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Measuring Simultaneous Fluxes from Soil of N_2O and N_2 in the Field Using the ^{15}N-Gas "Nonequilibrium" Technique

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Our purpose was to measure simultaneous fluxes from soil of both N_2O and N_2 from the same plot in the field using the ^{15}N-gas "nonequilibrium" technique (i.e., the "Hauck" technique) as used previously for N_2. We accommodated analysis of N_2O by modifying the head amplifier of our mass spectrometer. Our system accurately measured the ^{15}N enrichments of labeled soil slurries for both N_2 and N_2O. In the field, we measured flux of N_2 and N_2O during soil denitrification from a ^{15}N-labeled plot of winter wheat. Nine chamber incubations were conducted over 4 days. N_2 flux ranged from below detection limit (<0.022 g $\cdot$ m^{-2} $\cdot d^{-1}$) to 0.055 g $\cdot$ m^{-2} $\cdot$ d^{-1}. N_2O flux ranged from 0.0002 to 0.0027 g N_2O-N $\cdot$ m^{-2} $\cdot$ d^{-1}, with a detection limit of 1.0 $\cdot$ 10^{-6} g N_2O-N $\cdot$ m^{-2} $\cdot$ d^{-1}. For N_2O flux, the ^{15}N-gas technique and gas chromatography technique agreed well ($r = 0.98$). The ^{15}N enrichment of the soil mineral pool undergoing denitrification, measured nondestructively using the N_2O data, dropped from about 0.82 to 0.72 atom fraction ^{15}N over 4 days. Applying the ^{15}N-gas nonequilibrium technique to N_2O complements its use for $^{15}N-N_2$ analysis when studying the relative production of N_2O and N_2 during denitrification.

Introduction

Microbial denitrification in soil produces nitrous oxide (N_2O) and dinitrogen (N_2) from soil nitrate (NO_3^-). Loss of gaseous N from soil may contribute to nutrient limitation in terrestrial ecosystems. Furthermore, the rapid postindustrial increase of N_2O in Earth's atmosphere contributes to global warming and to the destruction of stratospheric ozone (*1*−*3*). High variability in the relative proportions of N_2O and N_2 produced during denitrification (*4, 5*) frustrates attempts to understand the contribution of denitrification to the growing atmospheric pool of N_2O. Measurement of simultaneous fluxes of N_2O and N_2 in the field helps characterize denitrification with respect to relative proportions of gases produced.

Gas flux from soil is commonly measured in the field by some chamber method: a soil cover traps evolving gases and a time-series of headspace samples is analyzed. For N_2O, change in headspace concentration is typically analyzed by gas chromatography (*6*). For N_2, however, the only suitable direct method for measuring flux in the field is ^{15}N analysis of N_2 collected over ^{15}N-labeled soil (*7*). If applied label (e.g. $^{15}NO_3^-$) is distributed uniformly in the soil mineral pool, the technique introduced by Hauck and others (*8*−*10*) measures not only flux but also nondestructively measures the enrichment of the pool undergoing denitrification, by extrapolation from the shifting headspace abundances of singly- and doubly-labeled molecules. The technique takes advantage of the fact that ^{15}N atoms are not distributed randomly among the molecules that comprise a mixture of soil-derived (labeled) N_2 and atmosphere-derived N_2. The mixture is therefore not in isotopic equilibrium. We introduce the descriptor "^{15}N-gas nonequilibrium technique" to distinguish this general strategy from others in the class of ^{15}N-gas evolution techniques reviewed by Myrold (*6*). "Nonequilibrium equations" (*9*−*15*) are systems of equations that calculate average ^{15}N enrichment of the soil mineral N pool and the fraction in the sample of N-gas derived from labeled soil, using information about all three molecular masses (whether provided by dual- or triple-collector mass spectrometers).

In principle, the ^{15}N-gas nonequilibrium technique can be used to measure N_2O flux as well as N_2 flux. However, no one has reported using the nonequilibrium technique directly on undiluted, unreduced N_2O for determination of field N_2O flux. Other methods have been reported (*16, 11, 17*) that can be used to measure flux of N_2O from ^{15}N-labeled soil (*16, 18, 19, 20*). The method of Brooks et al. (*16*) requires destructive sampling of soil to determine ^{15}N enrichment of the soil mineral N pool and flux. The method of Mulvaney and Kurtz (*11*) applies nonequilibrium equations (refined (*12, 13*)) to a mixture of sample N_2O diluted by laboratory standard N_2 and reduced to N_2 over hot copper. The method of Stevens et al. (*17*) analyzes N_2O directly (masses 44, 45, 46) but evaluates concentration change rather than isotope shift for calculation of flux (Appendix 1). The methods of both Mulvaney and Kurtz and Stevens et al. assume that atmospheric N_2O in chamber headspaces is negligible—frequently not the case for field fluxes.

Measuring N_2O flux by the ^{15}N-gas nonequilibrium technique is the natural complement to its use for measurement of N_2 flux, especially for studies of denitrification. N_2O flux by the nonequilibrium technique provides an independent, nondestructive measurement of ^{15}N enrichment of the denitrifying pool that can corroborate N_2 results. Furthermore, expressions of the relative proportions of N_2O and N_2 produced during denitrification may be accurate even when the denitrifying pool is not uniformly labeled, since underestimation of flux for the two gases will be similar. Extending the nonequilibrium technique to N_2O is hampered, however, by analytical constraints pertaining to the differences between N_2 and N_2O. Since N_2 is naturally abundant in the Earth's atmosphere (~79%) only small air samples (a few milliliters) are needed to obtain sufficient N for analysis; also, flux of labeled N_2 from soil is greatly diluted by ambient N_2 in chamber headspaces, such that resulting isotope ratios are not very different from ambient. In contrast, N_2O is a trace gas (~317 ppb_v) in the atmosphere, requiring large air samples to obtain sufficient N for analysis; and even small fluxes of labeled N_2O from soil easily perturb headspace isotope ratios, resulting in a need for a much greater analytical range than for N_2.

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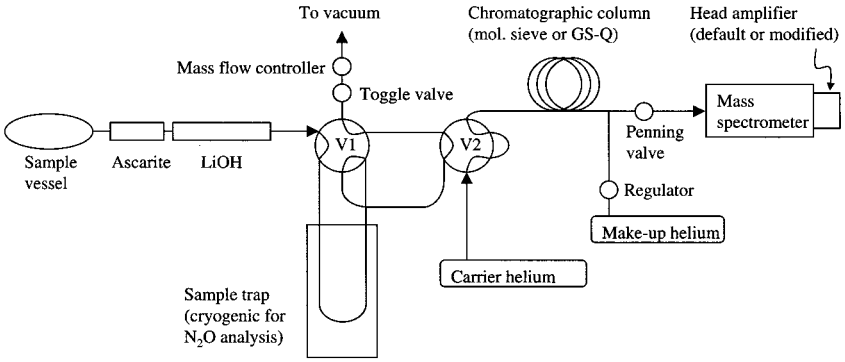

FIGURE 1. Sample preparation system connected to mass spectrometer. During purification, He bypasses the sample trap and travels onto the GC column, while the sample passes into the unchilled sample trap for N_2 or through the chilled sample trap and then to waste via the mass flow controller for N_2O. (Valves V1 and V2 are shown in positions 'a'; 60° rotation gives position 'b', with the complementary internal pairing of neighboring ports.) During analysis, helium passes through the sample trap and onto the GC column, while the sample vessel remains open to vacuum via the mass flow controller. Different columns are used for N_2O and N_2. Makeup helium is needed only during N_2 analysis.

Our work had three objectives. First, we sought to modify an isotope ratio mass spectrometer and associated hardware to permit the ^{15}N analysis of N_2O in air collected over labeled soil without compromising the ^{15}N analysis of N_2. Second, we wanted to confirm by means of laboratory denitrification experiments that our system of analysis and data processing is accurate and precise. Third, we wanted to measure simultaneous fluxes of N_2O and N_2 during soil denitrification from the same plot in the field, using the ^{15}N-gas nonequilibrium technique.

Experimental Methods

Analytical Procedure. We developed an analytical procedure permitting ^{15}N analysis of N_2O in samples of air collected over labeled soil. Existing equipment included a dual-inlet triple-collector isotope ratio mass spectrometer (Micromass PRISM). To the inlet of the mass spectrometer, we added a preparation system consisting of chemical traps, a cold trap, two six-port two-position rotary valves, and a gas chromatograph (Figure 1). The air sample is collected in a preevacuated 0.5 L Pyrex vessel and then attached to the preparation system. After evacuation of dead space (valve positions V1:a, V2:a, Toggle valve open) the sample is released (vessel stopcock opened), passing through an ascarite trap (removal of CO_2 and H_2O), a LiOH trap (removal of additional CO_2), and a high efficiency liquid nitrogen trap (hereafter the "sample trap": 0.5 m × 1/16 in. i.d. nickel tubing, coiled). The sample trap retains N_2O, while noncondensable gases are pumped away. Then the N_2O is isolated (V1:b, V2:a) and thawed. Finally, a stream of He (50 psi) carries the N_2O onto a chromatographic column (J. W. Scientific GS-Q) for separation from trace CO_2 and CO (V1:b, V2:b). The column delivers the N_2O to the mass spectrometer, where mass ratios 45/44 and 46/44 are compared for the sample and laboratory reference (0.83 per mil ^{15}N vs air).

The same sample can be analyzed for both N_2 and N_2O if the N_2 analysis is performed first on a subsample. For N_2, the sample vessel is attached to the preparation system as for N_2O (Figure 1). The sample trap is not chilled with liquid nitrogen but simply evacuated (valve positions V1:a, V2:a, Toggle valve open) and isolated (Toggle valve closed). The sample vessel stopcock is then opened briefly (10 s) to allow an aliquot of sample gas to pressurize the sample trap, which is then isolated (V1:b, V2:a). The remaining vessel sample is reserved for N_2O analysis. The N_2 sample is flushed onto a molecular sieve column (Alltech 8 m by 1/8 in. o.d., 5 Å) for separation from oxygen (V1:b, V2:b). The column delivers the sample to the mass spectrometer for analysis of mass ratios 29/28 and 30/28. Makeup helium is required to dilute the sample to within the detectable range and helps regulate pressure.

Our default spectrometer configuration is optimal for all analyses of N_2 in air and for analyses of ambient N_2O in air. However, higher-than-ambient mass ratios (i.e. enriched N_2O) may result in samples that are out of range for one or more of the spectrometer detectors. We solve this problem by maintaining separate head amplifiers for analysis of N_2 and (enriched) N_2O. The resistor values in the default head amplifier are $5 \cdot 10^8\ \Omega$, $5 \cdot 10^{10}\ \Omega$, and $5 \cdot 10^{10}\ \Omega$ for the major beam and two minor beams, respectively—a configuration that anticipates the relative rarity of the heavier (minor) isotopes. In a second head amplifier (hereafter, "the modified head amplifier"), we installed resistors with the value $1 \cdot 10^9\ \Omega$ in all three positions (making no assumptions about the relative abundance of masses 44−46). The head amplifiers are readily interchangeable. Typically, all sample vessels from an experiment are processed for N_2 with the default head amplifier, and then the modified head amplifier is installed and the spectrometer retuned for N_2O analysis.

Application of ^{15}N-gas nonequilibrium equations to N_2O is analogous to their use for N_2. However, because of naturally occurring isotopes of oxygen, the molecular fractions $^{44}N_2O$, $^{45}N_2O$, and $^{46}N_2O$ do not strictly correspond to the N_2 analogues $^{28}(N_2)O$, $^{29}(N_2)O$, and $^{30}(N_2)O$. We "oxygen-corrected" our spectrometer mass ratios 45/44 and 46/44 to 29/28 and 30/28 using equations derived elsewhere with different notation (*21*):

$$^{29}R = {}^{45}R - {}^{17}R$$

and

$$^{30}R = {}^{46}R - ({}^{29}R)({}^{17}R) - {}^{18}R$$

where ^{29}R and ^{30}R represent $^{29}(N_2)O/^{28}(N_2)O$ and $^{30}(N_2)O/^{28}(N_2)O$, ^{45}R and ^{46}R represent $^{45}N_2O/^{44}N_2O$ and $^{46}N_2O/^{44}N_2O$, and ^{17}R and ^{18}R represent $^{17}O/^{16}O$ and $^{18}O/^{16}O$. The value 0.000373 was used for ^{17}R, and 0.0020052 was used for ^{18}R (*22*). For both N_2 and N_2O, ^{29}R and ^{30}R were converted to molecular fractions ^{29}x and ^{30}x using

$$^{29}x = {}^{29}R/({}^{29}R + {}^{30}R + 1)$$

and

$$^{30}x = {}^{30}R/({}^{29}R + {}^{30}R + 1)$$

For paired (initial/final) headspace samples, molecular fractions ^{29}x and ^{30}x were used in ^{15}N-gas nonequilibrium

equations (*14*) to calculate the average enrichment of the soil mineral pool from which N_2O derives ($^{15}a_p$) and to calculate the fraction of sample that was derived from the soil (*d*). Flux was calculated from *d* (Appendix 2).

To assess performance of the modified head amplifier, we compared measured ^{29}x and ^{30}x with theoretical ^{29}x and ^{30}x for N_2O samples prepared as follows. We used microliter gastight syringes (Hamilton) to deliver aliquots (typically 1 μL) of N_2O to an evacuated mixing vessel on a standard vacuum line, immersed in liquid nitrogen. Mixtures of $^{44}N_2O$, $^{45}N_2O$, and $^{46}N_2O$ were prepared in the ratios 1:0:1, 1:1:0, 1:1:1, and 1:1:2. The laboratory standard was used for $^{44}N_2O$; $^{45}N_2O$ was $^{15}N-N-O$, >98% ^{15}N Cambridge Isotope Laboratories, Inc., Andover, MA; and $^{46}N_2O$ was > 99% $^{15}N-N_2O$, Isotec, Inc., Miamisburg, OH. The mixing vessel was isolated by a stopcock and thawed. Subsamples (~0.2 μL) were analyzed and measured molecular fractions were calculated as described above. Theoretical molecular fractions were calculated from the enrichments and purities of the N_2O source gases, accounting for minor species (e.g. trace mass 45 in $^{44}N_2O$).

Laboratory Tests. We tested whether our analysis of N_2O could accurately measure the enrichment of mineral nitrate pools ($^{15}a_p$) undergoing biological denitrification in the laboratory. Soil slurries were established in Erlenmeyer flasks; each of nine flasks received sieved soil (10 g of fresh soil, typic hapludalf, 8% gravimetric moisture, 2.8 μg of water-extractable $NO_3^--N \cdot g$ dry soil $^{-1}$), 1 g of steel wool activated with detergent solution to scrub trace O_2 (23), and sodium succinate as a nonlimiting carbon source for denitrifiers (10 mL, 1.0 mM). Flasks were flushed with high purity nitrogen (99.999%), C_2H_2 was injected (10% of headspace—inhibits the microbial reduction of N_2O to N_2), and N_2O production was monitored by gas chromatography (24). When accumulation of headspace N_2O ceased (i.e. denitrification of native soil N), each flask was flushed with pure N_2 and then received 1 mL of 0.36 mM KNO_3 solution with enrichment of ~10, 20, or 40% ^{15}N. When headspace N_2O concentrations reached 1−2 $\mu L \cdot L^{-1}$, gas samples were collected for isotopic analysis. Enrichment of the soil NO_3^- pool undergoing denitrification was calculated from ^{29}x and ^{30}x. Theoretical enrichment was calculated by mass balance of labeled and unlabeled N used to prepare the slurries, e.g.

$$M_1E_1 + M_2E_2 = M_3E_3$$

where M represents mass in grams, E represents atom fraction ^{15}N, 1 denotes stock nitrate (assumed 0.003663 atom fraction ^{15}N), 2 denotes ^{15}N KNO_3 (0.9993 atom fraction ^{15}N), and 3 denotes the mixture.

We conducted a similar denitrification experiment to test whether our analysis of N_2 accurately measures the enrichment of mineral nitrate pools ($^{15}a_p$) undergoing biological denitrification in the laboratory. Slurries were prepared using 20 g of fresh soil (1.4 μg $NO_3^--N \cdot g$ dry soil $^{-1}$), 1 mL of 0.1 M sodium succinate, 1 mL of 0.1 M KNO_3 solution, and 20 mL of deionized water. Flasks were flushed with purified N_2. Evolution and subsequent disappearance of N_2O (presumably consumed by denitrifiers) were monitored by gas chromatography. Headspace gas samples were collected after ~2 days and analyzed for N_2 by isotope ratio mass spectrometry. Enrichment of the soil NO_3^- pool undergoing denitrification was calculated from ^{29}x and ^{30}x, using an analysis of the N_2 flush gas to represent initial isotopic character of headspace N_2. Theoretical enrichment was calculated by mass balance, as above.

Field Test. We attempted to measure simultaneous fluxes of N_2O and N_2 during soil denitrification from the same plot in the field using the ^{15}N-gas nonequilibrium technique. In April 1998, a 0.25 m^2 plot of winter wheat (Kellogg Biological Station, Hickory Corners, MI, 42°24′N, 85°24′W) was fertilized with 30 kg $N \cdot ha^{-1}$, using 99% ^{15}N KNO_3. An aluminum frame (0.085 m^2, 6 cm deep, water channel on upper edge for sealing lid) was pressed into the soil (25). Over 4 days, nine 1-h incubations were conducted between and during precipitation events. For each incubation, a soil cover (30 cm × 30 cm × 14 cm deep) was fitted to the frame. Gas samples were collected at the start and end of each incubation for analysis of N_2O and N_2 by mass spectrometry and N_2O by gas chromatography. The soil cover had a 1-L polyethylene bag affixed to an internal wall, vented to external atmosphere, to minimize pressure artifacts at the soil surface during sample collection.

Results and Discussion

Analytical Procedure. We were able to modify our isotope ratio mass spectrometer to permit the ^{15}N analysis of N_2O as well as N_2 in air collected over labeled soil (Figure 1). The cryogenic sample trap was easy to add, and its equivalent is probably available commercially as an option on GC-IRMS packages. We switched GC columns manually; however, it should not be very difficult to configure automated valves for the purpose.

The most significant equipment modification was the substitution, as suggested by others (26), of range-appropriate resistor values in the head amplifier when analyzing N_2O rather than N_2. Convenience of analyzing both gases was facilitated by maintaining separate, interchangeable head amplifiers for N_2O and N_2. The problem is that N_2O collected over labeled soil may exhibit "excessive enrichment" (26), whereas N_2 collected over labeled soil—because of the high natural abundance background—will not. That is, minor species of N_2 (singly- and doubly-labeled) will almost always have abundances several orders of magnitude less than the major species, even after large flux. All three spectrometer detectors in many triple-collector isotope ratio mass spectrometers have the same fixed voltage range (10 V). Current at the detector is proportional to species abundance (beam intensity). Therefore, from Ohm's law (V = IR; V is voltage, I is current, and R is resistance) it is reasonable that detectors for minor species should have resistances several orders of magnitude greater than the major detector—as is usually the case—when analyzing N_2. For N_2O collected over labeled soil, however, minor species may rival or surpass the major species in abundance, causing the range of the detectors to be exceeded during analysis. In our modified head amplifier, we increased the value of the "minor" resistors relative to the "major" resistor to prevent highly enriched N_2O samples from exceeding detector range. Mulvaney and Kurtz (11) address essentially the same problem by diluting highly enriched N_2O samples with a very large quantity of laboratory N_2 (after which N_2O is reduced to N_2 for analysis of the mixture). Their dilution approach does avoid head amplifier modification but it reduces sensitivity (see Results and Discussion: Comments).

The novelty of our head amplifier modification justified a simple test of its performance. We wished to show whether ion beam ratios or their adjusted equivalents (i.e. the mole fractions ^{29}x and ^{30}x) would be unbiased across a broad range of enrichments. Figure 2 shows measured vs theoretical mole fractions ^{29}x and ^{30}x for 6 mixtures of $^{44}N_2O$, $^{45}N_2O$, and $^{46}N_2O$ in the ratios 1:0:1, 1:1:0, 1:1:1 (three independent mixtures), and 1:2:2. Results were analyzed by linear regression. For ^{29}x, $y = 1.02x - 0.010$; $R^2 = 0.996$. For ^{30}x, $y = 0.99x + 0.010$; $R^2 = 0.998$. For both fractions, slopes are close to 1, intercepts are small, and linearity (R^2) is high. The head amplifier modification was deemed suitable for our purposes.

Laboratory Tests. With respect to isotopic analysis, determination of gas flux by the ^{15}N-gas nonequilibrium technique depends on accurately measuring the enrichment

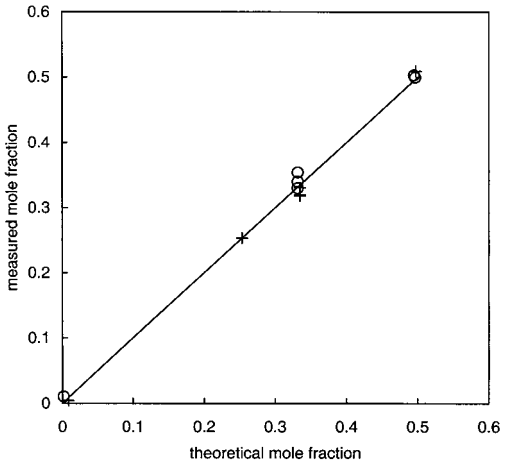

FIGURE 2. Measured vs theoretical mole fractions ^{29}x (circles) and ^{30}x (cross hairs) for six mixtures of $^{44}N_2O$, $^{45}N_2O$, and $^{46}N_2O$. The diagonal represents equivalence ($y = x$). See text for regression statistics.

TABLE 1. Measured and Theoretical Enrichment of the Soil NO_3^- Pool ($^{15}a_p$) for Laboratory Denitrification Experiments[a]

gas	measured $^{15}a_p$	theoretical $^{15}a_p$	$P > \|t\|$	n
N_2O	0.1026 ± 0.0003	0.1032	0.210	3
N_2O	0.2007 ± 0.0013	0.2028	0.034	3
N_2O	0.3976 ± 0.0012	0.4019	0.068	3
N_2	0.1072 ± 0.0053	0.1032	0.531	3
N_2	0.1969 ± 0.0031	0.2028	0.199	3
N_2	0.3827 ± 0.0007	0.4019	0.001	3

[a] Measured enrichment is calculated using mass spectrometric analysis for ^{29}x and ^{30}x and ^{15}N-gas nonequilibrium equations (*14*). Theoretical enrichment is calculated by mass balance, i.e., from the masses and enrichments of labeled and unlabeled N added to soil slurries. $^{15}a_p$ is reported as means and standard errors; P > |t| is the significance level for Student's *t*-test, and *n* is the number of samples.

of the soil mineral N pool undergoing denitrification. The measurement is nondestructive, since it infers enrichment from analysis of evolved gas. In parallel laboratory experiments, we tested whether our methods for N_2O and N_2 accurately measure the enrichment of the soil NO_3^- pool ($^{15}a_p$) for enrichments of approximately 0.1, 0.2, or 0.4 atom fraction ^{15}N. Results are given in Table 1. In most cases, mean measured $^{15}a_p$ was within 1% of theoretical $^{15}a_p$.

The N_2O experiment is also a test of equilibrium theory. Since background N_2O and N_2O from denitrification of soil N was purged from flasks, sampled N_2O should have derived from a single, uniformly enriched source: added KNO_3. When N_2O or N_2 (both diatomic with respect to N) derive from a single source, ^{15}N atoms should be randomly distributed among the mole fractions ^{29}x and ^{30}x. For example, ^{29}x should be a simple function of enrichment: $^{29}x = 2(^{15}a_p)(1-^{15}a_p)$ (15). Figure 3 shows a plot of ^{29}x vs $^{15}a_p$ for the laboratory N_2O experiment. The curve, representing theoretical equilibrium, is intersected by vertical bars at the theoretical enrichments for the experiment. Open circles represent measurements of ^{29}x and $^{15}a_p$ for the N_2O samples summarized in Table 1. One may infer by inspection of Figure 3 that the samples were in equilibrium as predicted: distance of sample clusters from the curve is small relative to the scatter within clusters.

Field Test. We were able to measure simultaneous flux of N_2O and N_2 from soil during denitrification in the field (Figure 4). Soil moisture was initially high, and about 2.4 cm rain fell during the 4-d period. N_2 fluxes ranged from 0.006 to 0.055 g · m^{-2} · d^{-1}; results are not included in Figure 4; however, since six of nine measurements were less than our

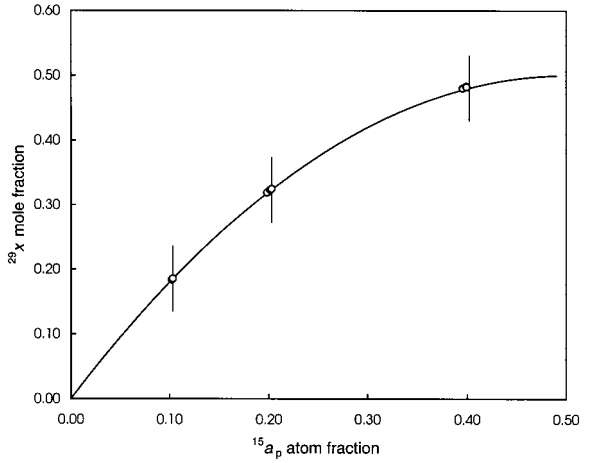

FIGURE 3. Measured and theoretical positions: ^{29}x vs $^{15}a_p$ for the N_2O samples from laboratory denitrification experiment. The curve represents equilibrium, i.e., $^{29}x = 2(^{15}a_p)(1 - {}^{15}a_p)$. Open circles are measured positions; theoretical positions are the intersections of vertical bars with the equilibrium curve.

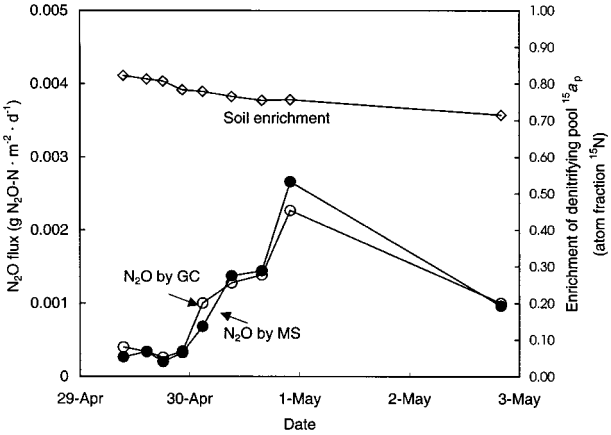

FIGURE 4. Flux of N_2O and enrichment of denitrifying pool during denitrification in the field. Flux is measured by gas chromatography (GC) and by the ^{15}N-gas nonequilibrium technique using mass spectrometry (MS). Enrichment for the mineral N pool undergoing denitrification is estimated nondestructively from N_2O isotopic data.

conservative estimate of detection limit (see below). N_2O flux ranged from 0.0002 to 0.0027 g $N_2O-N \cdot m^{-2} \cdot d^{-1}$; these values are in the low end of the range reported in a summary by Williams et al. (27) for agricultural fields (−2.2 to 234 ng $N_2O-N\ m^{-2}\ s^{-1}$, about −0.0002 to 0.02 g $N_2O-N \cdot m^{-2} \cdot d^{-1}$). N_2O flux measured by the ^{15}N-gas nonequilibrium technique agreed well with N_2O flux measured by gas chromatography: the Pearson product moment correlation coefficient (r) was 0.98. Enrichment of the soil mineral N pool undergoing denitrification, calculated nondestructively from the N_2O data, declined steadily throughout the experiment from about 0.82 to about 0.72 atom fraction ^{15}N. The decline is consistent with the conjecture that applied ^{15}N label was being diluted by unlabeled N from microbial nitrification. It is possible that nitrification and denitrification were occurring simultaneously in this soil.

We estimated detection limit for the ^{15}N-gas nonequilibrium technique as implemented here. Detection limit depends on spectrometer precision, chamber volume, chamber area at the soil surface, enrichment of the soil pool, and duration of the incubation. We defined detection limit conservatively as the smallest flux for which both 45/44 and 46/44 at the end of an incubation exceed their initial values by three standard deviations, where standard deviation is calculated from replicate analyses of ambient air. For the

field demonstration described, headspace was ~14 L, area was 0.085 m^2, enrichment averaged 0.77 ^{15}N, and duration was ~1 h. Analyses of replicate air samples (n = 3) using the modified head amplifier gave means and standard deviations of $8.02 \cdot 10^{-3} \pm 0.09 \cdot 10^{-3}$ for mass ratio 45/44 and $2.12 \cdot 10^{-3} \pm 0.03 \cdot 10^{-3}$ for mass ratio 46/44. Under these conditions, the N_2O detection limit for our method was $1.0 \cdot 10^{-6}$ g $N_2O-N \cdot m^{-2} \cdot d^{-1}$, well below our smallest measured flux. Detection limit calculated similarly for N_2 was 0.022 g $N_2 \cdot m^{-2} \cdot d^{-1}$ ($7.2230 \cdot 10^{-3} \pm 0.0005 \cdot 10^{-3}$ for mass ratio 29/28, and $1.56 \cdot 10^{-4} \pm 0.01 \cdot 10^{-4}$ for mass ratio 30/28, n = 12), falling within our range of measured fluxes.

Comments. Measurement of field fluxes of N_2O by the ^{15}N-gas nonequilibrium technique—based on analysis of undiluted, unreduced N_2O—has not been previously reported. The nonequilibrium model for N_2 involves the mixture of two components: N_2 evolved from labeled soil and atmospheric N_2 trapped in the chamber headspace. Similarly, our analysis of N_2O is based on the mixture of N_2O evolved from labeled soil and atmospheric N_2O trapped in the chamber headspace. The N_2O method of Mulvaney and Kurtz (11) involves a mixture of N_2O from labeled soil and a very large aliquot of laboratory N_2. (The N_2O component is reduced to N_2 over hot copper, after which the entire mixture is analyzed as N_2.) Nonequilibrium equations are used to determine the amount and enrichment of N_2O from the soil source. Since the equations resolve only two components, the method of Mulvaney and Kurtz is valid only if atmospheric N_2O in the chamber headspace is negligible relative to N_2O evolved from soil, as the authors indicate. In contrast, our method is valid even when N_2O evolved from soil is all-but-negligible relative to atmospheric N_2O, representing many orders of magnitude increase in sensitivity. Also, our method is perhaps simpler, not requiring the dilution and reduction steps.

The automated method of Stevens et al. (17) analyzes undiluted, unreduced N_2O by mass spectrometry to calculate its concentration in a sample. Applied to field fluxes of N_2O (e.g. ref 20), the method is conceptually based on a single component: N_2O evolved from labeled soil. Therefore, nonequilibrium equations do not apply, and analysis does not give a nondestructive estimate of soil ^{15}N enrichment. Flux is determined by using isotopic data to calculate change in N_2O concentration. Like the method of Mulvaney and Kurtz, the method of Stevens et al. requires that atmospheric N_2O must be negligible relative to evolved N_2O. Run times are shorter than ours (~10 min vs ~40 min) probably because sample sizes are smaller (12 mL vs 500 mL). However, minimum detectable change in headspace concentration (Δ_{min}) is reported as $2.1 \cdot 10^{-6}$ L N_2O L^{-1}, compared to 2.2 10^{-10} L N_2O L^{-1} for our field experiment. For reference, we calculate that gas chromatography gives a Δ_{min} of about $1 \cdot 10^{-8}$ L N_2O L^{-1} when CV of the analysis is 1% (Appendix 3).

One advantage of applying the ^{15}N-gas nonequilibrium technique to N_2O rather than just N_2 is that it gives an independent measure of the enrichment of the mineral N pool undergoing denitrification. The relative uncertainty of the N_2 data for our field experiment highlights the significance of having such an alternative: detailed information about the changing enrichment of the soil ^{15}N pool was still available from the N_2O data set, despite the weaknesses of the N_2 data set.

It is also significant that measurement of soil N enrichment by the ^{15}N-gas nonequilibrium technique is nondestructive. Others have measured flux of N_2O from ^{15}N-labeled soil by mass spectrometry, using a technique that relies on destructive sampling to measure soil ^{15}N (16). In our experiment, destructive sampling would have compromised our ability to measure flux repeatedly over the same plot. Furthermore, destructive sampling requires assumptions about how and where the denitrifying pool is distributed in the soil volume. In contrast, the nonequilibrium technique assumes only that the ^{15}N pool undergoing denitrification is uniformly labeled. For our field experiment we conclude that the denitrifying pool was uniformly labeled, since measurements of flux by the nonequilibrium technique agreed with measurements by gas chromatography. Significant failure of pool uniformity should result in flux measurements by the nonequilibrium technique that significantly underestimate those made by gas chromatography (15, 28).

Another advantage of applying the ^{15}N-gas nonequilibrium technique simultaneously to N_2O and N_2 is that it allows more robust estimates of the relative proportion of N_2O and N_2 produced than if N_2O is measured by gas chromatography. If the soil mineral N pool undergoing denitrification is not uniformly labeled, N_2 flux by the nonequilibrium technique will be underestimated. However, a theoretical model of underestimation (14) shows that N_2O flux by the nonequilibrium technique will be underestimated by about the same proportion as for N_2, assuming the two gases derive from a common soil mineral pool and that the ^{15}N enrichment of their atmospheric pools is similar. Therefore, a proportional quantity such as N_2O mole fraction ($N_2O/[N_2O + N_2]$) would be accurate, despite pool nonuniformity. The point is moot for our study, since the denitrifying pool was apparently uniform (see above). N_2O mole fraction ranged from 0.004 to 0.14 but was 0.008 ± 0.004 for the three incubations where N_2 was greater than the estimated detection limit.

Although N_2 may be measured indirectly using the acetylene inhibition technique (29), separate incubations must be used if N_2O is also to be measured. Since spatial variability of denitrification in the field can exceed 500% CV (30) use of separate incubations may be an important source of error when comparing relative production of N_2O and N_2 in the field. Use of the ^{15}N-gas nonequilibrium technique for both gases allows a comparison of relative flux from a single chamber, as in our study.

One weakness of the design of our field experiment was its relatively low detection limit for N_2 relative to N_2O. N_2 sensitivity might have been improved by decreasing the height of the soil chamber relative to area, but in our study the consequent reduction in chamber volume would have conflicted with our need for very large (0.5 L) headspace samples for $^{15}N-N_2O$ analysis. We anticipate that better designs will be facilitated by technological improvements that reduce minimum sample size and run time for ^{15}N analysis of N_2O. Preliminary data suggest that addition of a cryofocusing step to our analytical method reduces the required sample size to 0.1 L, when analyzing atmospheric air for the isotopic composition of N_2O.

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Appendix 1

Stevens et al. (17) use the same instrumental configuration (CF-IRMS) for analysis of N_2 and N_2O. For calculation of flux, however, the nonequilibrium approach is used for N_2, while concentration change is used for N_2O (20).

Appendix 2: Estimation of Flux from *d*

Since nonequilibrium equations estimate d (the fraction of mixed gas derived from the soil source), estimation of

absolute flux requires some estimate of absolute abundance. If a represents gas from the atmosphere (preexisting headspace gas) and p represents gas derived from the soil mineral pool, then

$$d = p/[a + p] \quad (1)$$

Therefore

$$p = d * [a + p] \quad (2)$$

alternatively

$$p = da/[1\text{-}d] \quad (3)$$

Since concentration and therefore actual volume of headspace N_2 hardly changes during a typical incubation

$$[a + p] \approx a \quad (5)$$

and therefore

$$p \approx da \quad (6)$$

For N_2O flux, however, eq 3 must be used rather than eq 6; a is the average abundance of N_2O in the atmosphere ($\sim$310 ppb_v) multiplied by chamber volume. Alternatively, concentration can be measured by gas chromatography at the beginning and end of an incubation, so exact values of a (beginning; eq 3) and [a + p] (end; eq 2) are known. In the reported field demonstration, similar results were obtained whether eq 3 was used with an estimated a, eq 3 was used with a measured a, or eq 2 was used with a measured [a + p].

Appendix 3

Following Stevens et al. (17) and their citation, we calculate minimum detectable change in headspace concentration for N_2O as three times the standard deviation of the analysis, based on an atmospheric concentration of 300 ppb_v. CVs (coefficient of variation = standard deviation/mean) for gas chromatographic analysis of N_2O may vary, but 1% seems conservative. "Change in headspace concentration" is taken as positive, since ^{15}N methods are generally not suitable for measuring negative flux (i.e., soil consumption of N_2O).

Literature Cited

(1) Bouwman, A. F. In Soils and the greenhouse effect; Bouwman, A. F., Ed.; John Wiley & Sons: New York, 1990; p 61.
(2) Hahn, J.; Crutzen, P. J. Philos. Trans. Royal Soc. London 1982, B296, 521.
(3) Schimel, D.; 26 others. In Climate Change 1995: The Science of Climate Change; Houghton, J. T., Meira Filho, L. G., Callander, B. A., Harris, N., Kattenberg, A., Maskell, K., Eds.; Cambridge University Press: Cambridge, 1996; p 65.
(4) Rolston, D. E.; Sharpley, A. N.; Toy, D. W.; Broadbent, F. E. Soil Sci. Soc. Am. J. 1982, 46, 289.
(5) Letey, J.; Valoras, N.; Hadas, A. J. Environ. Qual. 1980, 9, 227.
(6) Myrold, D. D. In Denitrification in Soil and Sediment; Revsbech, N. P., Sørensen, J., Eds.; Plenum Press: New York, 1990; p 181.
(7) Mosier, A. R.; Klemedtsson, L. In Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties; Weaver, R. W., Angle, J. S., Bottomley, P. S., Eds.; Soil Science Society of America: Madison, 1994; p 1047.
(8) Hauck, R. D.; Melsted, S. W.; Yankwich, P. E. Soil Sci. 1958, 86, 287.
(9) Hauck, R. D.; D. R. Bouldin. Nature (London) 1961, 191, 871.
(10) Siegel, R. S.; Hauck, R. D.; Kurtz, L. T. Soil Sci. Soc. Am. J. 1982, 46, 68.
(11) Mulvaney, R. L.; Kurtz, L. T. Soil Sci. Soc. Am. J. 1982, 46, 1178.
(12) Mulvaney, R. L. Soil Sci. Soc. Am. J. 1984, 48, 690.
(13) Mulvaney, R. L.; Boast, C. W. Soil Sci. Soc. Am. J. 1986, 50, 360.
(14) Bergsma, T. T.; Bergsma, Q. C.; Ostrom, N. E.; Robertson, G. P. Soil Sci. Soc. Am. J. 1999, 63, 1709.
(15) Arah, J. R. M. Soil Sci. Soc. Am. J. 1992, 56, 795.
(16) Brooks, P. D.; Herman, D. J.; Atkins, G. J.; Prosser, S. J.; Barrie, A. In Agricultural Ecosystem Effects on Trace Gases and Global Climate Change; American Society of Agronomy: Madison, 1993; p 193.
(17) Stevens, R. J.; Laughlin, R. J.; Atkins, G. J.; Prosser, S. J. Soil Sci. Soc. Am. J. 1993, 57, 981.
(18) Mulvaney, R. L.; Kurtz, L. T. Soil Sci. Soc. Am. J. 1984, 48, 596.
(19) Mulvaney, R. L.; Vanden Heuvel, R. M. Soil Sci. Soc. Am. J. 1988, 52, 1332.
(20) Stevens, R. J.; Laughlin, R. J.; Malone, J. P. Soil Biol. Biochem. 1998, 30, 541.
(21) Arah, J. R. M. Soil Biol. Biochem. 1997, 29, 1295.
(22) Hayes, J. M. In Organic Geochemistry of Contemporaneous and Ancient Sediments; Meinschein, W. G., Ed.; Society of Economic Paleontologists and Minerologists: Bloomington, 1993; p 5-1.
(23) Kaspar, H. F.; Tiedje, J. M. In Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties; Weaver, R. W., Angle, J. S., Bottomley, P. S., Eds.; Soil Science Society of America: Madison, 1994; p 223.
(24) Crill, P. M.; Butler, J. H.; Cooper, D. J.; Novelli, P. C. In Biogenic Trace Gases: Measuring Emissions from Soil and Water; Matson, P. A., Harriss, R. C., Eds.; Blackwell Science: Oxford, 1995; p 164.
(25) Livingston, G. P.; Hutchinson, G. L. In Biogenic Trace Gases: Measuring Emissions from Soil and Water; Matson, P. A., Harriss, R. C., Eds.; Blackwell Science: Oxford, 1995; p 14.
(26) Mulvaney, R. L.; Kurtz, L. T. Soil Sci. Soc. Am. J. 1985, 49, 787.
(27) Williams, E. J.; Hutchinson, G. L.; Fehsenfeld, F. C. Global Biogeochemical Cycles 1992, 6, 351.
(28) Boast, C. W.; Mulvaney, R. L.; Baveye, P. Soil Sci. Soc. Am. J. 1988, 52, 1317−1322.
(29) Knowles, R. In Denitrification in Soil and Sediment; Revsbech, N. P., Sørensen, J., Eds.; Plenum Press: New York, 1990; p 151.
(30) Folorunso, O. A.; Rolston, D. E. Soil Sci. Soc. Am. J. 1984, 48, 1214.

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