

Atmospheric Pollutants and Trace Gases

Influence of Soil Moisture and Land Use History on Denitrification End-Products

Timothy T. Bergsma,* G. Philip Robertson, and Nathaniel E. Ostrom

ABSTRACT

We investigated the effects of recent moisture history on the relative production of N_2O and N_2 during denitrification in soil from cropped and successional ecosystems. The soils were pedogenically identical but had been managed differently for the past decade. Sieved soils were amended with nitrate, glucose, and water. *Long-wet* and *short-wet* incubations received 80 and 0%, respectively, of prescribed water 2 d before incubation and the rest just before incubation. The N_2O and N_2 production and N_2O mole fraction ($N_2O/[N_2O + N_2]$) were measured using acetylene inhibition. The N_2 production and soil ^{15}N enrichment were measured by ^{15}N -gas evolution. The response of N_2O mole fraction to moisture history differed by ecosystem. Mean N_2O mole fraction in the successional system was about the same for long-wet and short-wet treatments (0.34 and 0.33, respectively). For the cropped system, however, the N_2O mole fraction was 0.36 for the long-wet and 0.90 for the short-wet treatment. Thus, in the cropped system a much smaller proportion of end product was N_2O if soil had been wet for 2 d. For N_2 fluxes, the isotope method gave the same pattern ($r = 0.92$) but only about one-third the magnitude, suggesting that N_2 derived from two distinct pools. Differences in response of N_2O mole fraction for successional and cropped soils may be due to differences in microbial communities. Further knowledge of ecosystem differences with respect to N_2O mole fraction and recent moisture history may improve modeled estimates of local and global N_2O fluxes.

NITROUS OXIDE IS A significant greenhouse gas (Intergovernmental Panel on Climate Change, 1996) and regulator of stratospheric ozone (Hahn and Crutzen, 1982). A major source of N_2O is microbial denitrification in soil, which produces dinitrogen and nitrous oxide in proportions that vary widely (Tiedje, 1988; Robertson, 1999). The proportion of denitrification evolved as nitrous oxide (N_2O mole fraction) affects the global N_2O budget; in most ecosystems, denitrification appears to be the major source of N_2O .

Numerous environmental factors can influence N_2O mole fraction, including soil moisture, nitrate and nitrite concentration, pH, aeration, temperature, carbon avail-

ability, relative activities of NO_2^- and N_2O reductases, and moisture history (Colbourn and Dowdell, 1984; Sahrawat and Keeney, 1986; Firestone and Davidson, 1989; Arah and Smith, 1990; Bouwman, 1990; Aulakh et al., 1992; Hutchinson and Davidson, 1993). Moisture history may be particularly important because soil moisture status in most ecosystems can change rapidly; if denitrifying enzymes are induced differentially in response to wetting, then both the overall rate of denitrification ($N_2O + N_2$) as well as N_2O mole fraction ($N_2O/[N_2O + N_2]$) will differ substantially among ecosystems.

Many studies have documented ecosystem differences in the rate of denitrification following wetting (e.g., Gilliam et al., 1978; Rice and Smith, 1982; Robertson and Tiedje, 1985, 1988; Sexstone et al., 1986; Groffman and Tiedje, 1989; Ambus and Lowrance, 1991; Groffman et al., 1993), and some have noted denitrification differences between the wet-up and dry-down phases of soil moisture following rainfall events (e.g., Groffman and Tiedje, 1988). Fewer studies have examined the influence of moisture history on the nitrous oxide mole fraction (e.g., Dendooven and Anderson, 1995; Dendooven et al., 1996), and we know of no study that has explained how moisture history effects may differ with ecosystem management.

Moisture history effects on N_2O mole fraction may help explain the differences in N_2O flux among ecosystems. At our site, N_2O flux from a cropped system was three times as high as flux from a successional field on the same soil series (Robertson et al., 2000). Denitrifier taxa—isolated from the cropped system and an adjacent never-tilled successional field—varied considerably in the sensitivity of nitrous oxide reductase (NOS) to oxygen, a soil factor that varies inversely with soil moisture (Cavigelli and Robertson, 2001). In whole soil slurry assays, denitrifying enzymes were more sensitive to oxygen in the cropped soil, and NOS was more active in the successional soil (Cavigelli and Robertson, 2000). We hypothesize that differences in microbial community enzyme activity influence responses of N_2O mole fraction to rain events, helping to explain N_2O flux differences. Since most N is lost from soils during brief

T.T. Bergsma and G.P. Robertson, W.K. Kellogg Biological Station and Dep. of Crop and Soil Sciences, Michigan State Univ., 3700 E. Gull Lake Drive, Hickory Corners, MI 49060. N.E. Ostrom, Dep. of Geological Sciences, 206 Natural Science Building, Michigan State University, East Lansing, MI 48823. Received 23 Apr. 2001. *Corresponding author (tbergsma@kbs.msu.edu).

Abbreviations: BD, bulk density; NOS, nitrous oxide reductase; WFPS, water-filled pore space.

periods following irrigation or rainfall (Smith and Tiedje, 1979; Sexstone et al., 1985; Rolston et al., 1982; Mummey et al., 1994; Davidson, 1991), variation among ecosystems in the response of N_2O mole fraction to moisture history could have widespread significance for the global N_2O budget.

In this study, we estimated nitrous oxide mole fraction for incubations of soil from two ecosystems (row crop agriculture and early native succession) and for two recent moisture histories in a factorial design. The primary objective was to determine if the effect of recent moisture history on N_2O mole fraction depends on ecosystem management. A secondary objective was to compare the use of a ^{15}N -gas evolution method with the acetylene (C_2H_2) inhibition method for estimating N_2 gas flux.

METHODS

Soil Collection and Preparation

Soil was collected from the Long-Term Ecological Research (LTER) site at the W.K. Kellogg Biological Station (KBS) in southwestern Michigan ($42^{\circ}24' N$, $85^{\circ}24' W$). The soil at this site is a Kalamazoo loam (fine-loamy, mixed, semiactive, mesic Typic Hapludalf) (Austin, 1979) derived from glacial till that was deposited approximately 14 500 yr BP. The Ap horizon has a depth of 30 cm; percent sand, silt, and clay are 43, 38, and 19, respectively; cation exchange capacity is 8.4 cmol kg^{-1} ; total C is 12.85 g kg^{-1} ; total N is 1.31 g kg^{-1} ; pH is 5.5; and bulk density is typically 1.6 Mg m^{-3} . Mean annual temperature is approximately $9^{\circ}C$ and precipitation is approximately 860 mm yr^{-1} . Native forests, probably oak savannah, were cleared for agriculture prior to 1900. During the 1950s and 1960s and perhaps earlier the site was cropped continuously to maize (*Zea mays* L.). During the 1970s and 1980s, the site was used to produce grain and forage for a local dairy herd. In 1988 the entire site was planted to soybean [*Glycine max* (L.) Merr.] prior to initiation of the current rotation system, implemented in 1989.

The ongoing LTER experiment is a randomized complete block design with six replicate blocks and seven treatments on the main site, for a total of 42 one-hectare plots. We sampled two treatments: a high-input corn-soybean-wheat rotation and a native succession treatment last plowed in 1988. The annual cropping system is tilled and receives conventional applications of fertilizer and pesticides. The successional treatment is managed by occasional burning and/or felling of woody biomass to maintain an "old field" herbaceous community.

Soil was collected in December 1999 from three replicate blocks of each treatment. For each of six plots (two treatments by three blocks), four soil cores ($2 \times 16 \text{ cm}$) were collected at each of five sampling stations. Soil was bulked by plot, passed through a 4-mm screen, air-dried for 2 wk to about 1% gravimetric moisture, and stored in plastic bags at room temperature until the start of the experiment.

Experiment and Treatments

We incubated soil treatments for 24 h in 1-L glass mason jars. Twenty-six jars each received 150 g dry soil from one of three replicates of ecosystem. Soil was packed to a volume of approximately 125 mL. Each jar within a replicate set was assigned to one of two moisture histories (*long-wet* or *short-wet*, defined below), and one of three gas sampling strategies (^{15}N -labeled soil, unlabeled soil, C_2H_2 -amended soil) or re-

served for mineral N analysis. Two additional jars were established without soil to serve as blanks for gas analysis.

Soil from the successional ecosystem was incubated 4 wk later than soil from the cropped system. Soil was sampled for mineral N availability at the start to test for storage effects. Successional soil did not pack as easily as cropped soil, possibly causing bias in repacked bulk density (BD) and water-filled pore space (WFPS). Bulk density and WFPS could not be measured directly. However, soil depth was measured on a subsample of jars (12 per ecosystem) at the conclusion of the experiment as an index of BD.

All soils received 9.75 mg of KNO_3 (about $9 \text{ mg NO}_3^-N \text{ kg}^{-1}$ dry soil), 20 mg glucose (about 53 mg C kg^{-1} dry soil), and 56.6 mL deionized water for a target WFPS of 85% at a BD of 1.2 g cm^{-3} . Gravimetric moisture was 39% on a dry-soil basis. Long-wet soil received 80% of prescribed water 48 h before the start of the incubation; nitrate and glucose were added with the remaining water just before incubation. Short-wet soil received all water, nitrate, and glucose just before incubation. Blank jars received only 56.6 mL water (no soil). Solutions were delivered as a slow trickle down the edge of a tipped jar to minimize soil disturbance and air entrapment. The delivery method produced a wetting front that moved laterally across the soil within 15 min.

The ^{15}N -labeled soil received 9.84 mg $K^{15}NO_3$, the molar equivalent of the 9.75 mg $K^{14}NO_3$ received under the other three strategies. The C_2H_2 jars received 80 mL C_2H_2 at the start of the incubation for a headspace concentration of 10 kPa (10% v/v), to inhibit NOS (Yoshinari and Knowles, 1976). All jars were fitted with air-tight lids; rubber septa and Cajon (Macedonia, OH) UltraTorr unions (custom o-ring seal) were added as necessary for syringe sampling and sampling to custom Pyrex vessels (0.5 L, preevacuated, with stopcocks) for ^{15}N analysis (Bergsma et al., 2001).

Sampling and Analysis

Jars for mineral-N analysis of soil were sampled destructively for nitrate and ammonium about 2 h after the start of the incubation (10 g soil, dry weight equivalent, extracted in 100 mL 1 M KCl, followed by analysis using an AlpKem [Wilsonville, OR] autoanalyzer). The N_2O concentrations in other jars were measured by gas chromatography at 0, 6, 12, and 24 h after the start of the incubation.

At the close of the incubation (24 h) gas samples were collected from ^{15}N -labeled and unlabeled treatments for analysis by isotope ratio mass spectrometry. The vessel stopcocks were opened for about 10 s and then sealed. Analyses were performed within 2 wk, using the ^{15}N -gas non-equilibrium technique (Bergsma et al., 2001). For N_2O we measured m/z ratios 46/44 and 45/44. For N_2 , we measured ratios 30/28 and 29/28. Equations for estimating the ^{15}N enrichment of the soil mineral N pool and the fraction of headspace gas derived from the soil mineral pool (d) require initial and final measurements of isotopic character (Arah, 1992; Bergsma et al., 1999). Because of the very large sample size, only a final sampling was possible. Therefore, each labeled sample was paired with its corresponding unlabeled sample to represent final and initial conditions, respectively. An advantage of this pairing is that it controls for slight biological and mechanical artifacts that could influence isotopic character under the experimental conditions described above. The N_2 flux was calculated as the difference in N_2O production between the C_2H_2 -amended and control jars, using controls with unlabeled nitrate for all tests of treatment effects and using controls with labeled nitrate for comparison with N_2 flux measured isotopically. Differences among treatment means were examined by analysis of variance

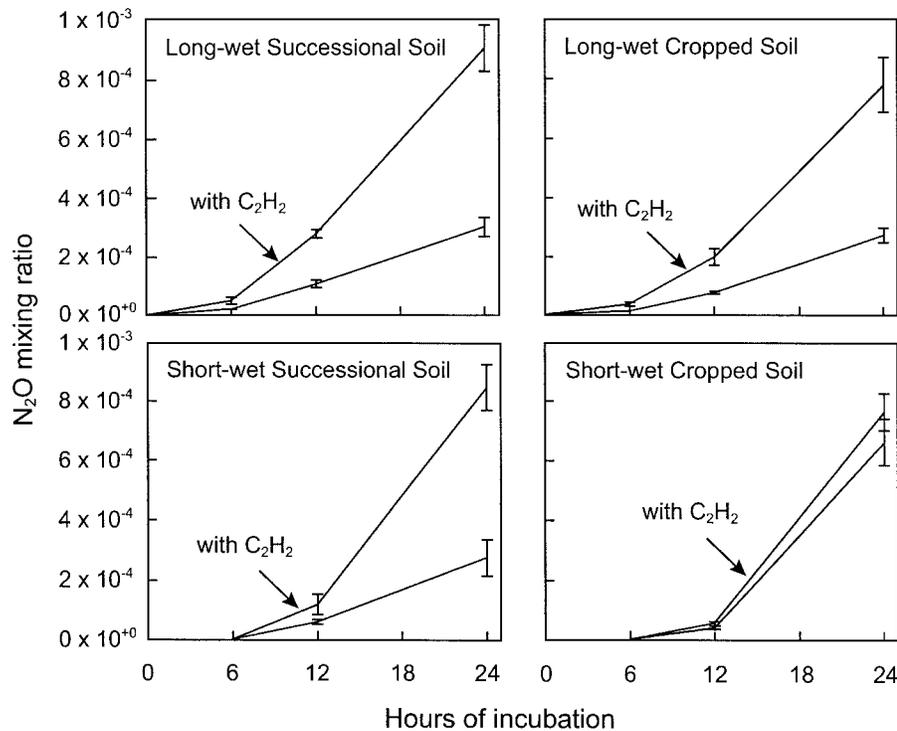


Fig. 1. Volumetric mixing ratios of nitrous oxide during soil incubations with C_2H_2 (arrows) or without. Successional incubations (left panels) show similar patterns regardless of moisture history. For short-wet cropped soil (lower right) accumulation of N_2O only (no arrow) was only slightly less than total denitrification (with arrow) in marked contrast to long-wet cropped soil (upper right). Error bars represent standard errors ($n = 3$).

(ANOVA), using JMPIN software version 3.1.5 (Sall and Lehman, 1996). The ^{15}N -based estimates of the enrichment of the soil mineral pool were compared with mass-balance estimates calculated from nitrate availability in stock soil and amount of labeled nitrate added.

RESULTS AND DISCUSSION

Nitrous Oxide Mole Fraction

All combinations of ecosystem and moisture history showed early accumulation of N_2 and N_2O , with N_2 estimated as the difference between jars with and without C_2H_2 (Fig. 1). Gas production began earlier for the long-wet treatments, but total denitrification was similar across all treatments at 24 h. Cropped short-wet soil produced very little N_2 , substantially less than did soil for the other three combinations of ecosystem and moisture history. Total denitrification, N_2O production, and N_2 production at 24 h were calculated (Fig. 2) and analyzed by ANOVA. Successional soil in general had higher total denitrification ($P < 0.05$). Cropped short-wet soil had significantly higher N_2O production ($P = 0.02$) and lower N_2 production ($P = 0.05$). Successional soil mineral N did not change appreciably between incubation dates. Soil mineral N at 2 h was similar for long-wet and short-wet treatments (although extractable NH_4^+ was lower for short-wet treatments); cropped soil had more NO_3^- and less NH_4^+ than successional soil.

Mean N_2O mole fractions were 0.34 and 0.33 respectively for successional long-wet and short-wet soil and were 0.36 and 0.90 respectively for cropped long-wet and short-wet soil (Table 1). Cropped soil showed a

strong effect of recent moisture history (ANOVA), while successional soil did not. The difference accounts for the highly significant interaction term (ecosystem by history: $P = 0.01$) for the whole model.

For the successional soil, the long-wet pretreatment apparently did not greatly enhance denitrification en-

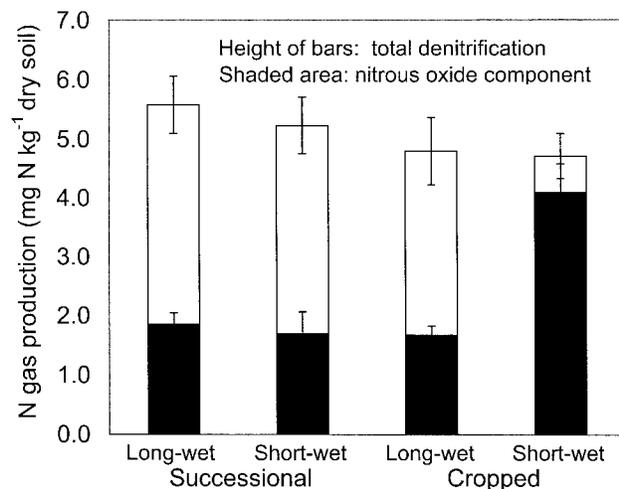


Fig. 2. Summary of N gas production. Total denitrification ($N_2O + N_2$ by C_2H_2 inhibition) is represented by the height of bars. The N_2O production (in the absence of C_2H_2) is represented by the shaded area. The N_2 production is inferred by difference (unshaded area). Even though total denitrification did not differ significantly between moisture histories for cropped soils, the short-wet incubations produced significantly more N_2O and less N_2 . Error bars represent standard error of total denitrification and N_2O production ($n = 3$).

Table 1. Results of analysis of variance (ANOVA) for nitrous oxide mole fraction[†] for successional and cropped soil with long-wet and short-wet moisture history.

Ecosystem	Long-wet‡	Short-wet‡	Combined‡	Block§	Moisture history§	Ecosystem§	Interaction§¶
Successional	0.34 ± 0.10	0.33 ± 0.07	–	0.0656	0.7157	–	–
Cropped	0.36 ± 0.08	0.90 ± 0.19	–	0.1335	0.0377	–	–
Combined	–	–	0.48 ± 0.09	0.0449	0.0150	0.0085	0.0122

[†] N₂O/[N₂ + N₂], C₂H₂ inhibition technique.

[‡] Treatment mean ± standard error.

[§] Effects in the model, prob. > F.

[¶] Moisture history by ecosystem interaction.

zyme status relative to the short-wet treatment. We conclude that denitrifying enzymes, especially NOS, persisted well in the successional soil during several months of air-dry conditions. Persistence of NO₂⁻ reductase (NIR) in dry soils has been observed by others (Smith and Parsons, 1985).

For the cropped soil, however, the long-wet pretreatment apparently enhanced the activity of NOS relative to the short-wet treatment, but not relative to successional soil. The N₂O mole fraction was more than doubled in the absence of prewetting. We suggest that NOS did not persist well in the cropped soil when air-dry, but was restored by 48 h of high soil moisture. Since total denitrification was only slightly less for cropped soil than for successional soil, NOS precursors (e.g., nitrate reductase [NAR], NIR) may have been less affected by drying than was NOS.

Procedural considerations do not account for the patterns of gas production. Actual WFPS, while not measured, could have been greater on average for cropped soils than for successional soil, since achieved BD was apparently greater (mean soil depths and standard deviations were 15.6 ± 0.7 for cropped soil and 17.0 ± 1.1 for the successional soil). Greater WFPS should have favored induction of NOS and total denitrification; however, cropped soil showed NOS limitation and lower total denitrification. High soil moisture in general could have limited the diffusion of C₂H₂, causing total denitrification and N₂ flux to be underestimated and N₂O mole fraction to be overestimated. While the cropped soil showed numerically smaller N₂ flux and greater N₂O mole fraction, its strong response to moisture history remains to be explained.

To the best of our knowledge, no other published study of N₂O mole fraction has examined the interaction between ecosystem management and moisture history. However, there are reports of effects of both ecosystem and moisture history on relative proportions of N₂ and N₂O. Merrill and Zak (1992) reported an N₂O mole fraction of 0.7 to 0.9 for well-drained sugar maple (*Acer saccharum* Marshall subsp. *saccharum*) forests in northern lower Michigan; in contrast, the N₂O mole fraction in a silver maple (*Acer saccharinum* L.)–red maple (*Acer rubrum* L.) swamp was 0.25. Dendooven et al. (1996) found an effect of moisture history on relative production of nitrous oxide and dinitrogen (N₂O to N₂) for pasture soil, but the difference was small: 0.54 for soil cores previously submerged for 96 h, and 0.4 for cores submerged for 6 h. Conversion to nitrous oxide mole fraction yields values of 0.35 and 0.29, similar to the

values presented here for successional soil and long-wet cropped soil. Mulvaney and Kurtz (1984) studied N₂O and N₂ flux for three ¹⁵N-amended soils subjected to wetting and drying cycles. We calculate from their Table 1 an average and standard error of 0.33 ± 0.02 (*n* = 12), similar to the result for our successional soils: 0.33 ± 0.04 (*n* = 6). In a study of three N-amended soils, Jacinthe et al. (2000) found that N₂O mole fraction was initially 0.68, increased to 0.95 with imposition of a water table at a depth of 10 cm, and decreased to 0.35 within 1 wk thereafter.

Our results show that the dependency of nitrous oxide mole fraction on recent moisture history may vary among ecosystems on the same soil series. The observation that N₂O mole fraction is higher, shortly after a moisture increase, for the cropped soil than for the successional soil may help explain field data showing three-fold greater annual flux of N₂O from the cropped system (3.5 ± 0.21 g N₂O-N ha⁻¹ d⁻¹) than from the successional system (1.1 ± 0.05 g N₂O-N ha⁻¹ d⁻¹; Robertson et al., 2000).

Differences in microbial community enzyme activity may influence responses of N₂O mole fraction to rain events. Perhaps lower availability of nitrate in the successional soil relative to the cropped soil (0.63 ± 0.04 and 6.54 ± 0.53 mg NO₃⁻-N kg⁻¹ dry soil, respectively; Robertson et al., 2000) has selected for a community of denitrifiers with the ability to maintain NOS status under dry (aerobic) conditions. Such a community could have a competitive advantage in exploiting the flush of carbon that occurs on soil wet-up (e.g., Groffman and Tiedje, 1988), since it could use N₂O as well as NO₃⁻ as a terminal electron acceptor if oxygen were limiting. In cropped soil, the incentive for NOS maintenance would be less, because of the abundance of the more energetically favorable electron acceptor NO₃⁻.

Our work using repacked soil advances the work of others at Kellogg Biological Station. Cavigelli and Robertson (2001) isolated 31 denitrifier taxa from two ecosystems: the cropped ecosystem studied here and a nearby never-tilled successional field. They showed that considerable variability exists among taxa for sensitivity of the NOS enzyme to varying levels of oxygen, a parameter related to soil drying. Furthermore, Cavigelli and Robertson (2000) found differences in denitrifying ability for whole soil microbial communities (slurry assay) for the cropped ecosystem and the never-tilled successional field. Denitrifying enzymes were more sensitive to oxygen levels in the agricultural soil, and NOS was more active in the successional soil. Their results are

consistent with our suggestion that the microbial community in the successional soil may have experienced selection for denitrifiers with the ability to maintain NOS status. The convergence of our results with previous results is noteworthy, given the additional structural complexity of repacked soil relative to soil slurries.

Moisture history, as a control on N_2O mole fraction, has an important place in the biogeochemistry of nitrogen. Many studies suggest that most N is lost from soils during brief periods following irrigation or rainfall (Smith and Tiedje, 1979; Sexstone et al., 1985; Rolston et al., 1982; Mummey et al., 1994; Davidson, 1991). Dependency of the N_2O mole fraction on short-term soil moisture history could have large consequences for the relationship between nitrous oxide production and total denitrification. Given our time scale of about 48 h, our finding is especially relevant for N_2O models with a daily time step (e.g., Li et al., 1992a,b), as well as models explicitly invoking the N_2O mole fraction (e.g., Parton et al., 1998). Improvement of biogeochemical models helps to constrain the global N_2O budget (Davidson, 1991).

Isotopic Analyses

The ^{15}N component of the experiment estimated production of N_2 and ^{15}N enrichment of the soil mineral N pool undergoing denitrification using either $^{15}N_2$ or $^{15}N_2O$ data. Both measures of enrichment agreed well with enrichment estimated by mass balance (Table 2). A well-known weakness of C_2H_2 inhibition is that C_2H_2 also inhibits nitrification, potentially leading to underestimation of N gas flux. Agreement of measured and estimated enrichment supports the view that NO_3^- was the principal substrate for N_2O production in this experiment, implying a negligible contribution from nitrification.

Production of $^{15}N_2O$ was so great relative to ambient N_2O that it could not be calculated accurately from the non-equilibrium mixing model (cf. Bergsma et al., 2001). However, since ambient N_2O was arguably negligible, $^{15}N_2O$ analyses represented soil-derived N_2O only (Fig. 3) and were therefore suitable for testing whether N_2O derived from a homogeneously labeled soil mineral N pool. In most cases, final N_2O was in equilibrium (on or near the equilibrium curve in Fig. 3), indicating homogeneity of the soil mineral pool from which it was derived (Bergsma et al., 1999; Bergsma et al., 2001). Ho-

mogeneity of the ^{15}N -labeled soil pool is a rarely tested assumption of ^{15}N -gas evolution methods that rely on a non-equilibrium model.

Production of N_2 measured by isotope methods was highly correlated with production of N_2 measured by C_2H_2 inhibition ($r = 0.92$), helping to confirm relative differences among ecosystems and moisture histories. However, the isotope method gave values consistently lower than the C_2H_2 method. For each combination of ecosystem, moisture history, and replicate, the ratio MS to GC was calculated, where MS refers to N_2 flux by mass spectrometry (^{15}N -gas non-equilibrium technique) and GC refers to N_2 flux by gas chromatography (C_2H_2 inhibition technique). Analysis of variance showed no effect of block, ecosystem, or moisture history, and no interaction of ecosystem and moisture history ($P > 0.4$ for all effects). With one outlier (negative) removed from 12 total values, mean and standard error for MS to GC is 0.34 ± 0.04 .

Methodological bias does not easily account for the differences in the two N_2 flux estimates. If the C_2H_2 had been diffusion-limited by high soil moisture, N_2 would have been underestimated rather than overestimated. It seems unlikely that C_2H_2 stimulated N_2O production (e.g., Klemetsson et al., 1988) in such a short interval, especially since C (glucose) was provided (see Topp and Germon, 1986). The ^{15}N -gas evolution techniques can underestimate N_2 flux when the soil mineral N pool undergoing denitrification is not uniform (Boast et al., 1988; Arah, 1992; Bergsma et al., 1999), but for most incubations, N_2O derived from soil was in equilibrium or nearly so, implying a well-mixed soil source (above). Since N_2O is the direct precursor of N_2 (Payne, 1981), one would expect N_2 from soil also to be in equilibrium (Focht, 1985) and therefore free of the underestimation ascribed to non-uniform pools. Furthermore, estimates of enrichment (of the soil mineral pool undergoing deni-

Table 2. Comparison of predicted soil NO_3^- enrichment (atom fraction ^{15}N) and apparent enrichment of the soil pool undergoing denitrification.

Ecosystem	Replicate	Mass balance [†]	From N_2 [‡]	From N_2O [‡]	Average [§]
Successional	A	0.90	0.87	0.87	0.87
Successional	B	0.78	0.83	0.83	0.83
Successional	C	0.84	0.84	0.86	0.85
Cropped	A	0.42	0.57	0.53	0.55
Cropped	B	0.35	0.31	0.38	0.34
Cropped	C	0.42	0.71	0.58	0.64

[†] Estimated from the extractable NO_3^- levels in stock soil and the known addition of KNO_3 .

[‡] Apparent enrichment estimated from N_2 or N_2O isotopic data.

[§] Average of N_2 - and N_2O -based estimates.

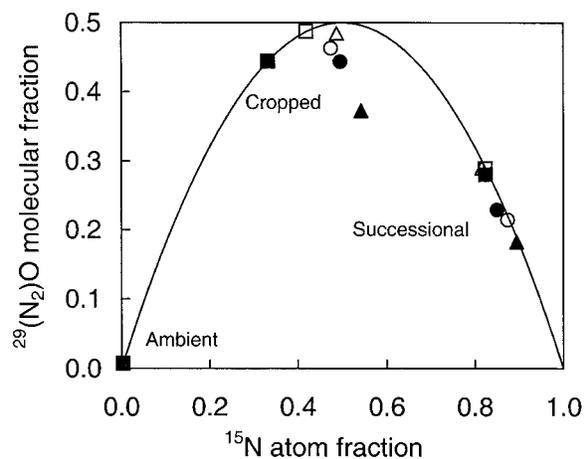


Fig. 3. Isotopic character of N_2O in labeled jars at the end of incubations (with ambient N_2O for reference). Filled and open symbols represent long-wet and short-wet treatments, respectively; shape corresponds to replicate. Points lying on or near the equilibrium curve indicate samples approximately in isotopic equilibrium with respect to ^{15}N distribution among molecular masses, suggesting derivation from an isotopically uniform pool. Higher effective enrichment of successional soils relative to cropped soils reflects a smaller contribution from native soil NO_3^- .

trification) based on N_2 in our study agreed well with estimates based on N_2O . Others have found similar results (Mulvaney and Kurtz, 1984; Mosier et al., 1986).

The two methods of calculating N_2 production may have reflected qualitatively different aspects of the experimental system. Possibly the C_2H_2 method represented gross N_2 production while the ^{15}N -gas evolution technique represented only production from a highly enriched, uniformly labeled pool (i.e., the enriched N_2O or its substrate). Apparently a second, unenriched soil mineral N pool was also a source of N_2 (indeed the major source), but not a net source of N_2O . Under these circumstances, N_2 production would have been underestimated without affecting the estimate of enrichment for the labeled pool (see Focht, 1985), explaining the agreement of N_2 and N_2O data for estimates of pool enrichment (Table 2). The agreement of the mass balance estimates and the ^{15}N estimates suggests further that the putative unlabeled source of N_2 is not a static, extractable NO_3^- pool. Additional study is needed to characterize potential mineral N sources for the experimental system described.

CONCLUSION

The N_2O mole fraction in successional soil was not affected by moisture history, but in cropped soil it was sharply lower when soil moisture had been high for 48 h prior to incubation. This suggests that microbial NOS in the successional soil is more persistent between wetting events, perhaps because a lower level of native soil nitrate selects for denitrifier taxa with enhanced capacity for enzyme maintenance. Explicit recognition of ecosystem differences in response of N_2O mole fraction to recent moisture history may improve modeled estimates of global N_2O flux. Furthermore, understanding the impact of soil management regimes on mole fraction dynamics within ecosystems may lead to strategies that can minimize flux of N_2O to the atmosphere.

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