Nitrate $\Delta^{17}O$ tracer method for determining gross nitrification rates

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ABSTRACT

Gross nitrification rate (GNR) can reflect the actual status of nitrification process but is difficult to constrain. To develop a practical method for assessing GNR, $\Delta^{17}O\text{-}NO_3^-$ tracers were applied to container systems under greenhouse conditions to test their feasibility. Two treatments with double- ($^{15}N^{\Delta 17}O_3^-$, negative $\Delta^{17}O$ value) or single-labeled ($N^{\Delta 17}O_3^-$, positive $\Delta^{17}O$ value) fertilizer nitrate salts were implemented to constrain nitrification fractions and GNRs in soilless media container systems. The nitrification fractions calculated from the $\Delta^{17}O\text{-}NO_3^-$ tracer method concurred with those from the $^{15}NO_3^-$ tracer method, with small deviations and a linear regression slope close to 1. This suggested the $\Delta^{17}O\text{-}NO_3^-$ tracer inherently works in the same way as the well-established $^{15}NO_3^-$ tracer. GNRs in the soilless media container systems ranged from 250.5($\pm$39.1) to 861.5($\pm$275.6) mg N/ (m^2·d), generally higher than in natural ecosystems. The discrepancies in GNRs between the two treatments were generally smaller than discrepancies in GNRs between replicate container systems, indicating the applicability of either negatively-labeled or positively-labeled $\Delta^{17}O\text{-}NO_3^-$ tracers. Commercial nitrate salts mined from the Atacama and Kumtag Deserts are proposed for use as $\Delta^{17}O\text{-}NO_3^-$ tracers for GNR estimation due to their abundant availability, low cost and distinct discrimination from nitrified NO_3^-.

1. Introduction

Gross nitrification rate (GNR) is the rate of NO_3^- production regardless of NO_3^- consumption, which can reflect the actual status of nitrification process and affect the potentials of NO_3^- availability or gaseous N losses (Hart et al., 1994). Despite its importance in constraining nitrogen losses in various systems, GNR is not extensively quantified due to limitations associated with current techniques. The traditional techniques for determining GNRs in soils (or soilless media) include the costly $^{15}NO_3^-$ tracer method (*e.g.* Davidson et al., 1991) and barometric process separation (BaPS) method that relies on some simplified assumptions (Ingwersen et al., 1999). The shortcomings have confined the use of these methods to laboratory conditions or small plots in the field.

Instead, we propose a new tracer for GNR quantification: $\Delta^{17}O\text{-}NO_3^-$. The isotopic abundances of three oxygen isotopes (^{16}O, ^{17}O and ^{18}O)

usually depend on the relative differences in isotope mass, which is referred as the mass-dependent isotopic fractionation, leading to $\delta^{17}O\sim0.52\cdot\delta^{18}O$ (Thiemens, 2006 and reference therein). However, mass-independent fractionation with "anomalous" ^{17}O excesses (quantified by $\Delta^{17}O=\delta^{17}O\text{-}0.52\cdot\delta^{18}O$) has been observed in photochemically-produced atmospheric NO_3^- with $\Delta^{17}O_{NO3atm}\sim20\text{‰}-35\text{‰}$ (*e.g.* Michalski et al., 2003; Morin et al., 2009). After deposition, the $\Delta^{17}O$ label derived from atmospheric NO_3^- is diluted by terrestrial NO_3^- produced *via* mass-dependent nitrification processes ($\Delta^{17}O_{nitrif}\sim0\text{‰}$), which is a function of ecosystem N turnover (Michalski and Thiemens, 2006; Riha et al., 2014) and water availability (Wang et al., 2016). This is similar to the $^{15}NO_3^-$ tracer method in principle as an isotope dilution experiment. This $\Delta^{17}O\text{-}NO_3^-$ tracer method was first developed to estimate GNRs in oligotrophic lake systems (Michalski and Thiemens, 2006; Tsunogai et al., 2011) and later successfully used in urban and forested catchments as well as other mesotrophic lakes (Riha

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et al., 2014; Fang et al., 2015; Rose et al., 2015; Tsunogai et al., 2010; Huang et al., 2020). Yu and Elliott (2018) recently used $NaNO_3$ fertilizer from the Atacama Desert (known as "Chilean Nitrate", $\Delta^{17}O = 18.6‰$) to amend soil samples in laboratory incubation experiments and calculated GNRs based on the $\Delta^{17}O$-NO_3^- tracer method, which were in good agreement with those predicted by the $^{15}NO_3^-$ tracer method. With recent progresses in $\Delta^{17}O$-NO_3^- measurements (Weigand et al., 2016), the $\Delta^{17}O$-labeled NO_3^- salts are a promising tracer for GNR quantification in various systems. However, the feasibility of $\Delta^{17}O$-NO_3^- tracer has not been widely verified. This study is designed to test the feasibility of the $\Delta^{17}O$-NO_3^- tracer method for GNR assessments by directly comparing the $\Delta^{17}O$-NO_3^- tracer method and the well-established $^{15}NO_3^-$ tracer method in soilless media container systems under controlled greenhouse conditions with double- ($^{15}N^{\Delta17}O_3^-$, negative $\Delta^{17}O$ value) and single-labeled ($N^{\Delta17}O_3^-$, positive $\Delta^{17}O$ value) nitrate salts. Specifically, the advantage of using a double-labeled nitrate salt is that the $\Delta^{17}O$-NO_3^- tracer and $^{15}NO_3^-$ tracer would experience the same procedure and hold the same assumptions with no discrimination.

2. Materials and methods

2.1. Experimental plants

A single one-year-old seedling of Red Sunset® red maple (*Acer rubrum* L.) was planted in 2 L containers (12.5 cm diameter top and 13 cm height). Each container was filled with Fafard® 2B Mix soilless media (Conrad Fafard, Agawam, MA) consisting of Canadian sphagnum peat moss, bark, perlite, vermiculite, dolomitic limestone, and wetting agent. Initial physical characteristics of the seedlings were: height of 22.64 ± 3.31 cm, leaf number of 17 ± 4, total leaf area of 192.8 ± 31.6 cm^2, stem diameter at 2 cm above media surface of 3.91 ± 0.58 mm, and leaf greenness of 18.3 ± 2.6 SPAD units (index for leaf chlorophyll concentration). Twenty seedlings were housed in a greenhouse (16 h/8 h light/darkness, 25 °C).

2.2. Preparation of fertilizer solution

$\Delta^{17}O$-labeled NO_3^- salts were first prepared using Hoffman® nitrate of soda 16−0−0 imported from Chile (purchased in the United States). Hoffman® $NaN^{\Delta17}O_3$ with $\delta^{15}N = 0.5‰$, $\delta^{17}O = 47.2‰$, $\delta^{18}O = 52.6‰$ and $\Delta^{17}O = 19.8‰$ (Michalski et al., 2015) was mixed with reagent grade KCl or $(NH_4)_2SO_4$ ($\delta^{15}N = 0‰$) in deionized (DI) water and purified by fractional crystallization *via* evaporation to synthesize $NH_4N^{\Delta17}O_3$ ($\Delta^{17}O = 19.8‰$) or $KN^{\Delta17}O_3$ ($\Delta^{17}O = 19.8‰$) salts to remove Na^+ that can inhibit plant growth and development.

Two mixture stock fertilizer solutions, with $\Delta^{17}O$ and/or ^{15}N enrichments, were then prepared and used throughout. Firstly, the $NH_4N^{\Delta17}O_3$ (described above), $KN^{\Delta17}O_3$ (described above), and $K^{15}N^{\Delta17}O_3$ (Sigma-Aldrich Inc., USA) were mixed in molar ratios of 66.2:32.1:1.7. The commercial $K^{15}N^{\Delta17}O_3$ was enriched in ^{15}N (99.0 atom%), as well as in both ^{17}O and ^{18}O but not mass dependently with a negative $\Delta^{17}O$ value. The enrichment in both nitrogen and oxygen isotopes might be due to fractional distillation of $H^{15}NO_3$ obtained *via* isotopic exchange with NO (Spindel and Taylor, 1955), while fractional distillation of NH_3 to produce $^{15}NH_3$ (Thode and Urey, 1939) that is then oxidized to $^{15}NO_3^-$ (Sant Ana Filho et al., 2008) would produce $^{15}NO_3^-$ without oxygen isotope enrichment. The mixture of $NH_4N^{\Delta17}O_3$, $KN^{\Delta17}O_3$ and $K^{15}N^{\Delta17}O_3$ were then amended according to Hoagland's nutrient recipe to yield "stock 1" fertilizer solution. The final isotopic composition of NO_3^- in "stock 1" fertilizer solution was $\delta^{15}N = 504.0 \pm 26.8‰$, $\delta^{18}O = 236.3 \pm 11.6‰$ and $\Delta^{17}O = -33.4 \pm 1.3‰$ (n = 3). Secondly, the mixture of $NH_4N^{\Delta17}O_3$ and $KN^{\Delta17}O_3$ were directly amended according to Hoagland's nutrient recipe to yield "stock 2" fertilizer solution with the same elemental compositions as "stock 1" fertilizer solution. The final isotopic composition of NO_3^- of "stock 2" fertilizer solution was $\delta^{15}N = -2.7 \pm 1.2‰$, $\delta^{18}O = 42.0 \pm 0.2‰$ and $\Delta^{17}O = 18.4 \pm$ 0.2‰ (n = 3).

2.3. Fertilizer application

Two treatments (10 replicates for each) were performed using "stock 1" (double-labeled $^{15}N^{\Delta17}O_3^-$) and "stock 2" (single-labeled $N^{\Delta17}O_3^-$) fertilizer solutions, respectively (Fig. 1 A and B). Irrigation occurred every seven days. At each irrigation event, container systems were first irrigated with 900 mL of DI water that was sufficient to leach out all NO_3^- remaining in the pore space from the previous irrigation event. Immediately after applying DI water, 400 mL of "stock 1" or "stock 2" fertilizer solution was applied to replenish the container systems. The leachate of each irrigation event (draining for 45 min) was collected in a plastic container fit under the container to prevent evaporation. The leachate volume (V_{leach}, mL) was measured and splits were immediately frozen for later chemical and isotopic analysis. Prior to each irrigation, samples of the DI water and stock fertilizer solutions were also collected for chemical and isotopic analysis as a test of blank and changes in fertilizer label with time (no change was found).

2.4. Chemical and isotopic analysis

NO_3^- concentrations of the stock fertilizer solutions ([NO_3-]$_{fert}$, mg/L) and leachates ([NO_3-]$_{leach}$, mg/L) were measured using WQ-NO_3 nitrate ion selective electrodes (reproducibility: $\pm 4‰$) (NexSens Technology Inc., USA). Another split of leachate was autoclaved at 121 °C for 30 min to prevent any microbial activities before the NO_3^- $\delta^{15}N$, $\delta^{18}O$ and $\Delta^{17}O$ isotope measurements using a recent bacterial reduction, gold redox method (Weigand et al., 2016). Briefly, NO_3^- was converted to N_2O using denitrifying bacteria (*Pseudomonas aureofaciens*) and the isolated N_2O was disproportionated over gold to N_2 and O_2 at 900 °C. Subsequently, the resulting N_2 and O_2 was analyzed by an isotope ratio mass spectrometer (Delta V Plus, Thermo Fisher Ltd., USA) at the Purdue Stable Isotope Facility for $\delta^{15}N$, $\delta^{18}O$ and $\Delta^{17}O$ with precisions of $\pm 0.4‰$, $\pm 1.0‰$, and $\pm 0.5‰$, respectively, based on replicate analysis of working standards and calibrations. All $\delta^{15}N$ values were reported *versus* air N_2, while $\delta^{18}O$ and $\Delta^{17}O$ values were reported *versus* Vienna Standard Mean Ocean Water (VSMOW).

2.5. Source apportionment and GNR quantification

By assuming that the labeled NO_3^- is linearly mixed with unlabeled NO_3^-, the relative contributions of the two NO_3^- sources (fertilizer and nitrification) can be solved based on the two-component isotope mixing models:

$$\Delta^{17}O_{leach} = (1-f_1) \times \Delta^{17}O_{fert} + f_1 \times \Delta^{17}O_{nitrif} \tag{1}$$

$$\delta^{15}N_{leach} = (1-f_2) \times \delta^{15}N_{fert} + f_2 \times \delta^{15}N_{nitrif} \tag{2}$$

where $\Delta^{17}O_{leach}$ and $\Delta^{17}O_{fert}$ are the measured $\Delta^{17}O$ of NO_3^- in the leachate and stock fertilizer solutions, respectively; the nitrified NO_3^- was obtained by leaching the container system with DI water on day 0, showing $\delta^{15}N_{nitrif}$ of $1.0‰ \pm 0.7‰$, $\delta^{18}O_{nitrif}$ of $13.6‰ \pm 2.9‰$ and $\Delta^{17}O_{nitrif}$ of $0‰ \pm 0.1‰$ (n = 10); f's are the mole fractions of nitrified NO_3^-; $1-f$'s are the mole fractions of fertilizer NO_3^-. Based on another assumption of no discrimination between $^{14}NO_3^-$ and $^{15}NO_3^-$, various N processes except nitrification would not affect f's.

The amount of retained fertilizer NO_3^- (A_{fert}) can be derived from subtracting the leached NO_3^- amount ([NO_3^-]$_{leach} \times V_{leach}$/1000, mg) from the applied fertilizer NO_3^- amount ([NO_3^-]$_{fert} \times 400$/1000, mg), and the amount of nitrified NO_3^- A_{nitrif} (mg) can be obtained based on A_{fert} and the relative contribution of the two sources derived from the $\Delta^{17}O$-NO_3^- tracer method:

$$A_{nitrif} = A_{fert} \times f_1/(1-f_1) = ([NO_3^-]_{fert} \times 400 - [NO_3^-]_{leach} \times V_{leach})/1000 \times f_1/(1-f_1) \tag{3}$$
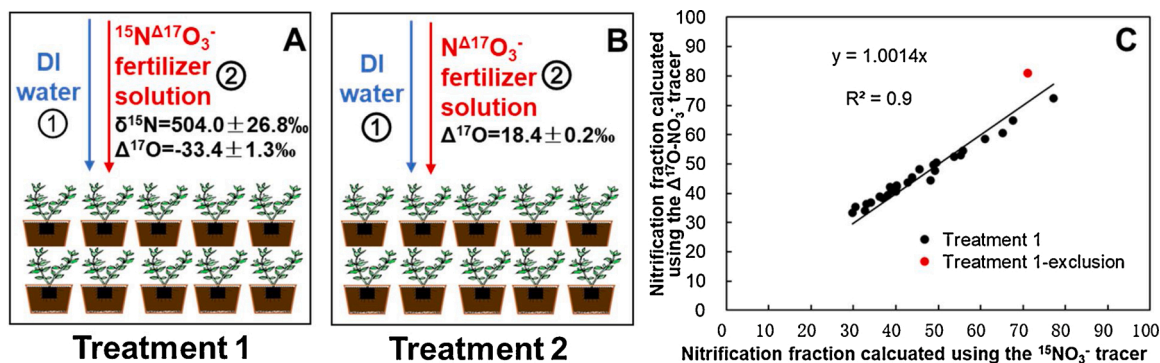
Fig. 1. The experimental setup (A and B) as well as the linear regression of gross nitrification fractions based on the $\Delta^{17}O\text{-}NO_3^-$ tracer method (f_1) relative to those based on the $^{15}NO_3^-$ tracer method (f_2) in Treatment 1 (C). The red dot was taken as an outlier $\Delta^{17}O\text{-}NO_3^-$ measurement and was excluded from the regression (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

GNRs (mg N/(m^2·day)) can then be obtained by dividing A$_{nitrif}$ by the plant container area (area, m^2) and the duration between two irrigations (*t*, days):

$$\text{GNR} = A_{nitrif} \times 14/(62 \times \text{area} \times t) \qquad (4)$$

3. Results and discussion

3.1. Comparison between the $\Delta^{17}O\text{-}NO_3^-$ and $^{15}NO_3^-$ tracer methods

The objective of this study was to compare the $\Delta^{17}O\text{-}NO_3^-$ tracer and $^{15}NO_3^-$ tracer methods and test for equivalence. In this sense, the comparative nitrification fractions (f_1, f_2) based on Eqs. (1) and (2) are more relevant than the actual GNRs, because there are inevitable uncertainties associated with GNR estimations due to uncertainties in measuring concentrations, container area, solution volumes and durations that all factor into GNRs. The comparative nitrification fractions (f_1, f_2), on the other hand, are insensitive to these variables and depend only on the relative changes in isotope abundance with time. The nitrification fractions calculated using the $\Delta^{17}O\text{-}NO_3^-$ tracer method (f_1) matched very well with those calculated from the $^{15}NO_3^-$ tracer method (f_2), particularly for Treatment 1 with very small deviations and a significant linear regression when a suspected outlier was excluded ($R^2 = 0.921$, slope of 0.9996, $p < 0.01$) (Table 1 and Fig. 1C). This suggests that the $\Delta^{17}O\text{-}NO_3^-$ tracer inherently works in the same way as the $^{15}NO_3^-$ tracer, suggesting the feasibility of the $\Delta^{17}O\text{-}NO_3^-$ tracer method for GNR quantification. The discrepancies in f_1 between Treatments 1 and 2 were generally smaller than discrepancies in f_1 between replicate container systems within the same treatment (Table 1), indicating the consistency in the $\Delta^{17}O\text{-}NO_3^-$ tracer method using either the negatively-labeled or positively-labeled $\Delta^{17}O\text{-}NO_3^-$ tracers. However, f_1 for irrigation period 2 in Treatment 1 is generally larger than those in Treatment 2, most likely due to systematic over- or under-estimations during volume recording or concentration analysis.

3.2. GNR quantification

GNRs could be calculated for each container system for each irrigation period based on the resolved nitrification fractions (f_1) and Eqs. (3) and (4) (Table 1). There were significant discrepancies in GNRs between replicate container systems and between irrigation periods, but GNRs between Treatments 1 and 2 were relatively consistent except for irrigation period 2 (Table 1), in line with the consistency in the growth rates, characterized by the percentage of the mass of new leaf and new stem in the total plant (including leaf, stem and root) mass gain over the experimental period, between Treatment 1 (24.0 %±2.7 %) and Treatment 2 (22.8 %±3.4 %) at the experimental endpoints.

The GNRs for our soilless media container systems ranged from 250.5(±39.1) to 861.5(±275.6) mg N/(m^2·d) (Table 1), and were largest during irrigation period 1 but decreased over time. GNRs in soilless media container systems have rarely been reported but our results indicate promotion of nitrification under our controlled greenhouse conditions, compared to that occurring in natural soils of 20−313 mg N/(m^2·d) (Hart and Gunther, 1989; McClaugherty et al., 1985; Aber et al., 1985; Castro et al., 1992; Ingwersen et al., 1999; Christenson et al., 2009; Cheng et al., 2015). The GNRs during irrigation period 1 were high, probably due to the stimulation of nitrifying microbes by the first-time addition of water and nutrients (Zaman and Chang, 2004). Later, the container systems were probably under water-saturated conditions between two irrigation periods that were not conducive to nitrification, leading to the decrease in the GNR.

3.3. Potential applications

Compared to the $^{15}NO_3^-$ tracer method, there are several advantages of using the $\Delta^{17}O\text{-}NO_3^-$ tracer method for GNR estimation. The $\Delta^{17}O\text{-}NO_3^-$ of an atmospheric origin is distinct from that of nitrified NO_3^-, easily identifying the risks of laboratory contamination during the handling of labeling material. Also, the commercial NO_3^- salts mined from the Atacama (Hoffman®/Hi-Yield/Bonide nitrate of soda, purity>97 %, $\Delta^{17}O\sim18.9‰\text{–}19.8‰$) (Michalski et al., 2015) and

Table 1
Analytical data and the calculated nitrification fractions and GNRs for two treatments.

Period	Treatment 1: $^{15}N^{\Delta^{17}O_3^-}$							Treatment 2: $N^{\Delta^{17}O_3^-}$				
	$\Delta^{17}O_{leach1}$, ‰	$\delta^{15}N_{leach1}$, ‰	f_1, %	f_2, %	A_{fert1}, mg	$A_{nitrif1}$, mg	GNR, mg N/(m^2•d)	$\Delta^{17}O_{leach2}$, ‰	f_1, %	A_{fert2}, mg	$A_{nitrif2}$, mg	GNR, mg N/(m^2•d)
Irrigation period 1	−14.5 ± 2.9	199.5 ± 48.7	56.5 ± 8.7	59.3 ± 9.4	423.8 ± 35.8	584.7 ± 187.1	861.5 ± 275.6	8.8 ± 1.5	52.0 ± 7.9	389.3 ± 35.5	432.4 ± 104.6	637.1 ± 154.1
Irrigation period 2	−18.8 ± 1.4	293.9 ± 23.0	43.7 ± 4.1	41.8 ± 4.6	475.3 ± 14.2	371.9 ± 56.8	547.9 ± 83.7	12.0 ± 0.5	34.9 ± 2.7	334.2 ± 18.3	179.3 ± 16.1	264.3 ± 23.8
Irrigation period 3	−20.7 ± 1.4	324.3 ± 25.5	38.1 ± 4.2	35.7 ± 5.1	351.8 ± 26.0	215.5 ± 31.8	317.5 ± 46.9	12.1 ± 0.4	34.4 ± 2.3	321.6 ± 27.3	170.0 ± 26.5	250.5 ± 39.1

Kumtag (SNM® Natural Sodium Nitrate, Industrial Grade 99.7 %, $\Delta^{17}O\sim17.6‰$, preliminary data) deserts can be used as the $\Delta^{17}O$-labeled material, which could be supplied in large quantities at low costs. These salts cost approximately USD $10/kg, compared to ~USD $120/kg for $Na^{15}NO_3$ with $\delta^{15}N = 350‰$ that was diluted from 5 atom% Sigma-Aldrich® $Na^{15}NO_3$, and 350‰ was selected with a similar differentiate from natural ^{15}N abundance in soil up to 20‰ to that for $\Delta^{17}O$ between mined $N^{17}O_3^-$ salts and nitrified NO_3^-. This cost difference makes field-scale application possible. The recently-developed bacterial reduction, gold redox method makes the $\Delta^{17}O$-NO_3^- analysis cheap, sensitive and rapid with no need of laborious sample preparation (Weigand et al., 2016), though good maintenance of the experimental bacterial culture and gold reduction conditions is not easy and has only been accomplished by a few labs over the globe. Finally, the soil (or soilless media) $\Delta^{17}O$-NO_3^- values are not altered by microbial assimilation, plant uptake, or denitrification processes that fractionate isotopes in mass-dependent manners, allowing for independent prediction of GNR in soils (or soilless media). Therefore, we highly recommend using $\Delta^{17}O$-NO_3^- as a tracer for GNR estimation to better understand the nitrification potentials in diverse systems, especially on large spatial and temporal scales. The major challenge of the $\Delta^{17}O$-NO_3^- tracer method occurs when N turnover is rapid. In these cases, the nitrified NO_3^- may overwhelmingly dominate the N pool and $\Delta^{17}O$-NO_3^- will be approximately zero, which can be compromised by the high application rates of $\Delta^{17}O$-labeled NO_3^- to some extent.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

References

Aber, J.D., Melillo, J.M., Nadelhoffer, K.J., McClaugherty, C.A., Pastor, J., 1985. Fine root turnover in forest ecosystems in relation to quantity and form of nitrogen availability: a comparison of two methods. Oecologia 66, 317–321.

Castro, M.S., Steudler, P.A., Melillo, J.M., Aber, J.D., Millham, S., 1992. Exchange of N_2O and CH_4 between the atmosphere and soils in spruce-fir forests in the northeastern United States. Biogeochemistry 18, 119–135.

Cheng, Y., Wang, J., Zhang, J.B., Wang, S.Q., Cai, Z.C., 2015. The different temperature sensitivity of gross N transformations between the coniferous and broad-leaved forests in subtropical China. Soil Sci. Plant Nutr. 61, 506–515.

Christensen, L.M., Lovett, G.M., Weathers, K.C., Arthur, M.A., 2009. The influence of tree species, nitrogen fertilization, and soil C to N ratio on gross soil nitrogen transformations. Soil Sci. Soc. Am. J. 73, 638.

Davidson, E., Hart, S., Shanks, C., Firestone, M., 1991. Measuring gross nitrogen mineralization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. J. Soil Sci. 42, 335–349.

Fang, Y., Koba, K., Makabe, A., Takahashi, C., Zhu, W., et al., 2015. Microbial denitrification dominates nitrate losses from forest ecosystems. Proc. Natl. Acad. Sci. 112, 1470–1474.

Hart, S., Gunther, A., 1989. *In situ* estimates of annual net nitrogen mineralization and nitrification in a subarctic watershed. Oecologia 80, 284–288.

Hart, S., Stark, J.M., Davidson, E., Firestone, M., et al., 1994. Nitrogen mineralization, immobilization and nitrification. In: Weaver, A.L. (Ed.), Methods of Soil Analysis Part 2, 2nd edn. Soil Science Society of America, Madison, WI, pp. 985–1018. SSSA book series number 5.

Huang, S.N., Wang, F., Elliott, E.M., Zhu, F.F., Zhu, W.X., et al., 2020. Multiyear measurements on $\Delta^{17}O$ of stream nitrate indicate high nitrate production in a temperate forest. Environ. Sci. Technol. 54, 4231–4239.

Ingwersen, J., Butterbach-Bahl, K., Gasche, R., Papen, H., Richter, O., 1999. Barometric process separation: new method for quantifying nitrification, denitrification, and nitrous oxide sources in soils. Soil Sci. Soc. Am. J. 63 (1), 117.

McClaugherty, C.A., Pastor, J., Aber, J.D., Melillo, J.M., 1985. Forest litter decomposition in relation to soil nitrogen dynamics and litter quality. Ecology 66, 266–275.

Michalski, G., Thiemens, M., 2006. The Use of Multi-isotope Ratio Measurements As a New and Unique Technique to Resolve NO_x Transformation, Transport and Nitrate, Deposition in the Lake Tahoe Basin. California Air Resources Board, Sacramento, CA.

Michalski, G., Scott, Z., Kabiling, M., Thiemens, M., 2003. First measurements and modeling of ^{17}O in atmospheric nitrate. Geophys. Res. Lett. 30, 1870.

Michalski, G., Kolanowski, M., Riha, K.M., 2015. Oxygen and nitrogen isotopic composition of nitrate in commercial fertilizers, nitric acid, and reagent salts. Isotopes Environ. Health Stud. https://doi.org/10.1080/10256016.2015.1054821.

Morin, S., Savarino, J., Frey, M.M., Domine, F., Jacobi, H.W., et al., 2009. Comprehensive isotopic composition of atmospheric nitrate in the Atlantic Ocean boundary layer from 65°S to 79°N. J. Geophys. Res. 114, D05303.

Riha, K.M., Michalski, G., Gallo, E.L., Lohse, K.A., Brooks, P.D., et al., 2014. High atmospheric nitrate inputs and nitrogen turnover in semi-arid urban catchments. Ecosystems 17, 1309–1325.

Rose, L.A., Elliott, E.M., Adams, M.B., 2015. Triple nitrate isotopes indicate differing nitrate source contributions to streams across a nitrogen saturation gradient. Ecosystems 18, 1209–1223.

Sant Ana Filho, C.R., Bendassolli, J.A., Rossete, A.L.R.M., Piedade, S.M.S., Prestes, C.V., 2008. Production of ^{15}N-enriched nitric acid ($H^{15}NO_3$). Braz. J. Chem. Eng. 25, 743–749.

Spindel, W., Taylor, T.I., 1955. Separation of nitrogen isotopes by chemical exchange between NO and HNO_3. J. Chem. Phys. 23 (5), 981–982.

Thiemens, M.H., 2006. History and applications of mass-independent isotope effects. Annu. Rev. Earth Planet. Sci. 34, 217–262.

Thode, H.G., Urey, H.C., 1939. The further concentration of N^{15}. J. Chem. Phys. 7 (1), 34–39.

Tsunogai, U., Komatsu, D.D., Daita, S., Kazemi, G.A., Nakagawa, F., et al., 2010. Tracing the fate of atmospheric nitrate deposited onto a forest ecosystem in Eastern Asia using $\Delta^{17}O$. Atmos. Chem. Phys. 10, 1809–1820.

Tsunogai, U., Daita, S., Komatsu, D.D., Nakagawa, F., Tanaka, A., 2011. Quantifying nitrate dynamics in an oligotrophic lake using $\Delta^{17}O$. Biogeosciences 8, 687–702.

Wang, F., Ge, W.S., Luo, H., Seo, J.H., Michalski, G., 2016. Oxygen-17 anomaly in soil nitrate: a new precipitation proxy for desert landscapes. Earth Planet. Sci. Lett. 438, 103–111.

Weigand, M.A., Foriel, J., Barnett, B., Oleynik, S., Sigman, D.M., 2016. Updates to instrumentation and protocols for isotopic analysis of nitrate by the denitrifier method. Rapid Commun. Mass Spectrom. 30 (12), 1365–1383.

Yu, Z., Elliott, E.M., 2018. Probing soil nitrification and nitrate consumption using $\Delta^{17}O$ of soil nitrate. Soil Biol. Biochem. 127, 187–199.

Zaman, M., Chang, S.X., 2004. Substrate type, temperature, and moisture content affect gross and net N mineralization and nitrification rates in agroforestry systems. Biol. Fert. Soils 39, 269–279.