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New high precision approach for measuring $^{15}N-N_2$ gas fluxes from terrestrial ecosystems

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ABSTRACT

Dinitrogen (N_2) production from denitrification and anaerobic ammonium oxidation represents a loss of reactive nitrogen (N) from terrestrial and aquatic ecosystems to the atmosphere. The large ^{15}N additions required to detect $^{15}N_2$ production against the high atmospheric background precludes the use of the ^{15}N tracer technique in natural terrestrial ecosystems. We present an isotope ratio mass spectrometry technique that dramatically improves the precision of $^{15}N_2$ measurements. The approach uses gas chromatography to remove oxygen and gas purification techniques to remove water vapor and trace gases that can interfere with $^{15}N_2$ analysis. The analytical precision for manual gas sample injection was 0.018‰ $\delta^{15}N$; this translates to a minimum detectable N_2 flux of 0.12 ng-N g^{-1} h^{-1} over a 24 h incubation using our experimental parameters. We measured denitrification-derived N_2 production rates of 0.67 ± 0.04 ng N g^{-1} dry soil h^{-1} following the addition of 0.1 μg 98 atom % $^{15}N-NO_3^-$ g^{-1} dry soil to anaerobically-incubated soils; rates were significantly higher with the addition of 1.0 μg 98 atom % $^{15}N-NO_3^-$ g^{-1} dry soil ($p < 0.001$, $n = 5$), averaging 1.3 ± 0.03 ng N g^{-1} dry soil h^{-1}. The N_2:N_2O ratio at 24 h after ^{15}N addition was 48 ± 12 and 5.4 ± 1.9 for the 0.1 μg ^{15}N g^{-1} and 1.0 μg ^{15}N g^{-1} treatments, respectively. The decrease in the N_2:N_2O with the addition of only 1 μg ^{15}N g^{-1} underscores the need to use very low rates of ^{15}N addition to accurately characterize denitrification dynamics. This analytical advance will allow us to better estimate the N_2:N_2O ratio of denitrification, constrain ecosystem N budgets, and explore the mechanisms of and controls on N_2 production.

1. Introduction

The largest pool of nitrogen (N) on Earth resides in the atmosphere as dinitrogen (N_2). Anaerobic ammonium (NH_4^+) oxidation and denitrification convert biologically available, reactive N to the unreactive atmospheric N_2 pool (Tiedje, 1988; Vandegraaf et al., 1995; Yang et al., 2012). Terrestrial N_2 fluxes are highly uncertain due to the difficulty in detecting N_2 production against the high atmospheric background N_2 concentration (Groffman et al., 2006), and thus, terrestrial N budgets are poorly constrained (Seitzinger et al., 2006). An obligate intermediate of denitrification that can be released to the atmosphere is nitrous oxide, (N_2O), a potent greenhouse gas and catalyst for stratospheric ozone depletion. The N_2:N_2O ratio of denitrification end-products is not well-characterized, and the controls on the ratio are poorly understood. The ability to quantify N_2 production rates in terrestrial ecosystems would improve our estimates of terrestrial ecosystem N budgets as well as our understanding of terrestrial N_2O dynamics. This is particularly important in the face of global changes such as increased N deposition that are altering N cycling on the global and ecosystem scales (Vitousek et al., 1997).

The ^{15}N tracer technique is a powerful approach for quantifying rates of N cycling in both terrestrial and aquatic ecosystems. When used for measuring gas fluxes, a ^{15}N-labeled substrate is added to a soil or sediment sample, and the N_2 or N_2O flux is calculated from the accumulation of the ^{15}N label in the gaseous product pool (Hauck and Melsted, 1956). In most cases, the gaseous product pool is not in isotopic equilibrium, and thus, the N_2 or N_2O flux must be calculated from the change in abundances of singly- and doubly-^{15}N-labeled molecules. The isotope pairing technique is a form of the ^{15}N gas flux method developed in aquatic systems in which the

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accumulation of $^{14}N–^{15}N$ versus $^{15}N–^{15}N$ is indicative of different processes producing N_2, such as coupled nitrification-denitrification in sediments versus denitrification in the water column (Nielsen, 1992). This technique has been modified to quantify rates of N_2 production from anaerobic NH_4^+ oxidation versus denitrification (Spott and Stange, 2007; Trimmer et al., 2006).

In terrestrial ecosystems, a large amount of ^{15}N label is generally required to achieve a $^{15}N_2$ flux measurable by the ^{15}N tracer technique, depending on denitrification rates and the N_2:N_2O ratio of denitrification end-products. Typically 8–30 g N m^{-2} is applied, an amount roughly equivalent to 80–300 μg ^{15}N g^{-1} if we assume soil bulk density of 1 g cm^{-3} and tracer application to the top 10 cm of soil (Schlesinger, 2009; Stevens and Laughlin, 1998). In agro-ecosystems that regularly receive fertilizer N inputs, this ^{15}N application rate may not cause a disturbance to N dynamics. In natural ecosystems, however, this high ^{15}N application rate would represent a large perturbation to the N cycle and likely change denitrification rates as well as the N_2:N_2O ratio of denitrification end-products (Firestone et al., 1979; Weier et al., 1993). Ideally a much smaller amount of ^{15}N-label would be added relative to the background substrate pool, but the precision of current $^{15}N–N_2$ gas analysis methods generally requires large N additions that would likely alter denitrification dynamics.

A major challenge in accurately measuring $^{15}N–N_2$ is anomalously high *m/z* 30 readings (Atkins et al., 1992; Stevens et al., 1993). Oxygen can react with N in the ion source to produce nitric oxide (NO), another species with *m/z* 30 (Eyre et al., 2002; Giese, 1966). Currently, the most common approach for addressing this issue is using continuous flow isotope ratio mass spectrometry (CF-IRMS) with a copper (Cu) reduction furnace to reduce O_2 in the gas sample. While this approach is relatively easy to implement, it yields relatively poor precision with a minimum detectable change in $^{15}N–N_2$ of 0.0006 atom % (Silver et al., 2001). The minimum detectable N_2 flux depends on both the sampling methodology and the analytical precision, but the detection limit reported for this approach ranges from 0.4 to 1.2 mg N m^{-2} d^{-1} at 60 atom % $^{15}N–NO_3^-$ enrichment up to 120 mg N m^{-2} d^{-1} at 10 atom % $^{15}N–NO_3^-$ enrichment (Stevens and Laughlin, 1998; Stevens et al., 1993). This detection limit is sufficient only for measuring N_2 production in heavily fertilized agricultural systems where rates are typically greater than 100 mg N m^{-2} d^{-1} (Stevens and Laughlin, 1998) and in natural ecosystems receiving high levels of anthropogenic N deposition where rates can reach as high as 22 mg N m^{-2} d^{-1} (Butterbach-Bahl et al., 2002).

A gas chromatograph-IRMS (GC-IRMS) approach utilizing a molecular sieve 5Å column can separate N_2 and O_2 in gas samples such that O_2 (*m/z* 32 and 34) does not co-elute with N_2. This approach has not previously been used to remove O_2 from gas samples but simply to separate O_2 and N_2 to avoid direct interference of O_2 with $^{15}N–N_2$ analysis. For example, Roberts et al. (2000) used this approach to separate O_2 and N_2 to analyze $\delta^{18}O–O_2$ in atmospheric air samples; $\delta^{15}N–N_2$ could be analyzed in the same run as O_2 with a precision of 0.2‰. Atkins et al. (1992) reported a precision of 0.00001 ^{15}N atom % for 13 mL gas samples analyzed using GC-IRMS to separate O_2 and N_2. Lewicka-Szczebak et al. (2013) used a Cu reduction furnace to remove O_2 from gas samples followed by a molecular sieve column to separate residual O_2 from N_2, yielding a precision of 1‰. These approaches represent substantial improvements in the precision of $^{15}N–N_2$ analysis over the commonly used Cu reduction CF-IRMS approach. However, the detection limits are low enough only to measure N_2 production in natural terrestrial ecosystems with relatively high denitrification rates, Moreover, the large sample volumes required may preclude its use, particularly in studies utilizing controlled laboratory experiments with small incubation chamber headspace volumes.

Here we present a new GC-IRMS approach for high precision $^{15}N_2$ gas analysis that yields improved precision while also using small sample volumes. The basic principle of this approach is to quantitatively remove O_2 to minimize *m/z* 30 productions in the ion source and to remove trace gases that could contribute to *m/z* 28, 29, or 30. For example, carbon monoxide (CO) can contribute *m/z* 28 ($^{12}C^{16}O$), *m/z* 29 ($^{13}C^{16}O$, $^{12}C^{17}O$) or *m/z* 30 ($^{12}C^{18}O$, $^{13}C^{17}O$). Our objective was to (1) determine the stability and detection limit of our GC-IRMS approach, (2) demonstrate our ability to detect $^{15}N_2$ fluxes from soil using small $^{15}NO_3^-$ additions, and (3) determine if small $^{15}NO_3^-$ additions (<1.0 μg 98 atom % $^{15}N–NO_3^-$ g^{-1} dry soil) alter soil denitrification dynamics.

2. Materials and methods

2.1. Mass spectrometry

We used an IsoPrime 100 continuous flow isotope ratio mass spectrometer (IRMS) (Isoprime Ltd, Cheadle Hulme, UK) configured with universal triple collectors to measure *m/z* 28, 29, and 30. This IsoPrime model has a 100 V head amplifier with gain switching such that at a low gain setting the beam saturates at 100 nA and at a high gain setting the beam saturates at 1 nA. The IRMS is tuned daily using ultra-high purity (UHP) N_2 (Praxair, Richmond, CA) as a reference gas to optimize the accelerating voltage, extraction voltage, half plate voltage, and ZV plate voltage. The IRMS is interfaced with an IsoPrime trace gas analyzer (TG) that utilizes cryo-trapping to analyze ^{13}C, ^{15}N, and ^{18}O of carbon dioxide (CO_2), methane (CH_4), or nitrous oxide (N_2O).

We modified the TG to provide helium (He) carrier gas for $^{15}N_2$ analysis. The TG is equipped with two He inlets: the prep flow is used to flush sample gas from vials or bottles into the TG for online sample preparation, and the gas chromatography (GC) flow is used to carry the sample gas from the GC column into the IRMS. The two inlets have separate flow controllers that we used to set the He flow rate to 9 mL min^{-1}. We inserted a two-way brass ball valve with 1/8″ tube fitting connections into the GC flow line upstream of the flow controller such that, depending on the valve position, the He flow would either enter the sample preparation lines for trace gas analysis or for $^{15}N_2$ analysis. The prep flow line has a 1/16″ Swagelok tube fitting connection that would typically connect the TG to an autosampler via 1/16″ stainless steel tubing. We attached a separate section of tubing to connect the TG to a 12-port valve (model #EC12WE, Valco Instruments Co. Inc, Houston, TX) for $^{15}N_2$ analysis.

The 12-port valve is operated either in load or inject position (Fig. 1). The sample is injected manually from a 500 μL gas-tight syringe into a septum port consisting of a 1/8″ to 1/16″ stainless steel reducing union connected by a 2 cm length of 1/16″ stainless steel tubing (0.01″ inner diameter) to a zero volume filter. On the 1/8″ side of the union, the ferrules are removed from the nut and a rubber septum is inserted. The filter prevents septum debris from entering the 12-port valve. In load position, the septum port is connected to a 50 μL sample loop consisting of 0.003″ inner diameter 1/16″ stainless tubing with Silco coating. The total dead volume of the septum port is 19 μL so 400 μL sample is injected to ensure that the sample loop and dead volume are flushed more than three times with sample gas. The sample loop is connected to a pigtail vent tube consisting of the same 1/16″ tubing (15 μL volume) with the open end pointing down to minimize the loss of He out of the vent tube. The sample loop volume was chosen to maximize the sample peak height at a trap current setting of 200 μA