In Situ Quantification of Biological N₂ Production Using Naturally Occurring $^{15}$N$^{15}$N

Laurence Y. Yeung,*† Joshua A. Haslun,‡ Nathaniel E. Ostrom,‡ Tao Sun,† Edward D. Young,§ Maartje A. H. J. van Kessel,∥ Sebastian Lücke,¶ and Mike S. M. Jetten‖

†Department of Earth, Environmental and Planetary Sciences, Rice University, Houston, Texas 77005, United States
‡Department of Integrative Biology and Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, Michigan 48824, United States
§Department of Earth, Planetary, and Space Sciences, University of California-Los Angeles, Los Angeles, California 90095, United States
∥Department of Microbiology, Radboud University, Nijmegen 6525 AJ, The Netherlands

ASDTRACTION: We describe an approach for determining biological N₂ production in soils based on the proportions of naturally occurring $^{15}$N$^{15}$N in N₂. Laboratory incubation experiments reveal that biological N₂ production, whether by denitrification or anaerobic ammonia oxidation, yields proportions of $^{15}$N$^{15}$N in N₂ that are within 1‰ of that predicted for a random distribution of $^{15}$N and $^{14}$N atoms. This relatively invariant isotopic signature contrasts with that of the atmosphere, which has $^{15}$N$^{15}$N proportions in excess of the random distribution by 19.1 ± 0.1‰. Depth profiles of gases in agricultural soils from the Kellogg Biological Station Long-Term Ecological Research site show biological N₂ accumulation that accounts for up to 1.6% of the soil N₂. One-dimensional reaction-diffusion modeling of these soil profiles suggests that subsurface N₂ pulses leading to surface emission rates as low as 0.3 mmol N₂ m⁻² d⁻¹ can be detected with current analytical precision, decoupled from N₂O production.

INTRODUCTION

Biological N₂ production constitutes the main mechanism through which fixed nitrogen is returned to the atmosphere. While many methods have been developed for measuring N₂ production in the field, obtaining accurate estimates of ecosystem fixed-nitrogen loss remains a challenge. Field-based techniques often require nutrient amendments (e.g., $^{15}$N-labeled nitrate), manipulation of biochemical pathways (e.g., C₂H₂ inhibition of nitrous oxide reductase), or sampling and incubation of soil cores, all of which introduce poorly constrained uncertainties. For example, in nutrient amendment studies, the fraction of extant nitrogen substrate utilized must be accounted for, but it is often difficult to constrain. Moreover, biological N₂ production can be stimulated by substrate addition, biasing measurements based on this approach. Soil-core incubations to evaluate N₂ production may not require nutrient amendments, but instead require that the extant gases be replaced with a gas mixture to reduce or replace the ambient N₂ background. Ultimately, this suite of methods for quantifying N₂ production rates can only probe short-term and potential rates of denitrification and other nitrogen-loss processes. Importantly, they may not integrate variation in activity that occurs over longer time scales at a given sampling site. Passive in situ measurements are rare, and fraught with a different set of complications: a recent attempt to use N₂/Ar ratios to probe excess N₂ production in situ found that physical fractionation of gases, combined with insufficient sensitivity, would likely preclude its widespread application. Stable isotopes of nitrogen at natural abundance levels could in principle be used to determine the amount of biologically produced N₂ in soil gases as well. Variations in the $^{15}$N/$^{14}$N ratio of N₂, reported as a δ-value in per mil (‰) relative to atmospheric N₂,

$$\delta^{15}N = \frac{^{15}R_{sample} / ^{15}R_{atm} - 1}{^{15}R_{atm}}$$

(1)

$$^{15}R = \frac{^{15}N}{^{14}N}$$

(2)

can be caused by variability in the chemistry of N₂ cycling, substrate δ$^{15}$N, and physical transport. Nevertheless, a large isotopic contrast may exist between biological and atmospheric...
**Experimental Methods**

Isotopic analyses were performed on the ultrahigh resolution Nu Instruments Panorama mass spectrometer at the University of California, Los Angeles according to methods described previously.\(^{14,18}\) The uniquely high resolution of the instrument allows the simultaneous measurement of \(^{14}\)N\(^{15}\)N/\(^{14}\)N\(^{14}\)N and \(^{15}\)N\(^{15}\)N/\(^{14}\)N\(^{14}\)N ratios at \(m/z = 29\) and 30, with near-baseline resolution of \(^{15}\)N\(^{15}\)N from \(^{15}\)O\(^{16}\)O and \(^{12}\)C\(^{16}\)O at \(m/z = 30\). N\(_2\) gas samples (20–50 \(\mu\)mol) were isolated from experimental headspace and soil-derived gases using cryogenic purification on a high-vacuum sample preparation line followed by gas chromatographic separation from O\(_2\) and Ar before isotopic analysis. Cryogenic purification removes condensable gases (e.g., CO\(_3\) and some hydrocarbons) and was accomplished by passing the gas through a stainless-steel U-trap submerged in liquid nitrogen (\(–196^\circ\)C). The gas was then condensed onto silica gel pellets at \(–196^\circ\)C within the sample-injection loop of the gas-chromatographic system. N\(_2\) gas was separated from O\(_2\) and Ar using a molecular sieve 5A column (3 m \(\times\) 1/8” OD) followed by a HayeSep D column (2 m \(\times\) 1/8 in. OD) inline, all with a 20 mL min\(^{-1}\) He flow rate at 25 \(^\circ\)C. The sample gases, air, and high-temperature standards of N\(_2\) (which were heated at 800 \(^\circ\)C for 24–48 h over strontium nitride) were purified the same way and analyzed during the same analytical sessions. Analytical precision for replicate air samples during these sessions was \(\pm 0.006\%e\) for \(\delta^{15}\)N and \(\pm 0.08\%e\) for \(\Delta_{30}\).

To determine the \(\Delta_{30}\) signatures of N\(_2\) produced by anammox bacteria, headspace outflows from several anammox bioreactors at Radboud University were sampled. Outflows from bioreactors containing enrichment cultures of the genera Candidatus Kuenenia\(^{19}\) and Ca. Brocadia\(^{20}\) (both freshwater genera), as well as Ca. Scalindua\(^{21}\) (a marine genus) were sampled using a 8 mL sampling loop made of a 1/4 in. OD stainless steel tube. The gas mixture was transferred cryogenically to a pre-evacuated sample finger filled with silica gel at \(–196^\circ\)C for 15 min before flame-sealing. All enrichment cultures at Radboud University were grown on the same NH\(_4\)SO\(_4\) + NaNO\(_2\) substrates, which had \(\delta^{15}\)N values of \(-0.5 \pm 0.3\%e\) and \(-26.2 \pm 0.3\%e\), respectively. Atmospheric contamination was monitored using gas chromatography–mass spectrometry of the outflow, using O\(_2\) (\(m/z = 32\)) as a proxy. A correction for air-N\(_2\) contamination in the bioreactor headspace was calculated from the O\(_2\) signal and a proportionality coefficient determined through a series of volumetrically calibrated mixtures of air in the 95% Ar/5% CO\(_2\) mixture used to flush the bioreactors. Measured air contamination varied between bioreactors, ranging from 0.6% for Kuenenia to 12.3% for Scalindua outflows, as a result of variable anammox activity compared to the flushing flow rate. After correction for background contamination (0.12–2.40\%e for \(\delta^{15}\)N and 0.1–2.3\%e for \(\Delta_{30}\)), duplicate collections showed reproducibility in \(\delta^{15}\)N and \(\Delta_{30}\) within \(\pm 0.01\%e\) and \(\pm 0.3\%e\), respectively.

Incubations of natural soils were performed to determine the \(\Delta_{30}\) signatures of N\(_2\) produced by natural biological communities. Soils from three agricultural treatments at the Kellogg Biological Station (KBS) Long-Term Ecological Research site were used for these experiments. Soils at the site belong to the Kalamazoo series, which are fine-loamy, mixed mesic Typic Hapludalfs.\(^{22}\) Soils T1 and T2 are agricultural soils that have been under an annual corn–soybean–winter wheat rotation since 1989, with T1 conventionally tilled with a chisel plow and T2 being no-till. Soil T7 comes from a native early successional old field community (containing grasses, shrubs, and trees) that was established in 1989 and has been maintained by an annual spring burn since 1997. Incubations of 25-g soil samples were conducted in 125 mL glass serum bottles that were crimp-sealed using butyl rubber caps.
rubber stoppers (Geomicrobial Technologies, Inc., Ochelata, OK, U.S.A.). Initially, after saturating the dried soils, an anaerobic headspace was created by sparging with He. The soils were then allowed to denitrify for 7–10 d to remove any initial oxidized N. At that point, the headspace was sparged again with He and then inoculated with glucose (0.3 mL, 1 M) and NaN3 substrate (1 mL, 0.3 M; $\delta^{15}N = 5.4\%$). Production of N2 was allowed to proceed for 96 h to ensure enrichment had occurred.

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Production of N2 was allowed to proceed for 96 h to ensure enrichment had occurred. Anammox enrichment cultures produced N2 with $\Delta_{30}$ values close to, but slightly different from the stochastic expression of hydrazine synthase enzyme. In any case, the $\Delta_{30}$ values for N2 produced by freshwater anammox genera are close to that expected from combinatorial isotope effects: the contrast in isotopic compositions between the NaN3 ($\delta^{15}N = -26.2\%$) and NH4SO4 ($\delta^{15}N = -0.2\%$) substrates, by itself, would yield $\Delta_{30} = -0.2\%$, close to the mean measured values of $-0.2 \pm 0.1\%$ and $-0.5 \pm 0.3\%$ (σ) for Ca. Kuenenia and Ca. Brocadia, respectively. Isotopic fractionation during biological uptake may cause additional variability in the $\delta^{15}N$ value of the assimilated substrates, but the $\Delta_{30}$ value of the N2 produced is not expected to deviate more than ~1% from zero because the combinatorial effect is a relatively weak function of the substrate $\delta^{15}N$ contrast.

An aerobic incubation of KBS soils yielded N2 with $\Delta_{30}$ values indistinguishable from the stochastic distribution of isotopes (i.e., all within 0.2%σ; see Table 1). Unlike in previous axenic laboratory cultures of denitrifying bacteria, no statistically significant dependence on reaction extent or $\delta^{15}N$ values was observed ($p = 0.2$–0.4 for a slope of zero, depending on the soil; see Table S1 of the Supporting Information, SI).

Compiling these results with those from earlier experiments on bacterial denitrifiers shows that biological N2 production yields $\Delta_{30}$ values between $-0.7\%$ and $+1.4\%$, with a weak dependence, if any, on bulk $\delta^{15}N$ values (Table S1). Moreover, the lack of $\Delta_{30}$ fractionation during biological nitrogen fixation suggests that it preserves $\Delta_{30}$ values in the N2 residue. Atmospheric N2, in contrast, is characterized by $\Delta_{30} = 19.1 \pm 0.1\%$ (Figure 1).

The origins of this correlation were not investigated, but deserve further scrutiny: the apparent difference in $\Delta_{30}$ value between freshwater and marine species may point to a different biochemistry related to the gene organization and subsequent expression of hydrazine synthase enzyme. In any case, the $\Delta_{30}$ values for N2 produced by freshwater anammox genera are close to that expected from combinatorial isotope effects: the contrast in isotopic compositions between the NaN3 ($\delta^{15}N = -26.2\%$) and NH4SO4 ($\delta^{15}N = -0.2\%$) substrates, by itself, would yield $\Delta_{30} = -0.2\%$, close to the mean measured values of $-0.2 \pm 0.1\%$ and $-0.5 \pm 0.3\%$ (σ) for Ca. Kuenenia and Ca. Brocadia, respectively. Isotopic fractionation during biological uptake may cause additional variability in the $\delta^{15}N$ value of the assimilated substrates, but the $\Delta_{30}$ value of the N2 produced is not expected to deviate more than ~1% from zero because the combinatorial effect is a relatively weak function of the substrate $\delta^{15}N$ contrast.

### RESULTS AND DISCUSSION

$\Delta_{30}$ Values from Biological N2 Production Are Near Zero. Anammox enrichment cultures produced N2 with $\Delta_{30}$ values close to, but slightly different from the stochastic distribution of isotopes (Table 1). Nitrogen gas produced by the two freshwater genera are characterized by $\Delta_{30} < 0$ (i.e., N2 was “anticlumped”), while N2 produced by the marine Ca. Scalindua enrichment had $\Delta_{30} = 1.0 \pm 0.3\%$, indistinguishable from an equilibrium distribution of $^{15}N$ isotopes at culturing temperatures (i.e., 1.0% at 35°C). A positive correlation between $\Delta_{30}$ and $\delta^{15}N$ values was observed when all anammox culture data are considered together ($R^2 = 0.86$, $p = 0.0009$).

### Table 1. Clumped-Isotope Composition of N2 ($\pm 1\sigma$) Derived from Experimental Cultures of Denitrifying or Anammox Bacteria

| Substrate                | $\Delta_{30}$ (%ε) | n  | Reference       |
|--------------------------|---------------------|----|-----------------|
| natural soils            |                     |    |                 |
| KBS T1 (conventional agricultural) | KNO$_3$            | $-0.1 \pm 0.1$ | 3   | this work       |
| KBS T2 (no-till agricultural)      | KNO$_3$            | $-0.1 \pm 0.3$ | 3   | this work       |
| KBS T7 (early successional)    | KNO$_3$            | $-0.2 \pm 0.2$ | 4   | this work       |
| anammox enrichment cultures|                     |    |                 |
| *Kuenenia* spp.           | NH$_4$SO$_4$ + NaNO$_2$ | $-0.2 \pm 0.1$ | 3   | this work       |
| *Brocadia* spp.           | NH$_4$SO$_4$ + NaNO$_2$ | $-0.5 \pm 0.3$ | 2   | this work       |
| *Scalindua* spp.          | NH$_4$SO$_4$ + NaNO$_2$ | $1.0 \pm 0.3$  | 3   | this work       |
| denitrifying bacteria     |                     |    |                 |
| *Pseudomonas* *stutzeri*  | KNO$_3$            | $0.9 \pm 0.4$  | 4   | 14              |
| *Paracoccus* *denitrificans* | KNO$_3$            | $0.6 \pm 0.2$  | 5   | 14              |

Using $\Delta_{30}$ Values to Detect Biological N2 Fraction in Soil Gas. Due to the large and relatively invariant $\Delta_{30}$ contrast between atmospheric and biologically produced N2, we suggest here that $\Delta_{30}$ values in N2 can be used to quantify biologically produced N2 in soils via mass balance. To illustrate this concept, we first write the two-component mixing equations for the N2 isotopologue ratios in soil, $^{29}R_{\text{soil}}$ and $^{30}R_{\text{soil}}$ in terms of the biological N2 fraction ($f_{\text{bio}}$) and the N2 isotopologue ratios of atmospheric and biological N2 (subscripts “atm” and “bio,” respectively):

$$^{29}R_{\text{soil}} = (1 - f_{\text{bio}})^{29}R_{\text{atm}} + f_{\text{bio}}^{29}R_{\text{bio}}$$

$$^{30}R_{\text{soil}} = (1 - f_{\text{bio}})^{30}R_{\text{atm}} + f_{\text{bio}}^{30}R_{\text{bio}}$$
Therefore, analytical uncertainty dominates coming from natural communities of biologically produced N2 has the same 15N/14N ratio as that of mates of when the true importance as of uncertainty of +36% and uncertainty of uncertainty of +36% when added in quadrature, but they quickly decrease in range of values close to different from bio decreases: for bio = 0.1 (resulting in a cumulative uncertainty of 0.1%). The expected range of 15N values close to the atmosphere, i.e., the range observed in laboratory experiments—results in an additional ±6% relative uncertainty in fbio (e.g., an error of ±0.006 for fbio = 0.1). Both errors are similar to that contributed by analytical uncertainty for fbio = 0.1 (resulting in a cumulative uncertainty of ±10% if added in quadrature), but they quickly decrease in importance as fbio decreases: for fbio = 0.01, analytical uncertainty of ±0.08% in Δ30 results in an asymmetrical uncertainty of +36% and −56% fbio i.e., fbio = 0.010±0.004. Therefore, analytical uncertainty dominates Δ30-based estimates of fbio for fbio < 0.1. Current analytical uncertainties suggest that soil gas containing ≥1% biological N2 will be detectable in Δ30-sul values.

To test this concept, we obtained two depth profiles of δ15N and Δ30 values in N2, along with N2O concentrations, from a monolith lysimeter installed in the KBS Interactions site. We found that many Δ30-sul values were less than or equal to Δ30-atm (Figure 3 and Table S2), ranging from 18.8‰ to 19.1‰. One sample analysis (34 cm depth on 10/11/17) was rejected based on apparent contamination that resulted in an abnormally elevated Δ30 value (4σ above the mean atmospheric value measured during the analytical session). The largest Δ30-sul depletions (−0.3 ± 0.1‰ relative to Δ30-atm), observed in both profiles, correspond to 16.5±0.5% of soil N2 at those depths being derived from biological processes. Soil-N2 δ15N values were equal to or slightly lower than the atmospheric value, although they differed between profiles: the profile obtained in July 2018 had δ15N values close to the atmospheric value, while the profile obtained in October 2017 had subatmospheric δ15N values ranging from −0.4 to −0.6‰. N2O concentrations increased nearly monotonically with increasing depth, with values exceeding 1000 parts per billion (ppb) at 170 cm depth (Figure 4). Taken together, these data imply an active nitrogen cycle and the presence of biological N2 in these soils.

Gas Diffusion and Denitrification Hot-Spots Can Explain Observed Soil Δ30 Profiles. A further understanding of the chemical and isotopic signatures measured in the soil gas can be obtained using a one-dimensional diffusion-reaction model based on Fick’s second law:

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial z^2} + J(z, t)
\]

where D is the effective gas diffusivity and J(z, t) is the production rate of a gas, which may be depth- and time-dependent. We treat the soil-gas system as a diffusive column ventilated to the atmosphere at the top (z = 0) and with zero permeability at the bottom (z = 180 cm). At steady state (\(\partial C/\partial t = 0\)) the depth profile is described by \(\delta C/\delta z = −J/D_2\), because J and D2 are positive as defined, concentration depth profiles at steady state should monotonically decrease toward the atmospheric value. Isotopic tracers may increase or
decrease toward the top depending how they are defined, but the change with depth should be monotonic toward the atmospheric value.

The depth profiles are not in steady state with respect to N₂. At steady state, deeper soil-gas would have accumulated low-Δ30 biological signals over time, resulting in Δ30,soil values increasing from depth to the surface. The N₂O depth profiles show accumulation at depth, but the N₂ profiles do not (Figures 3 and 4). Instead, Δ30,soil values are close to atmospheric values at depth, decrease at mid-depths, and return to atmospheric values at the surface. Pulsed biological N₂ production over a limited depth range is required to reproduce these mid-depth minima in Δ30,soil values. Specifically, a quiescent period with respect to N₂ production, which ventilates the soil down to 170 cm, must precede the pulse. Quantitative ventilation is not necessary, however; the quiescent period need only be long enough to dilute remnant Δ30,soil signals from earlier events beyond the limits of detection (∼5 days for the expected diffusivities; see below). Denitrification “hot moments” related to heterogeneities in soil moisture and organic carbon availability²⁸,²⁹ have the appropriate temporal and spatial variability. The contrast between N₂ and N₂O depth profiles suggest that their production during these hot moments can be temporally decoupled. Moreover, the accumulation of N₂O at depth argues against ventilation via gas exchange at the lysimeter—soil interface as the origin of the nonsteady-state Δ30,soil depth profile.

The shapes of the Δ30,soil depth profiles can be reproduced by solving eq 10 using a 0.1-day N₂ production pulse of Gaussian shape (1 cm full width at half-maximum), followed

![Figure 3](image1.png)

**Figure 3.** Depth profiles of Δ30 and δ¹⁵N values in N₂ drawn from the same monolith lysimeter at the KBS LTER Interactions Experiment site on 7/18/18 (A) and 10/11/17 (B). Mean measured atmospheric Δ30 values were 19.04 ± 0.03‰ (1 s.e.m., n = 5) during the analysis period (dashed lines). Solid lines show depth profiles calculated using a 1-D diffusion-reaction model for each sampling date that are consistent with the Δ30 data (10/11/17 profile offset by −0.4‰). Note that these best-fit profiles for Δ30 may not be unique solutions due to the number of adjustable parameters in the model, e.g., the duration, width, and depth distribution of the assumed biological N₂ pulse. Shaded areas therefore represent the range of N₂ production rates that describes the analytical 1σ of Δ30 values (i.e., +25% and −30% relative to the solid lines). Dashed lines denote isotopic compositions in the free atmosphere.

![Figure 4](image2.png)

**Figure 4.** Depth profiles of N₂O concentrations from the same samples as shown in Figure 3, along with illustrative steady-state profiles for uniform N₂O production rates and 5% gas-filled porosities (Dz = 0.0026 cm² s⁻¹)³¹,³².

| sampling date | center depth (cm) | pulse peak (nmol N₂ cm⁻³ s⁻¹) | sampling lag (h) | N₂ production (mmol N₂ m⁻²) |
|---------------|------------------|-------------------------------|-----------------|----------------------------|
| 10/11/17      | 59               | 1.2                           | 1.9             | 5.7                        |
| 7/18/18       | 37               | 2.6                           | 26.4            | 12.9                       |

“Pulses are Gaussian (1 cm full width at half maximum), occurring for 0.1 days.
by a small time lag between the N₂ pulse and sampling (see Table 2). Here, we assume an air-filled porosity, ε, of 0.05—resulting in a calculated soil diffusivity of \( D_z = 0.0039 \text{ cm}^2 \text{s}^{-1} \) for N₂—and \( \Delta_{30,\text{bio}} = 0 \). The assumed ε value is within the plausible range for these soils so it is appropriate for illustrative purposes. Using these parameters, the modeled \( \Delta_{30,\text{soil}} \) depth profile for July 2018 (the best-fit curve using a least-squares algorithm) reflects a depth-integrated gross production of 10.4 mmol N₂ m⁻² remaining in the soil after a 12.9 mmol N₂ m⁻² pulse (Figure 3A). The δ¹⁵N values of N₂ in that profile can be reproduced if the biological N₂ has δ¹⁵N = −11‰, on average. Note that the particular pulse shape, duration, and sampling lag used here (Table 2) is likely one of many that can explain the data and therefore not meant to be diagnostic; consequently, the profile is considered a local (rather than global) best fit. The depth-integrated gross production, however, should be robust for a given air-filled porosity. For example, the model can also yield a satisfactory fit of the data using a 10-fold longer initial N₂ pulse length of 1 day with a correspondingly weaker peak pulse peak of 0.3 mmol N₂ cm⁻³ s⁻¹ (instead of 2.6 mmol N₂ cm⁻³ s⁻¹). Both scenarios yield scaled-up N₂ pulse magnitudes (3–4 kg N ha⁻¹) that are consistent with peak N₂ fluxes observed in previous in lab and field experiments.

The modeled \( \Delta_{30} \) depth profile for October 2017 shown in Figure 3 implies a depth-integrated gross production of 5.7 mmol N₂ m⁻² using a pulse centered at 59 cm (Figure 3B, Table 2). Unlike for the July 2018 profile, the δ¹⁵N values of N₂ in that profile cannot be explained by biological N₂ production alone. Gravitational fractionation over this depth range would increase δ¹⁵N values by <0.01‰, so other physical mechanisms such as diffusive fractionation and/or water vapor flux fractionation may be especially important for this profile. Sampling took place the morning after a heavy overnight precipitation event (>40 mm), implicating a physical isotope effect such as a hydrologically driven diffusive influx of atmospheric N₂. These physical mechanisms will not affect \( \Delta_{30,\text{soil}} \) values significantly because they fractionate proportionately over a small δ¹⁵N range. In addition, solubility fractionation does not seem to affect clumped-isotope compositions of sparingly soluble gases, despite its effects on both elemental and bulk-isotope composition. Consequently, the \( \Delta_{30} \) tracer shows a clearer measure of biological N₂ production than the δ¹⁵N value of N₂.

If these biological N₂ pulses are isolated in time, then equivalent surface N₂ fluxes F can be derived from the reaction-diffusion models, and the results compared to previous measurements of KBS soils. For one-dimensional diffusion, the equation \( F = \left[ N_{2,\text{bio}} \right] \times D_z/z \) describes the instantaneous surface gas flux, where \( \left[ N_{2,\text{bio}} \right] \) is the concentration of biological N₂ and z is the depth from the surface. The results for \( z = 5 \) cm, the biological N₂ flux from the top 5 cm of soil, are shown in Figure 5. The flux \( F \) for the two profiles ranges from 0.1–2.9 mmol N₂ m⁻² d⁻¹ (3–81 mg N m⁻² d⁻¹) during the first 10 days after the pulse events, with a prolonged period of low, but nonzero flux lasting several times longer (e.g., \( F = 0.1–0.2 \) mmol N₂ m⁻² d⁻¹ for the 7/18/18 profile between 10 and 20 days after the pulse). These estimates are comparable to previous amendment-stimulated N₂ production rates from these soils. In particular, Bergma et al. (2001) reported surface N₂ fluxes of 0.2–2.0 mmol N₂ m⁻² d⁻¹ (6–55 mg N m⁻² d⁻¹) during a four-day experiment utilizing a surface flux chamber and an amendment of ¹⁵N-labeled KNO₃.

The model-derived fluxes strongly depend on the assumed air-filled porosity ε—which was not measured directly and can vary in time and space—so this agreement may be coincidental. Nevertheless, the two methods appear to yield results on the same order of magnitude. More well constrained in situ soil-atmosphere fluxes can be obtained with concurrent measurements of soil physical properties.

The only comparable in situ method for quantifying biological N₂ production in soils is the N₂/Ar method. Yang and Silver (2012) reported a relatively high detection limit of 3.9 mmol N₂ m⁻² d⁻¹ for surface-flux measurements, larger than the calculated peak surface fluxes shown in Figure 5. While the method can analytically resolve N₂ excesses of less than 0.1%, physical fractionation of N₂ and Ar in soils presents substantial systematic uncertainties in these environments. We hypothesize that measurements of N₂/Ar soil profiles may yield limited improvements in uncertainty because the physical mechanisms complicating the interpretation of δ¹⁵N values of N₂ (e.g., the water vapor flux fractionation) fractionate N₂/Ar ratios to a greater degree, offsetting any analytical sensitivity advantages. Soil \( \Delta_{30,\text{soil}} \) depth profiles, in contrast, are insensitive to physical fractionation, revealing evidence for biological N₂ production in soil profiles despite the lower analytical sensitivity of the method.

N₂ fluxes into the atmosphere can be derived from \( \Delta_{30,\text{soil}} \) profiles if soil physical properties (i.e., air-filled porosity and diffusivity) are determined independently. The method could be used to compare in situ production rates to incubation- and amendment-based methods in field studies, or to obtain independent estimates using an array of spatially dispersed observations across soil types and conditions. Time series of soil-gas profiles similar to those shown here, sampled through
lusiness or air-permeable tubing, would provide a long-term perspective on soil N₂ production dynamics, which is presently difficult to access without perturbing soil biogeochemistry and is useful for models.²¹ Analytical throughput (2–3 samples/day) and availability of instrumentation are currently limiting factors for the Δ30 approach, but the relatively long ventilation time scales of certain soils may still allow weekly to-monthly sampling to capture the impacts of hot moments. The initial results reported here suggest that Δ30,soil signals are sufficiently large that the approach can be used in future assessments of site- and ecosystem-scale loss of fixed nitrogen. Furthermore, the approach can also be applied to marine environments to investigate both the magnitude and mechanisms of fixed-nitrogen loss in low-oxygen zones.⁴² Finally, constraining biological N₂ production globally using Δ30,atm appears possible in principle if the terms related to upper-atmospheric chemistry in the global Δ30 budget—both the isotopic reordering rates and Δ30 endmembers—can be refined.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b00812.

Compilation of isotopic composition data for N₂ produced during pure- and enrichment-culture experiments reported here and in ref 14; isotopic composition data for N₂ and concentrations of N₂O in soil gases (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: leyung@rice.edu.

ORCID

Laurence Y. Yeung: 0000-0001-9901-2607

Notes

The authors declare no competing financial interest.

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