H.B. and Sorensen, J. (1984) Nitrous oxide production from nitrification and denitrification in marine sediment at low oxygen concentrations. Can. J. Microbiol. 30, 1073–1078.
[21] Folorunso, O.A. and Rolston, D E. (1984) Spatial variability of field-measured denitrification gas flux. Soil Sci. Soc. Am. J. 48, 1214–1219.
[22] Parkin, T.B. (1987) Soil microsites as a source of denitrification variability. Soil Sci. Soc. Am. J. 51, 1194–1199.
[23] Parkin, T.B. and Robinson, J.A. (1989) Stochastic models of soil denitrification. Appl. Environ. Microbiol. 55, 72–77.
[24] Martin, K., Parsons, L.L., Murray, R.E. and Smith, M.S. (1988) Dynamics of soil denitrifier populations: Relationships between enzyme activity, most probably number counts, and actual N gas loss. Appl. Environ. Microbiol. 54, 2711–2716.
[25] Birch, H.F. (1954) The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil 10, 9–31.
[26] Davidson, E.A., Galloway, L.F. and Strand, M.K. (1987) Assessing available carbon: comparison of techniques across selected forest soils. Commun. Soil Sci. Plant Anal. 18, 45–64.