Soil dinitrogen (N$_2$) emissions represent the loss of nitrogen (N) from the biosphere to the atmospheric pool, thus completing the N cycle. Despite the importance of this flux, terrestrial ecosystem N budgets often do not include it$^{[1,2]}$ because it is difficult to measure against the high background atmospheric N$_2$ concentration.$^{[3]}$ In terrestrial ecosystems, N$_2$ is primarily produced via denitrification, a process by which nitrate (NO$_3$) is reduced to nitrous oxide (N$_2$O) and then subsequently to N$_2$. Nitrous oxide is a potent greenhouse gas and a catalyst for stratospheric ozone depletion. Thus, the relative amounts of N$_2$O and N$_2$ released to the atmosphere via denitrification can have major implications for atmospheric chemistry. Humans have drastically increased the amount of reactive N in the environment, stimulating soil N$_2$O emissions.$^{[4]}$ However, the effect of anthropogenic N loading on soil N$_2$ emissions is largely unknown.$^{[5,6]}$ In most managed ecosystems, N inputs greatly exceed the measured outputs, suggesting that a large amount of anthropogenic N is either stored in the ecosystem or released as N$_2$.$^{[7]}$ Accurate measurements of soil N$_2$ emissions are needed to better constrain ecosystem N budgets, elucidate controls on denitrification, and provide the data necessary to validate and improve models that predict ecosystem N losses as N$_2$O and N$_2$ emissions.$^{[8]}$

A wide range of approaches has been used to estimate soil N$_2$ emissions, but each approach has well-recognized limitations.$^{[3]}$ Acetylene (C$_2$H$_2$) inhibits the reduction of N$_2$O to N$_2$ via denitrification. Thus, N$_2$ emissions can be estimated from the difference in N$_2$O production in the presence and absence of C$_2$H$_2$. The C$_2$H$_2$ inhibition technique can underestimate N$_2$ emissions by decreasing the supply of NO$_3$ substrate via nitrification, through diffusion limitation of C$_2$H$_2$ to microsites of denitrification activity, and due to the ineffectiveness of C$_2$H$_2$ on some denitrifiers and under low NO$_3$ conditions.$^{[9,10]}$ The addition of $^{15}$N-labeled NO$_3$, the substrate for denitrification, can be used to trace the rate of NO$_3$ reduction to N$_2$. The use of this method is typically restricted to systems with high soil NO$_3$ concentrations, such as agricultural systems,$^{[11]}$ because a large amount of $^{15}$NO$_3$ is needed to detect a change in the $^{15}$N enrichment of the atmospheric N$_2$ pool. This approach can potentially stimulate, and thus overestimate, denitrification rates as well as change the relative amounts of N$_2$O and N$_2$ produced. The gas flow soil core method has been used to estimate N$_2$ emissions from intact soil cores.$^{[12,13]}$ While this method has been successfully applied in a laboratory setting, the technical requirements make this method impractical in a field setting.$^{[14]}$ Finally, attempts have been made to characterize the N$_2$/N$_2$O ratio of denitrification.
end-products for specific study sites so that soil N\textsubscript{2} emissions can be estimated from soil N\textsubscript{2}O emissions alone, which are relatively easy to measure with high resolution.\textsuperscript{[15,16]} However, the N\textsubscript{2}/N\textsubscript{2}O ratios are often highly variable\textsuperscript{[17]} and dependent on environmental conditions,\textsuperscript{[17,18]} resulting in large uncertainty in estimates of soil N\textsubscript{2} emissions derived by this method.\textsuperscript{[5]}

Several recent advances have contributed to our understanding of soil N\textsubscript{2} emissions. First, \textsuperscript{15}N isotope budgets can be used to constrain gaseous N losses based on mass balance models and, with measurements of NO\textsubscript{x} losses, soil N\textsubscript{2} emissions can be estimated at the ecosystem scale\textsuperscript{[15,19]} and at the global scale.\textsuperscript{[20]} This method integrates over time and space to avoid the need for high-resolution measurements of soil N\textsubscript{2} emissions but, as a result, it cannot provide insights into smaller scale patterns in emissions within ecosystems or with changing environmental conditions. Second, the \textsuperscript{15}N\textsubscript{2}O pool dilution method provides field measurements of gross N\textsubscript{2}O production and consumption that can be related to soil characteristics, such as soil O\textsubscript{2} and mineral N concentrations.\textsuperscript{[21]} Because this method measures N\textsubscript{2}O consumption from the loss of \textsuperscript{15}N\textsubscript{2}O tracer, it may not capture complete denitrification that occurs intracellularly. Thus, the measured N\textsubscript{2}O consumption rates may only be an index of soil N\textsubscript{2} emissions. These methods can be powerful tools for answering specific questions about N budgets or denitrification and, if used in conjunction with complementary methods that compensate for their limitations, can be used to answer a broader set of questions about denitrification to N\textsubscript{2} and the controls on the N\textsubscript{2}O/N\textsubscript{2} ratio.

All the methods presented thus far, except for the gas flow soil core technique, specifically measure N\textsubscript{2} production via denitrification, but recent studies suggest that there may be other important terrestrial N\textsubscript{2} production pathways. In marine and freshwater ecosystems, anaerobic ammonium (NH\textsubscript{4}) oxidation using nitrite (NO\textsubscript{2}) as an electron acceptor (termed anammox) accounts for up to 67% of N\textsubscript{2} production.\textsuperscript{[22–24]} Anammox has not been demonstrated to occur in soils,\textsuperscript{[25]} but the Planctomycetes responsible for anammox have been detected in soils.\textsuperscript{[26,27]} Recently, the production of N\textsubscript{2} by iron (Fe) reduction coupled to anaerobic NH\textsubscript{4} oxidation was demonstrated in humid tropical forest soils.\textsuperscript{[28]} This process, termed Feammox, may be important in highly weathered soils rich in poorly crystalline Fe. Thus, methods that specifically measure N\textsubscript{2} production via denitrification could underestimate gross N\textsubscript{2} production, but they would remain useful for studying rates of and controls on denitrification.

The N\textsubscript{2}/Ar technique is used widely in aquatic systems to measure net N\textsubscript{2} production, but is more challenging to apply in terrestrial ecosystems. This method uses argon (Ar), an inert gas, as a conservative tracer. Thus, measured changes in measured N\textsubscript{2}/Ar ratios can be attributed to changes in the abundance of N\textsubscript{2}. Membrane inlet mass spectrometry (MIMS) can be used to measure N\textsubscript{2}/Ar ratios with high throughput (20–30 samples per hour) and with 0.05% precision (in terms of coefficient of variation) so that N\textsubscript{2} fluxes as low as 0.7 mg N m\textsuperscript{-2} day\textsuperscript{-1} can be measured.\textsuperscript{[29]} The N\textsubscript{2}/Ar technique is more challenging to apply in terrestrial ecosystems because higher precision is necessary to detect changes in the N\textsubscript{2}/Ar ratio in air. The concentration of N\textsubscript{2} in air is approximately 50 times more than that at saturation in fresh water at 20 °C. In addition, soils on average exhibit 10 times lower denitrification rates than aquatic sediments.\textsuperscript{[30]} These factors together suggest that minimum detectable soil N\textsubscript{2} fluxes are approximately 500 times lower than minimum detectable sediment N\textsubscript{2} fluxes, and that MIMS does not have the precision to detect changes in N\textsubscript{2}/Ar ratios from soil gas fluxes. Paleoclimatologists have developed an analytical approach to measure N\textsubscript{2}/Ar ratios with per meg level precision (i.e., 10\textsuperscript{-3} per mil) by using isotope ratio mass spectrometry (IRMS) to reconstruct past climate from ice core bubbles.\textsuperscript{[31]} In addition, expected changes in the N\textsubscript{2}/Ar ratio due to soil N\textsubscript{2} emissions are so small that physical fractionation effects on the N\textsubscript{2}/Ar ratio that can confound estimates of soil N\textsubscript{2} emissions due to biological processes. In terrestrial ecosystems, these effects include thermal fractionation, solubility fractionation, and water vapor flux fractionation. Because these fractionation effects on the N\textsubscript{2}/Ar ratio are governed by physical effects, they can be modeled to remove their confounding effect on measured shifts in N\textsubscript{2}/Ar ratios. In this paper, we present the theoretical considerations of the N\textsubscript{2}/Ar approach for terrestrial N\textsubscript{2} fluxes and provide a preliminary test of the validity and accuracy of the N\textsubscript{2}/Ar technique for measuring surface soil N\textsubscript{2} fluxes under controlled laboratory conditions. Our goal was to explore the theoretical and analytical feasibility of this approach. This is a necessary first step to determine if the approach holds promise prior to the significant effort involved in optimizing the method for deployment under real-world conditions. The objectives of this study were to determine a preliminary detection limit of the N\textsubscript{2}/Ar method for soil fluxes and also to determine if observed changes in the N\textsubscript{2}/Ar ratio can be corrected for physical fractionation effects. While we refer to this method as the ‘N\textsubscript{2}/Ar method’ because N\textsubscript{2} is the gas of interest, when presenting methods and data, we will refer to δ Ar/N\textsubscript{2} according to the tradition of expressing isotope ratios (or in this case, elemental ratios) with the heavier isotope in the numerator.

**THEORY AND CALCULATIONS**

**Thermal fractionation**

In the presence of a temperature gradient, a mixture of N\textsubscript{2} and Ar will become fractionated because the heavier molecule, Ar, tends to reside on the colder end of the gradient while the lighter molecule, N\textsubscript{2}, tends to reside on the warmer end of the gradient.\textsuperscript{[32]} This phenomenon is known as thermal diffusion.\textsuperscript{[33,34]} Temperature changes during a surface flux measurement can affect the N\textsubscript{2}/Ar ratio via thermal diffusion by disturbing the temperature gradient from the soil to the atmosphere. We assume that the soil column and air are at thermal steady state initially and that they reach a new steady state by the time of the final gas sampling. We define thermal steady state as one in which the gas composition has come into equilibrium with the soil temperature profile. Because gas diffusion is typically faster than heat diffusion in soils, this equilibrium can be approximately attained even if the temperature profile itself is not at steady state. Thus, the thermal fractionation effect (expressed as δ Ar/N\textsubscript{2} in units of per meg) can be calculated as the difference in the thermal diffusion effect at the initial and final sampling time points.
At each time point, the thermal diffusion effect (in per meg units), $\delta_d$, can be calculated using the following equation,$^{[38]}$

$$\delta_d = \left[ \left( \frac{T_f}{T_i} \right)^2 - 1 \right] \times 10^6 \quad (1)$$

where $T_f$ is the soil temperature at bottom of soil (K), $T_i$ is the chamber air temperature (K), and $z$ is the thermal diffusion factor for N$_2$/Ar (Eqn. (1)).

We used an $z$-value of 0.078 for a mixture of N$_2$-Ar in atmospheric proportions at 293 K based on empirically determined values for different N$_2$-Ar mixtures and at different temperatures reported in the literature (see Supporting Information). This $z$-value translates to a thermal diffusion effect of 286 per meg per K.

**Solubility fractionation**

Temperature changes during surface flux measurements can also affect the N$_2$/Ar ratio because of the differing temperature-solubility curves for different gases dissolved in water (Fig. 1). We call this the solubility fractionation effect. Note that this is a transient solubility effect different from equilibrium solubility fractionation defined by geochemists. The magnitude of the solubility fractionation effect depends on soil moisture and the change from a given equilibrium state (Fig. 2). For example, at 10% gravimetric soil moisture, up to 0.5 °C changes in temperature from 20 °C result in <50 per meg shifts in the N$_2$/Ar ratio. However, at 40% gravimetric soil moisture, the solubility fractionation effect is 100 per meg for every 0.1 °C change in temperature.

To estimate the solubility fractionation effect, we first calculated the proportion of soil that is particles ($f_p$), water ($f_w$), and air ($f_a$) from the gravimetric soil water content ($\theta$, kg water kg soil$^{-1}$), bulk density ($\rho_b$, g cm$^{-3}$), and total soil porosity ($P_T$, m$^3$ voids m$^{-3}$ soil) using Eqs. (2)–(4). The sum of $f_p$, $f_w$, and $f_a$ must equal 1. Thus, in 1 m$^3$ of soil at standard temperature and pressure (STP), the amount of soil particles ($n_p$), water ($n_w$), and air ($n_a$) is equivalent to $f_p$, $f_w$, and $f_a$, respectively, in units of m$^3$ STP.

$$f_w = \theta * \rho_b \quad (2)$$

$$f_a = P_T - f_w \quad (3)$$

$$f_p = 1 - P_T \quad (4)$$

The total porosity was calculated from $\rho_b$, 1.42 g cm$^{-3}$, and particle density ($\rho_p$, 2.55 g cm$^{-3}$) determined for the diffusion box sand used in this experiment$^{[36]}$ (Eqn. (5)).

$$P_T = 1 - \frac{\rho_b}{\rho_p} \quad (5)$$

Hamme and Emerson$^{[37]}$ made high-precision measurements of N$_2$ and Ar solubility in distilled water from 0 to 30 °C. We used their equations to calculate N$_2$ and Ar Bunsen coefficients ($\beta$, L of gas dissolved per L of water) at the temperatures measured when the initial and final gas samples were taken. We calculated the change in N$_2$ contained in soil water, $\Delta n_{N2,w}$, using Eqn. (6) where $X_{N2}$ is the dry air mole fraction of N$_2$ (0.78084 m$^3$ STP N$_2$ m$^{-3}$ STP) from Glueckauf$^{[38]}$:

$$\Delta n_{N2,w} = (X_{N2} * \beta_{N2,final} * n_w) - (X_{N2} * \beta_{N2,initial} * n_w) \quad (6)$$

We calculated the change in N$_2$ contained in soil air, $\Delta n_{N2,a}$, using Eqn. (7) where $T_{STP}$ is the temperature at STP, 273.15 K; and $T_{initial}$ (K) is the average initial soil temperature from the five thermocouples weighted by soil depth:

$$\Delta n_{N2,a} = \left( \frac{X_{N2} * n_a * T_{STP}}{T_{initial}} \right) - \Delta n_{N2,w} \quad (7)$$

We scaled $\Delta n_{N2,a}$ to the volume of soil beneath the chamber, $\Delta n_{N2,a,*}$, using Eqn. (8), where $A$ is the basal area of the chamber in m$^2$ and $z$ is the soil depth in m:

$$\Delta n_{N2,a,*} = \Delta n_{N2,a} * A * z \quad (8)$$

![Figure 1](image1.png)

**Figure 1.** Temperature-solubility curves for N$_2$ (solid line) and Ar (dashed line). Bunsen coefficients were calculated from the fitting curves reported by Hamme and Emerson.$^{[37]}$

![Figure 2](image2.png)

**Figure 2.** Isolines and shading represent the solubility fractionation effect (in per meg units of $\delta Ar/N_2$) as a function of soil moisture (g H$_2$O g$^{-1}$ soil) and a change in soil temperature (°C) from an initial value of 20.0 °C.
We calculated the initial amount of N₂ in the chamber headspace, \( n_{N₂,\text{initial}} \) in m³ STP, using Eqn. (9) where V is the chamber volume in m³:

\[
n_{N₂,\text{initial}} = \left(\frac{V \times T_{\text{STP}}}{T_{\text{initial}}}\right) \times X_{N₂}
\] (9)

We then determined the final amount of N₂ in the chamber headspace, \( n_{N₂,\text{final}} \) in m³ STP, using Eqn. (10):

\[
n_{N₂,\text{final}} = n_{N₂,\text{initial}} + \Delta n_{N₂,\text{final}}
\] (10)

We performed the same calculations for Ar to determine \( n_{Ar,\text{initial}} \) and \( n_{Ar,\text{final}} \) in m³ STP. The dry air mole fraction of Ar was 0.009340 m³ STP Ar m⁻³ STP.

We estimated the initial Ar/N₂ ratio, in per meg, using Eqn. (11):

\[
\left(\frac{\delta \text{Ar}}{\text{N}_2}\right)_{\text{initial}} = \left(\frac{n_{Ar,\text{initial}} / X_{Ar}}{n_{N₂,\text{initial}} / X_{N₂}} - 1\right) \times 10^6
\] (11)

We performed the same calculation for the final Ar/N₂ ratio and determined the solubility fractionation effect as the difference between the final and initial Ar/N₂ ratios in units of per meg.

**Water vapor flux fractionation**

Water vapor flux fractionation occurs when evaporation causes air to diffuse into the soil to replace the water vapor lost; N₂ is a lighter molecule than Ar so the N₂/Ar ratio decreases in response to evaporation. This effect is smaller in environments with low soil evaporation rates. The water vapor flux fractionation effect can be limited to less than 100 per meg if the relative humidity changes from an initial value of 95% to a final value of 100% with no accompanying changes in temperature (Fig. 3). Even if the relative humidity begins and ends at 100%, up to a 0.5 °C change in temperature can result in up to a 48 per meg shift in the

\[
\delta_w = \left[\frac{1 - X_{H₂O}}{1 - X_{H₂O}} (\frac{n_{H₂O}}{n_{H₂O}}) - 1\right] \times 10^6
\] (12)

The ratio of the binary diffusion coefficients of N₂ and Ar in water vapor was estimated to be 1.0163 using the Fuller method.

The water vapor mole fraction was calculated from water vapor pressure (\( p_{H₂O}, \text{ atm} \)) and atmospheric pressure (\( P, \text{ mbar} \)) using Eqn. (13):

\[
X_{H₂O} = p_{H₂O} \times 1013.25 \div P
\] (13)

The saturation vapor pressure (\( p_s, \text{ atm} \)) was calculated from the measured air temperature (\( T_{\text{air}}, \text{ °C} \)) using Eqn. (14):

\[
p_{H₂O} = \frac{1.69803084 + (0.7568078 \times T_{\text{air}}) - (0.0109724 \times T_{\text{air}}^2) + (0.00063967 \times T_{\text{air}}^3)}{760}
\] (14)

The water vapor pressure was then determined from the measured relative humidity (RH, %) and \( p_s \) (Eqn. (15)):

\[
p_{H₂O} = \frac{RH}{100} \times p_s
\] (15)

**Soil surface N₂ flux calculation**

Soil surface N₂ fluxes can be calculated from the change in the \( \delta \text{Ar/N}_2 \) values of gas sampled from a surface flux chamber headspace over time. The fundamental assumption of
the N2/Ar technique is that the headspace Ar concentration remains constant so that changes in δAr/N2 are due solely to changes in the headspace N2 concentration. For soil surface N2 fluxes, the physical fractionation effects discussed earlier also cause changes in the δAr/N2 values so these effects must be removed from the measured change in the δAr/N2 values to obtain the biological change in δAr/N2 values. The thermal and solubility fractionation effects enhance the measured changes in δAr/N2 values when the temperatures increase and lower measured fluxes in δAr/N2 values when the temperatures decrease. Thus, these effects are subtracted from the measured δAr/N2 value for the final headspace sample. The water vapor fractionation effect causes an apparent N2 flux into the soil, so this effect was added to the measured δAr/N2 value for the final headspace sample. We used the resulting biological δAr/N2 value for the final headspace sample in the following calculations to estimate the actual N2 flux over the 1-h sampling period.

We determined the N2 concentration in each sample (mole fraction), c_sample, using the following equation where c_atm is the atmospheric N2 concentration (assumed to be 0.78084 mole fraction in air [38]). δAr/N2 is the measured sample value (per meg), and δAr/N2 is the reference standard value (per meg) (Eqn. (16)). The reference standard is La Jolla air sampled from Scripps Pier at the University of California, San Diego (UCSD). The reference standard and samples were analyzed against a working standard consisting of ultra-purity (UHP) N2 and Ar in near atmospheric proportions.

\[
c_{\text{sample}} = \left\{ \frac{c_{\text{atm}}}{c_{\text{atm}}} + 1 \right\} \left\{ \frac{c_{\text{atm}}}{c_{\text{atm}}} + 1 \right\} * c_{\text{atm}}
\]

The soil surface N2 flux (mg N m\(^{-2}\) day\(^{-1}\)), \(F\), was calculated as the linear change in N2 concentration in the chamber headspace between the two time points using the following equation where \(m\) is the molecular weight of N2 (g mol\(^{-1}\)), \(t\) is the time elapsed (days), and \(R\) is the ideal gas constant (8.2057 * 10\(^{-5}\) m\(^3\) atm K\(^{-1}\) mol\(^{-1}\)) (Eqn. (17)):

\[
F = \left[ (c_{\text{final}} - c_{\text{initial}}) * V * m_{\text{N2}} * 10^3 \right] / a * t * P / R * \text{air}
\]

LABORATORY TEST

Experimental design

We tested the N2/Ar method by inducing known N2 fluxes in a diffusion box located in a climate-controlled laboratory at the UCSD. The box consisted of 61 × 61 cm mirror-finished aluminum sheets welded together to leave an open top. A 10.2 cm tall perforated aluminum platform was placed in the diffusion box to create a void space in the bottom. The platform was lined with window screening to minimize sand falling through into the void space. Two 50 × 50 mm fans were mounted on opposite sides of the diffusion box and offset from the center to promote air circulation within the void space. A septum port was installed in the center of a third side to allow injection of N2 into the void space. The box was filled with air-dried Felton sand (American Soil and Stone, Richmond, CA, USA) to 30 cm depth.

We performed ten consecutive control measurements of N2 fluxes in dry sand (no N2 injected) as well as ten consecutive measurements with 2 mL UHP N2 injected. We also performed ten consecutive measurements of N2 fluxes in wet sand with no N2 injected and five consecutive measurements with 10 mL UHP N2 injected. After each measurement with N2 injections, we opened the septum port and waited at least 20 h before performing the next measurement. To wet the sand, we added 20 L deionized water to the diffusion box to bring the soil moisture to 10% gravimetric water content. lysimeters containing 400 g wet sand were used to monitor changes in soil moisture daily. Typically, 1 L deionized water was added every other day to maintain the soil moisture. The sand was mixed to create homogeneous soil moisture throughout the diffusion box, and it was then allowed to equilibrate for at least 12 h before the next measurement. After each flux measurement, triplicate 30-g samples of sand were removed from the diffusion box to determine the gravimetric water content.

The expected N2 flux induced by the injection of N2 into the bottom void space of the diffusion box was calculated using Fick’s law (Eqn. (18)), where \(D_s\) is the soil diffusivity of N2 (m\(^2\) s\(^{-1}\)), \(d_c\) is the difference in N2 concentration between the top and bottom of the soil column (mole m\(^{-3}\)), and \(dz\) is the height of the soil column (m):

\[
F = D_s \frac{dc}{dz}
\]

Unit conversions are required to obtain \(F\) in units of mg N m\(^{-2}\) day\(^{-1}\). The soil diffusivity of N2 was estimated as the product of the free air diffusivity of N2 and soil tortuosity. The free air diffusivity of N2 at 20 °C, the ambient air temperature in the laboratory, was 1.92 * 10\(^{-5}\) m\(^2\) s\(^{-1}\) according to the Fuller equation [40]. Soil tortuosity, \(\tau\) (unitless), was described using the Millington and Quirk [41] model where \(f_a\) is air-filled porosity (m\(^3\) air-filled voids m\(^{-3}\) soil) and \(P_f\) is total soil porosity (m\(^3\) voids m\(^{-3}\) soil) (Eqn. (19)):

\[
\tau = \frac{f_a \times 10 / 3}{P_f}
\]

Sample collection

Gas samples were collected from a custom-made two-piece 18-L aluminum chamber covered with two layers of reflective bubble wrap. Two internal mixing fans (50 × 50 mm) were attached to the chamber lid. The lid also had inlet and outlet ports consisting of stainless steel Swagelok bulkhead tube fittings sealed on viton. The lip of the chamber lid sealed against a viton strip lining the 1.5” wide lip of the chamber base when clamped together using either six spring clamps or four bar clamps. The chamber was deployed over water and leak checked with SF\(_6\) the measured leak rate over 3 h was 0.3% per hour.

Gas samples were stored in custom-made 2-L cylindrical glass flasks with two parallel 9-mm (o.d.) high-vacuum
of the hand instead of finger-turning them. This minimizes the fractionation that occurs when a small orifice is left before the valve is completely closed.

For each measurement, we inserted the chamber base 3 cm into the sand in the center of the box and then inserted T-type thermocouples to five depths (0, 5, 10, 15, 26 cm) next to the chamber. The thermocouples were connected to a CR10X datalogger (Campbell Scientific, Logan, UT, USA) that recorded temperature every 30 s. We turned on the void fans just before injecting the desired amount of UHP N2 through the septum port using a polypropylene syringe equipped with a zero-volume stopcock. We allowed the void air to continue circulating for 5 min while placing the lid on the chamber base and sealing the chamber. We then turned off the void fans, turned on the chamber fans, and started sampling using the pumping module. We measured the initial relative humidity by placing a thermohygrometer probe (Oakton, Vernon Hills, IL, USA) on the sand surface next to the chamber while the initial gas sample was taken, and we measured the final relative humidity by quickly placing the probe inside the chamber after the final gas sample was taken.

Sample preparation

Gas samples were prepared for mass spectrometric analysis on a vacuum line using a method developed for the Ar/N2 analysis of gas bubbles trapped in ice core samples. The stainless steel vacuum line included a series of traps: (1) a glass U-trap in ethanol/liquid N2 mix to –90 °C to remove water vapor, (2) a glass U-trap in liquid N2 to remove N2O, carbon dioxide, and hydrocarbons, (3) copper (Cu) mesh in a 5 cm long quartz tube heated to 500 °C to remove O2, and (4) a glass U-trap in liquid N2 to remove any hydrocarbons produced in the Cu oven. The Cu was regenerated after every 4–8 samples by passing hydrogen through the heated Cu oven at greater than 5 Torr pressure for 5–10 min.

The sample flask was placed horizontally in the center of an isothermal box with a small opening to allow a 1/4” stainless steel tube to protrude to the exterior of the box. This tube connected the flask side port to the end of the vacuum line via Ultra-torr fittings. The flask pipette was pumped down until enough water vapor was removed to leak check the vacuum line. If no leaks were detected, a sample aliquot was transferred from the sample flask to the pipette by closing the vertical valve and then opening the horizontal valve to the flask side port. The gas was allowed to equilibrate for 30 min before the horizontal valve was closed to seal off the bulk sample in the flask.

Sample aliquots were quantitatively transferred from the sample flask pipette to an evacuated dip tube cooled in a liquid helium Dewar. To transfer the aliquot, the vertical valve was opened very slowly to keep the pressure in the vacuum line below 1 Torr; this ensured that all the O2 in the sample was removed in the Cu oven. The leak check procedure was also used to determine that the entire sample had been transferred into the dip tube and that no leaks had occurred during the transfer. Samples were then allowed to homogenize in the dip tubes for 2–18 h before analysis on the mass spectrometer. As a precaution against variability in measured N2/Ar ratios caused by differences in

Figure 4. Photograph of sample flask valves.
homogenization times, all samples for a given flux measurement were homogenized for a similar amount of time (i.e., no greater than 4 h difference in homogenization time).

Sample analysis

Samples were analyzed on a dual inlet ThermoFinnigan DeltaPlus XP isotope ratio mass spectrometer (Bremen, Germany) with multiple collectors for N, Ar, and O isotopes. Samples containing O$_2$ are normally analyzed on this instrument, but, during this study, no O$_2$ was introduced into the mass spectrometer. Samples were analyzed for 6 blocks of 16 cycles using a 12-s integration window. At the beginning of each block, the bellows were compressed to achieve a 4.2 V m/z 36 signal to minimize potential errors due to non-linearity. The mass spectrometer output was corrected for pressure imbalance between the two bellows using a linear regression equation based on the 6 block averaged data. For the flux calculations, we used the average Ar/N$_2$ ratio of 2–3 analytical replicates from each flask.

A working standard gas consisting of high-purity N$_2$ and Ar in near atmospheric proportions was standardized against La Jolla air collected from the Scripps Pier (La Jolla, CA, USA). An aliquot of gas was transferred from the standard can to a stainless steel pipette (bracketed by 4 H bellows valves) that had been pumped down online. The gas was allowed to equilibrate between the standard can and the pipette for 20 min before the inner valve was closed; the outer valve was then opened to fill the evacuated standard bellows. Meanwhile, the closed dip tube containing the prepared sample was pumped down online for at least 1 h, and the dip tube was then opened to fill the evacuated sample bellows. The sample and standard are allowed to equilibrate in the completely expanded bellows for 10 min. The standard can, dip tube, and bellows were contained in an isothermal box to minimize N$_2$/Ar fractionation due to thermal diffusion as well as variability in measured N$_2$/Ar due to temperature changes during bellows equilibration and/or sample analysis.

We evaluated the external precision of the δAr/N$_2$ measurements by calculating the pooled standard deviation for all analytical replicates within each treatment (e.g., N$_2$ injected in dry sand). The flux measurements in dry sand with an induced N$_2$ flux were performed with samples analyzed in triplicates whereas the samples for the other treatments were analyzed in duplicate. The use of duplicates rather than triplicates allowed us to analyze samples for two flux measurements each day rather than only one per day.

RESULTS AND DISCUSSION

Precision and detection limit

We consistently measured N$_2$/Ar ratios with very high precision. For the flux measurements in dry sand with an induced N$_2$ flux, the precision was 5 per meg for triplicate sample analysis. For the other treatments, the precision ranged from 12 to 13 per meg for duplicate sample analysis. The poorest reproducibility among analytical replicates for all samples was a standard deviation of 42 per meg for a duplicate set. There were no indications of leaks or other reasons for the poor reproducibility of some replicates, so none of the data were rejected. Despite the higher precision yielded by analyzing triplicates, time constraints forced us to analyze duplicates for most of the flux measurements performed. This allowed us to approximately double our throughput, analyzing samples for two flux measurements per 14-h work day versus one flux measurement per 12-h work day. Using our sampling equipment and procedure, a 1 per meg change in δAr/N$_2$ was equivalent to an N$_2$ flux of approximately 3 mg N m$^{-2}$ day$^{-1}$. If the detection limit of the method is calculated as three times the precision in δAr/N$_2$ analysis, the detection limit is 45 mg N m$^{-2}$ day$^{-1}$ using triplicates whereas it is 108 mg N m$^{-2}$ day$^{-1}$ using duplicates.

The correction for the physical fractionation effects on N$_2$/Ar ratios can create additional errors in the flux measurements if temperature and relative humidity are not accurately measured. These errors would probably overwhelm the errors associated with the models used to estimate the physical fractionation effects. For example, if the chamber temperature was initially 0.1 °C warmer than measured, the thermal fractionation effect would be 28.6 per meg greater and the water vapor flux fractionation effect would be 9 per meg less than measured for a total underestimate of 19.6 per meg for these fractionation effects. This would translate to a 78 mg N m$^{-2}$ day$^{-1}$ error in the corrected N$_2$ flux.

Measurements in dry sand

The control measurements in dry sand demonstrate that flux values below the calculated detection limit of 108 mg N m$^{-2}$ day$^{-1}$ are highly variable and can be inaccurate. For three of the ten measurements, we observed a 1 per meg change in δAr/N$_2$, which is consistent with no induced N$_2$ flux. However, overall we measured changes in δAr/N$_2$ ranging from −71 to 18 per meg corresponding to an average N$_2$ flux of 43 ± 27 mg N m$^{-2}$ day$^{-1}$ (± SE, n = 10). When corrected for thermal fractionation, which ranged in magnitude from 19 to 96 per meg, the N$_2$ flux was 46 ± 45 mg N m$^{-2}$ day$^{-1}$. This could suggest that the sampling protocol causes an artifact of approximately 45 mg N m$^{-2}$ day$^{-1}$. However, the average measured N$_2$ flux of 111 ± 19 mg N m$^{-2}$ day$^{-1}$ (n = 10) closely matched an induced N$_2$ flux of 108 mg N m$^{-2}$ day$^{-1}$ in dry sand. Therefore, the control measurements probably do not represent a sampling artifact but simply reflect our inability to accurately measure N$_2$ fluxes below the detection limit.

The induced N$_2$ flux measurements in dry sand suggest that thermal fractionation may not be manifested within the 1-h sampling period that we used. In dry soil, solubility and water vapor flux fractionation do not occur, allowing us to separately test thermal fractionation effects. The measured changes in δAr/N$_2$ ranged from −59 to 2 per meg while the thermal fractionation effect ranged from −34 to 34 per meg. When thermal fractionation was taken into account, the average corrected N$_2$ flux was 91 ± 36 mg N m$^{-2}$ day$^{-1}$. The correction for thermal fractionation not only caused the measured N$_2$ flux to diverge from the expected N$_2$ flux, but it also increased the variability in the measured N$_2$ fluxes. The coefficient of variation (CV) for the uncorrected N$_2$ fluxes was 53% whereas the CV for the...
corrected N2 fluxes was 124%. This suggests that the correction for thermal fractionation does not improve the N2 flux estimation and does not accurately represent the effects of thermal diffusion on the N2/Ar ratio. For these reasons we do not recommend the use of a thermal fractionation correction for measurements on short time scales.

There are several reasons why the correction for thermal fractionation may not be valid or necessary for our N2 flux measurements. First, changes in the temperature differential between the chamber headspace and soil depth were ≤0.3 °C. Although thermal fractionation causes a 286 per meg shift in δAr/N2 per degree of temperature change, it is possible that small changes in the temperature gradient within 1 h do not cause a detectable shift in the δAr/N2 of the chamber headspace gas. Second, where thermal fractionation has been observed – in sand dunes, polar firn, and ice cores – the gases had reached steady-state equilibria. In the diffusion box, thermal steady-state conditions were not reached because the temperature changed throughout the sampling period, violating a fundamental assumption of the thermal diffusion correction. Thus, it is possible that thermal fractionation may not have been manifested because the gas molecules had not reached equilibrium with respect to the temperature gradient in the sand. Third, the temperature profiles show that the temperature change was often non-linear with depth, making it difficult to model a shift in the δAr/N2 values of the chamber headspace due to thermal fractionation. Here we simply assumed a linear temperature gradient between the chamber headspace and the bottom of the diffusion box to calculate the thermal fractionation effect. This simplified approach may not have been valid.

Measurements in wet sand

When we wet the diffusion box sand to 10% gravimetric soil water content, we observed evidence of the water vapor fractionation effect, which causes a decrease in the N2/Ar ratio to create an apparent net uptake of N2 by the soil. For the control measurements, we observed N2 fluxes of −45 ± 82 mg N m−2 day−1 (n = 5) when the water vapor flux fractionation effect was not taken into account. We also performed these flux measurements after allowing for an initial 10-min period with the chamber lid set but not sealed on the chamber base before the sampling procedure began. This additional time would be needed for the added N2 to move through the wet sand for the induced N2 flux measurements. This change in procedure did not significantly change the measured N2 fluxes, with a measured N2 flux of −48 ± 78 mg N m−2 day−1 (n = 5). We used this modified sampling protocol for flux measurements with a known N2 flux of 160 mg N m−2 day−1 (from the injection of 10 mL UHP N2) in wet sand; the measured N2 flux was 62 ± 25 mg N m−2 day−1. As expected, the measured N2 fluxes were lower than the expected N2 fluxes for all treatments when the water vapor flux fractionation effect was not considered.

The water vapor flux fractionation effect was difficult to quantify because the thermohygrometer readout took approximately 5 min to stabilize, and, during this time, the relative humidity inside the chamber was changing in response to ongoing evaporation. The measurement was also complicated by the fact that we could not place the thermohygrometer probe inside the chamber during the flux measurement due to the design of the chamber. Measurements of changes in relative humidity inside the chamber taken when gas was not being sampled show that the relative humidity reached 98.8% in 10 min and 99.4% in 20 min after the chamber lid was placed on the deployed chamber base. Assuming that the initial air inside connecting tubing and the flasks was at ambient relative humidity (50%) and that the chamber headspace was at 99.9% relative humidity, we calculate a relative humidity of 90% at the initial time point, and an assumed relative humidity of 99.9% at the final time point. A 1% error in relative humidity translates into a 15 per meg error in the water vapor flux fractionation effect.

Solubility fractionation had a relatively small effect on δAr/N2 compared with the other fractionation effects. With the assumed relative humidity and measured air temperature, we estimated that the water vapor flux fractionation effect ranged from 28 to 42 per meg for all the measurements in wet sand, the solubility fractionation effect ranged from −17 to 15 per meg, and the thermal fractionation effect ranged from −64 to 32 per meg. We calculated solubility fractionation based on the average soil temperature. If the solubility fractionation effect was calculated for each depth increment and then summed, it only produced a 1 per meg difference compared with the approach that we used. With the relatively low soil moisture in the diffusion box and the small temperature changes during our flux measurements, the solubility fractionation effect was only −17 to 15 per meg, with the direction of the effect dependent on whether the bulk soil cooled or warmed during the measurement. This effect could be larger when soil moisture is higher or when temperature changes are greater. For example, Fig. 2 shows that at 40% gravimetric soil water content, the solubility fractionation effect is greater than 100 per meg per 0.1 °C change in temperature. This underscores the importance of limiting temperature changes during N2/Ar measurements, which can be accomplished by shading the chamber and avoiding the times of day with large changes in soil temperature (e.g., just after sunrise).

The flux measurements in wet sand support the implications from the flux measurements in dry sand. For the control measurements, the average measured N2 flux corrected for all physical fractionation factors was 86 ± 45 mg N m−2 day−1 whereas the average N2 flux was 67 ± 68 mg N m−2 day−1 when only corrected for solubility and water vapor fractionation. These measurements demonstrate that we cannot measure N2 fluxes below the calculated detection limit. For the induced N2 flux measurements, the average N2 flux was 134 ± 38 mg N m−2 day−1 when all physical fractionation effects were accounted for and 146 ± 20 mg N m−2 day−1 when only solubility and water vapor flux fractionation were considered. Without correcting for thermal fractionation, the average measured N2 flux was more similar to the expected N2 flux of 160 mg N m−2 day−1 and also less variable. Thus, this provides additional evidence that the correction for thermal fractionation is not useful as currently implemented. Water vapor flux fractionation was the most poorly constrained physical fractionation effect, but we were able to effectively account for its effect on the N2/Ar ratio. Our approximation of the initial relative humidity in the chamber headspace appears to be valid because the measured N2 fluxes corrected for the water vapor flux and solubility fractionation effects agreed well with the expected values for...
the induced N₂ fluxes. However, it is preferable for water vapor pressure to be measured in the chamber headspace in real time with an instrument that stabilizes more quickly.

We tested the N₂/Ar technique in sand, but we expect that in general the physical fractionation effects on soils with finer textures will be similar. However, in clayey soils with very small pore spaces, gas diffusion is characterized by Knudsen diffusion in which gas molecules are more likely to collide with soil grains than with other gas molecules. The addition of a Knudsen diffusion term would complicate the modeling of water vapor flux fractionation as presented here.

**Future improvements to the method**

The detection limit for this method at present is very high, but it can easily be lowered proportionally by increasing the ratio of chamber basal area to volume as well as by increasing the sampling time period. For example, doubling the ratio of chamber basal area to volume or the sampling time period would lower the detection limit by 50%. Chambers with large basal areas can be unwieldy and difficult to deploy in ecosystems with dense vegetation. Theoretically, the ratio of chamber basal area to volume can also be increased by decreasing the chamber height from the 12 cm that we used. Short chambers (<10 cm tall) can suppress soil surface trace gas fluxes because of gas storage in the soil surface. A longer sampling time period can also lower the detection limit, but, like short chambers, it can cause changes in the concentration gradients of trace gases in the soil and suppress the surface flux. While N₂ is not a trace gas, changes in the diffusion of trace gases could cause corresponding non-trivial changes in N₂ diffusion. Thus, these measures for lowering the detection limit should be employed with caution.

The N₂/Ar technique can be applied to soil depth profiles which may exhibit greater differences in N₂/Ar that can be measured accurately using the gas analysis approach described here. This approach creates the challenge of sampling soil gas without causing fractionation at the per meg level. We have attempted passive sampling in laboratory tests with no induced change in N₂, but we have measured approximately 500 per meg fractionation in δAr/N₂ (data not shown). Additional investigation is necessary to understand the cause of this fractionation. The sampling procedure that we used in this study involved pumping air through large-volume flasks and resulted from many years of testing to minimize sampling artifacts associated with this approach. For example, Blaine et al. found that temperature gradients within the sampling equipment can cause N₂/Ar fractionation. Another factor in using the soil profile approach is the sample volume of soil gas that can be collected. A large sample volume is required for running analytical replicates without fractionating the remaining sample in the flask. If analytical replicates are not desired, entire small samples could be analyzed to avoid fractionation. Beyond these practical considerations, the physical fractionation effects will also need to be constrained along the soil profile. At steady state, solubility fractionation will not affect the observed soil air N₂/Ar gradient through the soil profile. However, the thermal and water vapor flux fractionation effects will affect the observed N₂/Ar soil profile under both steady-state and transient conditions. Thus, constraining the physical effects on N₂/Ar gradients in soil air differs from constraining the physical effects on the N₂/Ar ratio in the headspace of surface flux chambers, which is subject only to the transient aspects of the fractionating processes.

Theoretically, the observed fractionation of inert gas ratios or isotope ratios of inert gases can be used to constrain the physical fractionation effects on N₂/Ar. For example, δ⁴⁰Ar and δ¹⁵N values are often used together in paleoclimate work to separate different physical fractionation effects. Argon is the most abundant naturally occurring inert gas, and the isotopic composition of Ar can be measured with higher precision than that of other inert gases. However, the precision for Ar isotopic analysis is lower than that for δAr/N₂ so it cannot be used to accurately constrain physical fractionation effects on N₂/Ar. Future improvements in δ⁴⁰Ar analysis could allow this approach to be used as validation of the modeling approach that we employed to estimate the physical fractionation effects.

Gas analysis using a dual inlet IRMS instrument with offline sample preparation had a very low throughput that precludes its use for high-resolution measurements of soil N₂ emissions. This analytical approach was chosen for this study because the instrumentation and protocols were already set up and tested for our analyses. Keeling et al. presented a higher throughput method on a continuous flow IRMS system with online removal of water vapor and carbon dioxide that provides δAr/N₂ analysis with per meg level precision similar to that presented here. The addition of online O₂ removal would permit their method to be applied to our approach. With a higher throughput method, the analysis of more replicates per sample could lower the detection limit considerably. For example, we observed 5 per meg precision for δAr/N₂ values when analyzing analytical triplicates and 12 per meg precision when analyzing analytical duplicates. Thus, the detection limit would be lowered from 108 mg N m⁻² day⁻¹ to 45 mg N m⁻² day⁻¹ simply by analyzing analytical triplicates instead of duplicates. Furthermore, gas handling from offline sample preparation probably introduces a large proportion of the variability among analytical replicates and, as such, online sample preparation would probably improve the external precision. It could be possible to achieve 1 per meg precision, which would yield a detection limit of 3 mg N m⁻² day⁻¹. This is comparable with the detection limit reported for the gas flow core method and the gas flux method. Thus, a continuous flow IRMS approach could not only increase sample throughput, but also lower the detection limit of the method so that it can be used in ecosystems with low soil N₂ emissions.

**CONCLUSIONS**

We demonstrated that the N₂/Ar technique can be used to measure soil N₂ emissions without manipulating soil conditions. Given the current high detection limit and throughput limitations of the method, it is currently best suited as a verification tool for other methods in ecosystems with high soil N₂ emissions rather than a technique for high-resolution field measurements. Physical fractionation effects on the N₂/Ar ratio can be constrained, but accurate measurements of temperature and relative humidity are necessary. Moreover,
temperature changes and evaporation during the sampling period should be minimized to limit the physical fractionation effects. With technological and methodological advances we can lower the detection limit, better constrain the physical fractionation effects, and increase sample throughput so that the N₂/Ar technique can be used in a wider range of ecosystems for high resolution field measurements of soil N₂ emissions.

**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of this article.

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