

# Triple Oxygen Isotope Analysis of Nitrate Using the Denitrifier Method and Thermal Decomposition of N<sub>2</sub>O

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Interaction with ozone transfers its anomalous (non-mass-dependent) <sup>17</sup>O enrichment to atmospheric nitrogen oxides and nitrate. The <sup>17</sup>O anomaly ( $\Delta^{17}\text{O}$ ) in nitrate can be used to identify atmospheric nitrate inputs into terrestrial and aquatic environments as well as to study the role of ozone in the atmosphere's reactive nitrogen cycle. We report here on an online method for analysis of the <sup>17</sup>O anomaly, using a strain of denitrifiers to convert nitrate to N<sub>2</sub>O, which decomposes quantitatively to N<sub>2</sub> and O<sub>2</sub> in a gold furnace at 800 °C, followed by gas chromatographic separation and isotope analysis of O<sub>2</sub>. This method requires ~50 nmol of nitrate, 2–3 orders of magnitude less than previous offline thermal decomposition methods to achieve a similar analytical precision of 0.5‰ for  $\Delta^{17}\text{O}$ . There is no significant memory effect, but calibration via nitrate or N<sub>2</sub>O reference materials is required for scale normalization. The N<sub>2</sub>O decomposition method is shown to be well-suited for nitrate analysis in freshwater and seawater samples from various environments.

Nitrogen oxides (NO<sub>x</sub> ≡ NO + NO<sub>2</sub>) play a key role in atmospheric chemistry, since they control destruction and production of ozone and affect the concentration of the hydroxyl radical (OH), dubbed the “detergent of the atmosphere”.<sup>1</sup> The abundance of NO<sub>x</sub> is linked to other reactive nitrogen (NO<sub>y</sub>) compounds including nitric acid (HNO<sub>3</sub>) and organic nitrates. Tropospheric HNO<sub>3</sub> is efficiently removed by precipitation and contributes to acid rain, but might also be a significant source of fixed nitrogen in many terrestrial ecosystems<sup>2</sup> or coastal waters.<sup>3</sup> Stratospheric

aerosol nitrate participates in heterogeneous reactions on polar stratospheric clouds, leading to ozone destruction in the Antarctic polar vortex. Deposition of stratospheric aerosol nitrate might be the dominant nitrate source for Antarctic firn and ice<sup>4</sup> and knowing its contribution provides an important constraint on polar chemistry and climate in the past.

Nitrogen inputs into freshwater and coastal areas through river runoff lead to eutrophication and associated adverse effects.<sup>5</sup> Understanding the relative importance of different nitrogen sources and subsequent microbial nitrogen conversion reactions such as nitrification and denitrification is important to evaluate the impact of such anthropogenic perturbations on the nitrogen budget as direct fertilizer inputs and increased NO<sub>x</sub> emissions from fossil fuel combustion and biomass burning.<sup>6</sup>

Isotope measurements can help elucidate some of the above processes.<sup>7</sup> For example, dual <sup>15</sup>N/<sup>14</sup>N and <sup>18</sup>O/<sup>16</sup>O isotope ratio measurements have been used to separate the contributions of various nitrate sources to freshwater ecosystems and groundwater. However, some ambiguity remains because both denitrification and atmospheric nitrate inputs can lead to enriched <sup>18</sup>O/<sup>16</sup>O isotope ratios. Michalski et al.<sup>8</sup> recently presented a method to determine the triple oxygen isotope composition of nitrate. Large non-mass-dependent oxygen isotope enrichments were found for atmospheric aerosols.<sup>9</sup> This provides a unique signature for quantifying atmospheric nitrate deposition and for studying atmospheric NO<sub>x</sub> cycling.<sup>10,11</sup> The method of Michalski et al.<sup>8</sup> is based on the conversion of nitrate to oxygen for isotope mass spectrometric analysis. It requires high-purity nitrate because

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organic contaminations lead to strong interferences.<sup>12</sup> Therefore, before mass spectrometric analysis, a range of preparation steps are needed, including multiple ion chromatographic purification and fractionated collection of nitrate, conversion to AgNO<sub>3</sub>, filtration, drying, thermal decomposition, and cryogenic separation of the decomposition products. Relatively large sample sizes between 10 and 40 μmol of nitrate are best suited for this method; otherwise, large blank corrections are required. Seawater samples cannot be analyzed because the high salt content is incompatible with ion chromatographic separation.

Here, we present a novel method to measure the triple oxygen isotope composition of nitrate, which does not suffer from the above problems. It is based on the denitrifier method, originally developed for <sup>15</sup>N/<sup>14</sup>N and <sup>18</sup>O/<sup>16</sup>O isotope ratio of seawater and freshwater nitrate.<sup>13,14</sup> A bacterial denitrifier strain that lacks an active nitrous oxide reductase enzyme is used to convert nitrate to nitrous oxide (N<sub>2</sub>O). The specific strain used (*Pseudomonas aureofaciens*) typically shows less than 5% isotope exchange with water and is therefore suitable for oxygen isotope analysis. In an online coupled gas chromatography/isotope ratio mass spectrometry system N<sub>2</sub>O is then converted to O<sub>2</sub> and N<sub>2</sub>, using a gold catalyst at 800 °C. O<sub>2</sub> and N<sub>2</sub> are separated on a molecular sieve column and the <sup>17</sup>O/<sup>16</sup>O and <sup>18</sup>O/<sup>16</sup>O isotope ratios of the O<sub>2</sub> peak are measured by an isotope ratio mass spectrometer.

Below, we provide a detailed description of the method and subsequent data analysis. The method is rapid, can be fully automated, and only requires ~50 nmol of nitrate, 2–3 orders of magnitude less nitrate than the AgNO<sub>3</sub> method, at a similar analytical precision. We then show results from an application of the method to measure the triple oxygen isotopic composition of nitrate in precipitation, streamwater, soil water, and seawater samples.

## EXPERIMENTAL SECTION

**Bacterial Conversion of Sample Nitrate to Nitrous Oxide.** Sigman et al.<sup>13</sup> and Casciotti et al.<sup>14</sup> described medium preparation, culturing conditions, and preparation of N<sub>2</sub>O from individual nitrate samples. Briefly, the denitrifier *P. aureofaciens* (ATCC 13985, recently reclassified as a strain of *Pseudomonas chlororaphis*) is grown in 130-mL batches of tryptic soy broth medium, amended with 10 mmol/L KNO<sub>3</sub>, 15 mmol/L NH<sub>4</sub>Cl and 1 mL/L antifoaming agent. After 6–10 days, the cells are harvested and concentrated 5–7.5-fold and split into 2-mL aliquots in 20-mL headspace vials (instead of 10-fold as used by Casciotti et al.<sup>14</sup>; no adverse effect on the N<sub>2</sub>O yield or reproducibility of the isotopic measurements was noted for the lower cell concentration). The vials were then purged for at least 6 h with N<sub>2</sub>, increased from 3 h,<sup>14</sup> to further reduce the size of any N<sub>2</sub>O blank. A sample amount corresponding to ~50 nmol of nitrate is added to the sample vials and incubated overnight. Then, 0.1–0.2 mL of 10 mol/L NaOH is added to lyse the cells and scavenge CO<sub>2</sub>. A constant sample size is desirable to avoid corrections for mass spectrometer nonlinearity at different

amounts of N<sub>2</sub>O or O<sub>2</sub> (see Sample Size Dependence of Δ<sup>17</sup>O and δ<sup>18</sup>O in Results and Discussion).

**N<sub>2</sub>O Extraction and Conversion to O<sub>2</sub>.** N<sub>2</sub>O is extracted from the sample vials using a modified version of the manual extraction system<sup>13</sup> (see Casciotti et al.<sup>14</sup> for an automated version). The N<sub>2</sub>O from the sample vial is purged with a helium carrier stream at 15–20 mL/min, trapped in a U-tube filled with glass beads, and immersed in liquid nitrogen. A water trap filled with glass beads at –70 to –100 °C (ethanol/liquid nitrogen cooling mixture) is used to remove most of the water from the sample stream. Rather than using a manual switch between purging and extraction modes described by Sigman et al.,<sup>13</sup> the previous sample vial is left connected while the U-tube for the next sample is purged at a high helium flow rate (80–100 mL/min).

The U-tube containing the N<sub>2</sub>O sample is then transferred to a modified Finnigan MAT PreCon device,<sup>15</sup> connected via a Finnigan MAT gas chromatograph and a Finnigan MAT Conflo II injection device to a Finnigan MAT 252 isotope ratio mass spectrometer. In the PreCon device, the N<sub>2</sub>O sample is purified by passing it through a Supelco type F hydrocarbon purge trap, which replaces the standard Mg(ClO<sub>4</sub>)<sub>2</sub>/ascarite trap of the PreCon. The trap is conditioned at 180 °C overnight with a helium flow of 100 mL/min in reverse flow direction and used for ~200 analyses before reconditioning. This additional purification is necessary to remove volatile organic compounds (VOCs) formed during the bacterial incubation step, which otherwise lead to partial O<sub>2</sub> consumption in the gold furnace and spurious results. We tried different Supelco hydrocarbon purge traps and identified type F traps as the most suitable, because they appear to remove VOCs most efficiently and give the expected near-zero Δ<sup>17</sup>O value for reference material USGS-34 (Figure 1). The purified N<sub>2</sub>O sample is preconcentrated and cryofocused using the standard PreCon configuration. However, a gold furnace is placed between the final fused-silica trap and the gas chromatograph (see Figure 1 in ref 15). The gold furnace consists of the original methane oxidation furnace provided with the PreCon device, fitted with a 500-mm-long, 1.6-mm-o.d., 0.6-mm-i. d. pure gold tube (C. Hafner, Pforzheim, Germany), held at 800 °C.<sup>16</sup> The ends of the gold tube are fitted through 13-mm-thick copper plates, cooled by forced air ventilation, to avoid excessive heating of the unions joining the gold tube to the fused-silica tubing. Upon release of an N<sub>2</sub>O sample from the fused-silica trap, it travels with the helium flow through the gold furnace and decomposes to N<sub>2</sub> and O<sub>2</sub>. These two gases are then separated directly (without further cryofocusing) on a molecular sieve fused-silica column (Chrompack, 0.5 nm, 25 m × 0.32 mm, 40 °C, 1.3 mL/min, Δ*p* = 1.3 bar). The O<sub>2</sub> peak elutes after ~200 s and is analyzed isotopically. The N<sub>2</sub> peak elutes after ~300 s and is only analyzed for its magnitude; the mass spectrometer resistor configuration did not allow for precise determination of its isotope ratio. The quantitative reaction of N<sub>2</sub>O was verified by removing the GC column and testing for residual N<sub>2</sub>O with the mass spectrometer. Production of both NO<sub>2</sub> and NO, estimated from direct mass spectrometric analysis without GC separation of the outflow from the gold tube and comparison to the *m/z* 46 and 30 intensities of nonheated N<sub>2</sub>/O<sub>2</sub> mixtures,

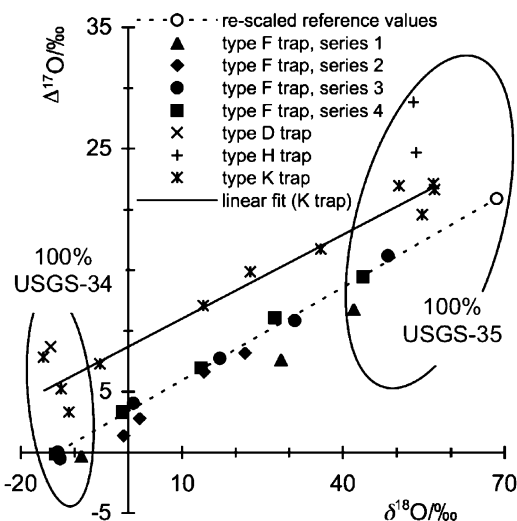
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**Figure 1.** Test of different Supelco purge traps for suitability of cleaning up  $N_2O$  produced by the denitrifier method. The  $\delta^{18}O$  and  $\Delta^{17}O$  values are with respect to the  $O_2$  working reference tank. The rescaled reference values are based on the  $\delta^{18}O(NO_3^-)$  and  $\Delta^{17}O(NO_3^-)$  values of USGS-34 and USGS-35 (Table 1), but rescaled to the same  $\delta^{18}O_{USGS-34}$  value as measured for type F trap, series 3 and 4. No scale normalization or blank and isotope exchange correction has been applied. Intermediate points for the type D trap series are for mixtures of 20%/40%/60%/80% USGS-34 and 80%/60%/40%/20% USGS-35. Intermediate points for the type F trap series are for 25%/50%/75% USGS-34 and 75%/50%/25% USGS-35. Only the results obtained with the type F trap were deemed satisfactory, because all other traps showed nonzero  $\Delta^{17}O$  values for USGS-34, in disagreement with the expectation of a near-zero  $^{17}O$  anomaly for this reference material (see Table 1). The traps are packed with the following materials: type D trap—16 cm Tenax TA, 7.7 cm activated charcoal; type F trap—1 cm 3% SP-1200/Chromosorb W AW, 15 cm Tenax TA, 7.7 cm silica gel 15, type H trap—7.6 cm Carboxen B, 1.3 cm Carboxen S-III, type K trap—10 cm Carboxen B, 6 cm Carboxen 1000, 1 cm Carboxen 1001. Note: The forward slash (“/”) in the axis legends of this and the following figures means “divided by”, using internationally recommended notation for unit calculus (see section 5.3.1 in ref 32). For example,  $\delta^{18}O/‰ = 30$  corresponds to  $\delta^{18}O = 30‰$ .

was insignificant ( $<0.001$  and  $<0.02\%$  of the initial  $N_2O$ , respectively).

**Isotopic Analysis.** In line with the “identical treatment principle”,<sup>17</sup> the method for triple oxygen isotope analysis of nitrate follows the approach of Casciotti et al.<sup>14</sup> in using nitrate reference materials and an  $^{18}O$ -labeled water spike to quantify the degree of isotope exchange in the bacterial denitrification step. To place the isotopic analyses on the Vienna Standard Mean Ocean Water (VSMOW) scale, IAEA-NO-3 is used, as it is the most frequently used and best characterized international nitrate reference material. To define the isotope scale contraction (see below), the additional reference materials USGS-34 and USGS-35<sup>18</sup> are used, as they have a larger  $\delta^{18}O$  difference values and are therefore more suitable for this purpose. The isotopic compositions adopted for the reference materials are given in Table 1. In practice, the nitrate reference materials are converted to  $N_2O$  and then to  $N_2$  and  $O_2$  alongside the nitrate samples. Then, the gas chromatographic

$O_2$  peaks are measured relative to an  $O_2$  working reference gas tank ( $\delta^{17}O = 10.1‰$ ,  $\delta^{18}O = 19.4‰$  vs VSMOW) that is injected into the mass spectrometer via the reference open split. The working reference gas is injected into the mass spectrometer during the time intervals 10–40, 70–100, and 130–160 s after release of the  $N_2O$  sample from the fused-silica cryofocussing trap. After 170 s, the sample capillary is lowered into the open split, in anticipation of the  $O_2$  sample peak. The third reference gas peak (130–160 s) is used for comparison to the sample  $O_2$  peak. After the sample peak has passed through (270 s after release of the  $N_2O$  sample), the ion beam is adjusted to  $m/z$  28, to measure the  $N_2$  peak area for verification purposes. We use Finnigan Isodat NT 2.0 software to integrate the peak areas and determine relative isotopic enrichments. The mass spectrometer has a triple Faraday cup configuration for simultaneous registration of  $m/z$  32, 33, and 34 ion currents, with  $10^9$ ,  $10^{12}$ , and  $10^{11}$   $\Omega$  resistors, respectively. A typical peak area for a 50-nmol nitrate sample (12.5 nmol of  $O_2$ ) is 40 V·s.

**Definitions.**  $\delta$  values are defined as relative isotopic enrichments of a sample with respect to a reference material, i.e.,  $\delta = R_{\text{sample}}/R_{\text{ref}} - 1$ , where  $R$  in the case of oxygen represents the  $^{17}O/^{16}O$  or  $^{18}O/^{16}O$  isotope ratio. Following Miller<sup>19</sup> and Kaiser et al.,<sup>20</sup> we define the oxygen isotope anomaly of nitrate ( $\Delta^{17}O$ ) as

$$\Delta^{17}O \equiv \frac{1 + \delta^{17}O}{(1 + \delta^{18}O)^\beta} - 1 \quad (1)$$

with a three-isotope exponent of  $\beta \equiv 0.5279$  exactly, corresponding to the mass-dependent fractionation line determined from the isotopic water reference materials VSMOW, SLAP, and GISP<sup>21</sup> with a precision of  $\pm 0.0001$ . Because of the good solubility of nitrate, water is more appropriate than rocks to define the  $\beta$  value of nitrate isotope anomalies. Li and Meijer<sup>22</sup> measured a similar value of  $0.5281 \pm 0.0015$  for a range of meteoric waters, with the larger uncertainty being due to possible peak overlap on the  $m/z$  33 collector. Miller<sup>19</sup> reported a three-isotope exponent for unpublished results on meteoric waters equal to the value of  $0.5247 \pm 0.008$  based on terrestrial silicates. Equation 1 with a  $\beta$  value of 0.525 was subsequently adopted for the definition of nitrate oxygen isotope anomalies by one group of authors.<sup>18,23</sup> However, as the details of Miller’s measurements are not published and as the other two values agree well within another, we have adopted the more precise value of 0.5279 here. Note that the definition of  $\Delta^{17}O$  and choice of  $\beta$  is a mere convention. For example, other authors<sup>8</sup> define the oxygen isotope anomaly of nitrate as  $\Delta^{17}O \equiv \delta^{17}O - 0.52 \delta^{18}O$ . The latter definition has the advantage to be linear in terms of mixing, but suffers from reference gas-dependent  $\Delta^{17}O$  values and from spurious nonzero  $\Delta^{17}O$  values even for a mass-dependent relationship between  $^{17}O/^{16}O$  and  $^{18}O/^{16}O$  fractionation factors with the same value for  $\beta$  as in eq 1,<sup>20</sup> i.e.,  $^{17}\alpha = ^{18}\alpha^\beta$ . Anyway,  $\Delta^{17}O$  values close to zero should not be construed as indications of non-mass-dependent processes,

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**Table 1. Isotopic Composition of Nitrate Reference Materials**

$\delta$ value	$\delta^{15}\text{N}$ (in ‰)	$\delta^{18}\text{O}$ (in ‰)	$\delta^{17}\text{O}^a$ (in ‰)	$\Delta^{17}\text{O}^{+b}$ (in ‰)	$\Delta^{17}\text{O}^c$ (in ‰)
reference source	air-N <sub>2</sub>	VSMOW	VSMOW	VSMOW	VSMOW
IAEA-NO-3	ref 18	ref 18	calculated	refs 8, 18	calculated
USGS-34	4.7 ± 0.1	25.61 ± 0.2	13.18 ± 0.16	-0.14 ± 0.12	-0.25 ± 0.12
USGS-35	-1.8 ± 0.1	-27.93 ± 0.3	-14.80 ± 0.19	-0.28 ± 0.10	0.04 ± 0.10
	2.7 ± 0.1	57.50 ± 0.2	51.46 ± 0.15	21.56 ± 0.11	20.88 ± 0.11

<sup>a</sup> Calculated from  $\Delta^{17}\text{O}^+$  and  $\delta^{18}\text{O}$  measurements of Böhlke et al.<sup>18</sup> <sup>b</sup> Defined as  $\Delta^{17}\text{O}^+ = \delta^{17}\text{O} - 0.52 \delta^{18}\text{O}$ .<sup>8</sup> <sup>c</sup> Calculated using eq 1.

because  $\beta$  can range from 0.5 to 0.53 for different mass-dependent processes<sup>24</sup> and even beyond that for special cases.<sup>25</sup> Thus, using eq 1 and a  $\delta^{18}\text{O}$  value of 100‰,  $\Delta^{17}\text{O}$  values between -2.7 and +0.2‰ could be caused by mass-dependent isotope effects. Similarly, oxygen isotope anomalies ( $\Delta^{17}\text{O}$  values) should not be considered as anomalous in an absolute sense, but rather as anomalous with respect to the operational definition given in eq 1.

**O<sub>2</sub> Isotope Data Reduction.** The atomic  $\delta^{17}\text{O}(\text{O}_2)$  and  $\delta^{18}\text{O}(\text{O}_2)$  values can be approximated without significant error by the molecular raw <sup>33</sup> $\delta$  and <sup>34</sup> $\delta$  values, measured against the O<sub>2</sub> working reference tank (see Supporting Information). Using the isotopic scale normalization described below,  $\delta^{17}\text{O}(\text{O}_2)$  and  $\delta^{18}\text{O}(\text{O}_2)$  are then transferred to the same scale as the N<sub>2</sub>O isotope measurements. After correcting the O<sub>2</sub> isotope data for blank and isotope exchange effects, the isotopic composition of the initial nitrate sample is obtained. The latter correction is realized through eq S4 derived in the context of the N<sub>2</sub>O isotope data reduction (Supporting Information), taking into account the different reference materials and the fact that O<sub>2</sub>, not N<sub>2</sub>O, is analyzed.

**N<sub>2</sub>O Isotope Data Reduction.** In the Supporting Information to this paper, we revisit published data reduction procedures for N<sub>2</sub>O isotope measurements,<sup>14,18,23</sup> with the aim to incorporate possible <sup>17</sup>O isotope anomalies and to provide a self-consistent way of calculating atomic isotope ratios (i.e., <sup>15</sup>N/<sup>14</sup>N, <sup>17</sup>O/<sup>16</sup>O, and <sup>18</sup>O/<sup>16</sup>O) from the measured 45/44 and 46/44 ion current ratios. We also use the N<sub>2</sub>O isotope measurements for comparison with the  $\delta^{18}\text{O}$  measured in O<sub>2</sub>. Direct N<sub>2</sub>O isotope measurements were performed with a Finnigan Delta Plus mass spectrometer.<sup>14</sup> The calculations are based on Casciotti et al.<sup>14</sup> and Kaiser et al.<sup>26</sup> Corrections are included for isobaric interferences (e.g., <sup>14</sup>N<sub>2</sub><sup>17</sup>O and <sup>15</sup>N<sup>14</sup>N<sup>16</sup>O, or <sup>15</sup>N<sub>2</sub><sup>16</sup>O and <sup>14</sup>N<sub>2</sub><sup>18</sup>O),  $\Delta^{17}\text{O}$  anomalies of sample and reference, isotope exchange between nitrogen oxyanions and water during bacterial denitrification of nitrate to N<sub>2</sub>O, and blanks in the denitrifier method.

**Isotopic Scale Normalization.** Isotopic scale normalizations are often applied to bring measurements from different laboratories to a common scale. The normalization is achieved by stretching or compressing the measurement scale based on the measured isotopic compositions of two reference materials and their assigned values. In the present case, we apply a similar normalization to bring the O<sub>2</sub> isotope measurements to the same

scale as the N<sub>2</sub>O isotope measurements. For this purpose, we use two nitrate reference materials, USGS-34 and USGS-35, with a large difference in their  $\delta^{18}\text{O}$  values (see Table 1). The measured isotopic enrichment of the USGS-35-derived N<sub>2</sub>O relative to the USGS-34-derived N<sub>2</sub>O in comparison to the relative enrichment of the corresponding O<sub>2</sub> samples is used to bring the measured  $\delta^{18}\text{O}(\text{O}_2)$  values to the same scale as the measured  $\delta^{18}\text{O}(\text{N}_2\text{O})$  values. The normalization used to convert measured to normalized  $\delta$  values is defined as

$$1 + \text{normalized } \delta_{\text{sample}}^{\text{USGS-34}}(\text{O}_2) = [1 + \text{measured } \delta_{\text{sample}}^{\text{USGS-34}}(\text{O}_2)]^\lambda \text{ with } 1 + \text{measured } \delta_{\text{USGS-35}}^{\text{USGS-34}}(\text{N}_2\text{O}) = [1 + \text{measured } \delta_{\text{USGS-35}}^{\text{USGS-34}}(\text{O}_2)]^\lambda, \text{ i.e., } \lambda = \frac{\ln[1 + \text{measured } \delta_{\text{USGS-35}}^{\text{USGS-34}}(\text{N}_2\text{O})]}{\ln[1 + \text{measured } \delta_{\text{USGS-35}}^{\text{USGS-34}}(\text{O}_2)]} \quad (2)$$

Indices to the right of the  $\delta$  symbol indicate sample (subscript) and reference (superscript) identity. As explained in the Supporting Information, this logarithmic definition differs slightly from the linear definition often used in the literature, but has the advantage of being scale-invariant and universally applicable.

We assume that the scale normalization parameter  $\lambda$  found for  $\delta^{18}\text{O}$  also applies for  $\delta^{17}\text{O}$ . It follows from eq 1 that the same  $\lambda$  needs to be used for  $\Delta^{17}\text{O}$ :

$$1 + \text{normalized } \Delta^{17}\text{O}_{\text{sample}}^{\text{USGS-34}}(\text{O}_2) = [1 + \text{measured } \Delta^{17}\text{O}_{\text{sample}}^{\text{USGS-34}}(\text{O}_2)]^\lambda \quad (3)$$

The validity of this approach is demonstrated under Accuracy in Results and Discussion, which also shows that the  $\Delta^{17}\text{O}$  value of USGS-35 measured by the new N<sub>2</sub>O decomposition method agrees with the offline conversion result.<sup>8</sup>

## RESULTS AND DISCUSSION

In this section, we first explore precision and accuracy of the method, for both pure N<sub>2</sub>O and pure nitrate samples and for both their  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  values. Then, we discuss a sample size dependence that was found for  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  and explain how it can be corrected for by scale normalization. Finally, we show results from triple oxygen isotope measurements of nitrate in environmental precipitation, streamwater, soil water, and seawater samples.

**Precision and Absence of Memory Effects.** As discussed above, the denitrifier method is used to convert nitrate samples

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to  $\text{N}_2\text{O}$ .  $\text{N}_2\text{O}$  is subsequently converted to  $\text{O}_2$  to determine the isotopic composition. The  $\delta^{18}\text{O}$  precision of each step of the method is estimated from a series of experiments: (a)  $\delta^{18}\text{O}(\text{N}_2\text{O})$  analysis, 0.06‰ (from  $\text{N}_2\text{O}$  tank reference gas on–off experiments); (b) conversion of nitrate sample to  $\text{N}_2\text{O}$  and  $\delta^{18}\text{O}(\text{N}_2\text{O})$  analysis,<sup>14</sup> 0.34‰; (c)  $\delta^{18}\text{O}(\text{O}_2)$  analysis: 0.10‰ (from  $\text{O}_2$  tank reference gas on–off experiments); (d) conversion of  $\text{N}_2\text{O}$  sample to  $\text{O}_2$  and  $\delta^{18}\text{O}(\text{O}_2)$  analysis, 0.28‰; (e) conversion of nitrate sample to  $\text{N}_2\text{O}$ ,  $\text{N}_2\text{O}$  to  $\text{O}_2$  and  $\delta^{18}\text{O}(\text{O}_2)$  analysis, 0.9‰ (whole method).

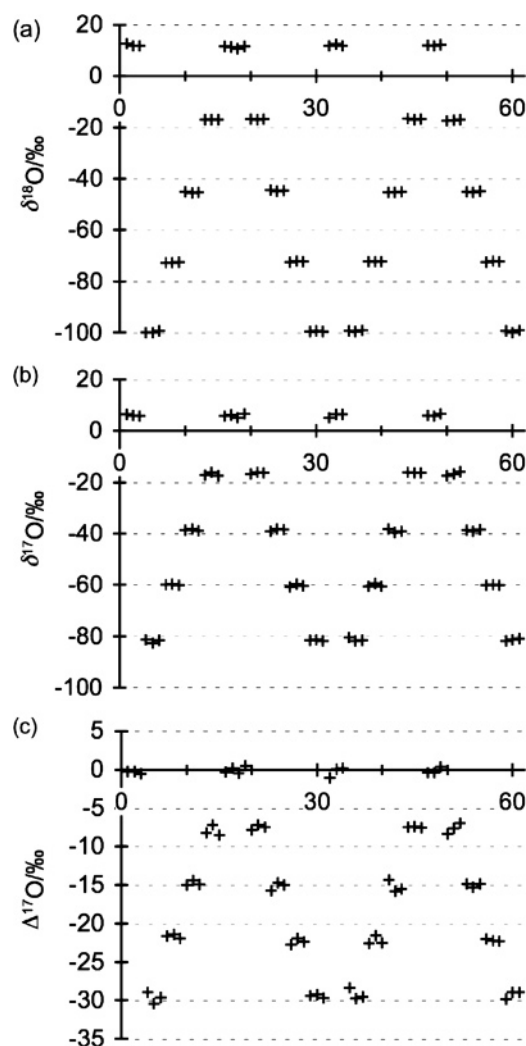
From experiments a and b, it follows that the  $\delta^{18}\text{O}$  precision of the denitrifier conversion alone is  $(0.34^2 - 0.06^2)^{1/2}\text{‰} = 0.33\text{‰}$ . Together with the precision for experiment d, the expected precision for experiment e is therefore  $(0.33^2 + 0.28^2)^{1/2}\text{‰} = 0.44\text{‰}$ . The measured precision is a factor of  $\sim 2$  higher, and the additional uncertainty of  $(0.9^2 - 0.44^2)^{1/2}\text{‰} = 0.8\text{‰}$  must be due to the  $\text{N}_2\text{O}$  purification (VOC removal) step.

The precision of the  $\text{N}_2\text{O}$  to  $\text{O}_2$  conversion step and subsequent  $\delta^{18}\text{O}(\text{O}_2)$  analysis (experiment d) is tested with a sequence of samples with changing isotopic composition, generated by mixing two  $\text{N}_2\text{O}$  samples of very different isotopic compositions (Figure 2). There was no indication of a memory effect in the gold furnace (Figure 2). Even when switching from the most depleted sample ( $\text{N}_2\text{O}$ -discharge-3,  $\delta^{18}\text{O} \approx -100\text{‰}$  with respect to the tank reference peak) to the most enriched sample ( $\text{N}_2\text{O}$ -normal,  $\delta^{18}\text{O} \approx 12\text{‰}$ ), the first sample in a series of three is not significantly different from the other two.

The precision of experiment e is established by analyses of mixtures of the nitrate reference materials USGS-34 and USGS-35. The results give an average standard deviation of  $0.9 \pm 0.4\text{‰}$  for  $\delta^{17}\text{O}$ , of  $0.9 \pm 0.7\text{‰}$  for  $\delta^{18}\text{O}$ , and of  $0.5 \pm 0.1\text{‰}$  for  $\Delta^{17}\text{O}$  (Table 2). The lower standard deviation of the oxygen isotope anomaly  $\Delta^{17}\text{O}$  compared to the individual  $\delta$  values indicates that the  $\delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  errors are correlated. The standard deviations achieved in experiment e are typical for the method.

**Accuracy.** As noted in the previous section, an isotopic scale normalization is required to correct the  $\delta^{18}\text{O}(\text{O}_2)$  scale (designated “measured”) to the  $\delta^{18}\text{O}(\text{N}_2\text{O})$  scale (“normalized”). This is illustrated in Figure 3a, which shows a comparison between measured and expected  $\delta^{18}\text{O}(\text{O}_2)$  values, based on the  $\delta^{18}\text{O}(\text{N}_2\text{O})$  values of the range of  $\text{N}_2\text{O}$  mixtures shown in Figure 2. The success of the scale normalization is depicted in Figure 3b, showing vanishing residuals for the unknown three intermediate  $\text{N}_2\text{O}$  mixtures. Normalized  $\delta^{18}\text{O}$  values were calculated for these intermediate mixtures based on the  $\delta^{18}\text{O}(\text{N}_2\text{O})$  value of the  $\text{N}_2\text{O}$ -discharge-3 sample in the lower left corner of Figure 3a.

To verify the accuracy of the method for  $\Delta^{17}\text{O}(\text{NO}_3^-)$ , we compare the  $\Delta^{17}\text{O}(\text{NO}_3^-)$  value measured for USGS-35 versus USGS-34 with the result of  $20.83 \pm 0.15\text{‰}$  derived from data by Michalski et al.<sup>8</sup> and Böhlke et al.<sup>18</sup> (Table 1). The measured  $\delta^{18}\text{O}(\text{O}_2)$  value for USGS-35 versus USGS-34 of  $77.4 \pm 0.9\text{‰}$  (Table 2) was normalized with respect to the  $\delta^{18}\text{O}(\text{N}_2\text{O})$  value of  $82.8 \pm 0.4\text{‰}$  measured for USGS-35 versus USGS-34 on the same day. The resulting scale normalization parameter of 1.066 was applied to both the  $\delta^{17}\text{O}(\text{O}_2)$  and  $\delta^{18}\text{O}(\text{O}_2)$  values. The derived  $\Delta^{17}\text{O}(\text{O}_2)$  anomaly is  $20.2 \pm 0.7\text{‰}$ . This value has yet to be corrected for the method blank and isotope exchange. For this particular data set, we measured a blank fraction  $b_{\text{sample}}$  of 0.25% and an isotope



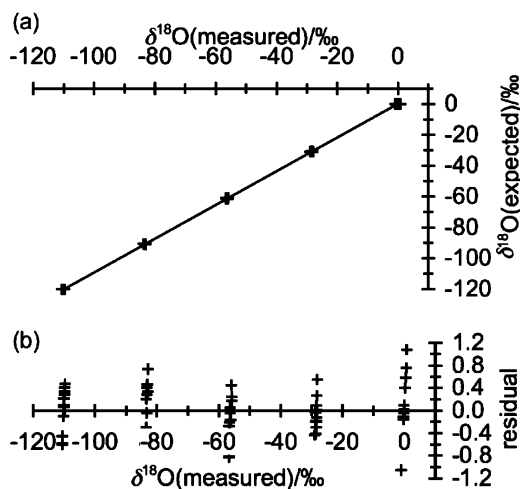
**Figure 2.** Repeated analysis of mixtures of  $\text{N}_2\text{O}$ -discharge-3 (anomalously depleted  $\text{N}_2\text{O}$  produced in an electric discharge tube,  $6 \mu\text{mol/mol}$   $\text{N}_2\text{O}$  in  $\text{N}_2$ ) and  $\text{N}_2\text{O}$ -normal (commercial mixture of  $10 \mu\text{mol/mol}$   $\text{N}_2\text{O}$  in  $\text{N}_2$ , BOC Gases) as a test for precision and absence of memory effects (amount of  $\text{N}_2\text{O}$  per sample,  $15 \text{ nmol}$ ). The (a)  $\delta^{18}\text{O}$ , (b)  $\delta^{17}\text{O}$ , and (c)  $\Delta^{17}\text{O}$  values are raw (not normalized) data with respect to the  $\text{O}_2$  working reference tank. The average standard deviations are  $0.5 \pm 0.2$ ,  $0.3 \pm 0.1$ , and  $0.5 \pm 0.2\text{‰}$  for  $\delta^{17}\text{O}$ ,  $\delta^{18}\text{O}$ , and  $\Delta^{17}\text{O}$ , respectively. The  $\text{N}_2\text{O}$ -discharge-3 sample was prepared by isolation and subsequent extraction of  $\text{N}_2\text{O}$  from the  $\text{NO}_x$ -dominated product gas mixture of an electric discharge in artificial air (79%  $\text{N}_2$ , 21%  $\text{O}_2$ ). The byproducts  $\text{O}_3$ ,  $\text{NO}_2$ , and  $\text{NO}$  were removed using silver wool, an alkaline Ascarite trap and cryodistillation at  $-186 \text{ }^\circ\text{C}$ . The purity of the final  $\text{N}_2\text{O}$  product was verified mass spectrometrically. The  $\text{N}_2\text{O}$ -discharge-3 sample is strongly enriched in  $^{15}\text{N}$  and strongly depleted in both  $\delta^{17}\text{O}$  and  $\delta^{18}\text{O}$ , with a non-mass-dependent oxygen triple isotope composition. The  $\delta^{15}\text{N}$  value of  $\text{N}_2\text{O}$ -discharge-3 relative to  $\text{N}_2\text{O}$ -normal is  $186.9 \pm 0.7\text{‰}$ ; the  $\delta^{18}\text{O}$  value is  $-120.1 \pm 0.5\text{‰}$ . Both were calculated from measurements by a Delta Plus XL mass spectrometer using a normalized  $\Delta^{17}\text{O}$  value of  $-32.3\text{‰}$  (see Accuracy in Results and Discussion).

exchange parameter  $\alpha'$  of 2.8%. These corrections are applied to both  $\delta^{17}\text{O}(\text{O}_2)$  and  $\delta^{18}\text{O}(\text{O}_2)$ , from which we derive a final  $\Delta^{17}\text{O}(\text{NO}_3^-)$  value of  $20.9 \pm 0.7\text{‰}$  for USGS-35 versus USGS-34, in agreement with the literature value. The  $\delta^{18}\text{O}(\text{NO}_3^-)$  difference between USGS-35 and USGS-34 is  $85.6 \pm 1.0\text{‰}$ , slightly smaller than the literature value of  $87.9 \pm 0.4\text{‰}$ .

**Table 2. Measurement Results from Analysis of USGS-34/USGS-35 Mixtures**

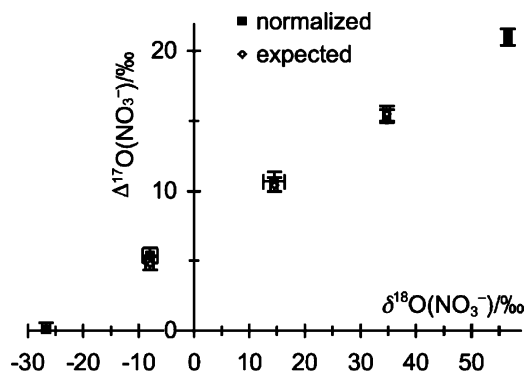
sample identity	peak area (in V s)	$\delta^{17}\text{O}(\text{O}_2)^a$ (in ‰)	$\delta^{18}\text{O}(\text{O}_2)^a$ (in ‰)	$\Delta^{17}\text{O}(\text{O}_2)^c$ (in ‰)	$\delta^{17}\text{O}(\text{NO}_3^-)^b$ (in ‰)	$\delta^{18}\text{O}(\text{NO}_3^-)^b$ (in ‰)	$\Delta^{17}\text{O}(\text{NO}_3^-)^c$ (in ‰)
USGS-34	30.9	-7.5	-13.4	-0.4	-14.4	-26.3	-0.5
USGS-34	41.5	-7.2	-14.2	0.3	-14.1	-27.1	0.3
USGS-34	38.2	-6.3	-13.1	0.6	-13.1	-26.0	0.7
USGS-34	40.7	-7.2	-13.6	0.0	-14.0	-26.5	0.0
USGS-34	33.6	-7.9	-14.8	0.0	-14.8	-27.8	0.0
average	37.0 ± 4.6	-7.2 ± 0.6	-13.8 ± 0.7	0.1 ± 0.4	-14.1 ± 0.6	-26.7 ± 0.7	0.1 ± 0.4
USGS-35	38.8	53.2	63.2	19.7	52.2	57.3	21.8
USGS-35	38.2	52.0	62.6	18.8	50.9	56.7	20.7
USGS-35	31.7	51.9	61.8	19.1	50.8	55.8	21.1
USGS-35	32.8	51.6	62.6	18.4	50.5	56.6	20.4
average	35.4 ± 3.6	52.2 ± 0.7	62.5 ± 0.6	19.0 ± 0.5	51.1 ± 0.8	56.5 ± 0.6	21.0 ± 0.6
75% USGS-34, 25% USGS-35	41.4	6.4	3.8	4.3	0.8	-7.5	4.8
75% USGS-34, 25% USGS-35	42.1	7.6	4.4	5.3	2.2	-6.9	5.9
75% USGS-34, 25% USGS-35	32.4	6.0	2.0	5.0	0.4	-9.5	5.5
average	38.6 ± 5.4	6.7 ± 0.8	3.4 ± 1.3	4.9 ± 0.5	1.1 ± 0.9	-8.0 ± 1.4	5.4 ± 0.5
50% USGS-34, 50% USGS-35	39.9	23.9	25.7	10.3	20.1	16.3	11.4
50% USGS-34, 50% USGS-35	40.2	22.2	23.8	9.6	18.2	14.3	10.6
50% USGS-34, 50% USGS-35	34.8	20.9	22.3	9.1	16.8	12.6	10.1
average	38.3 ± 3.0	22.3 ± 1.5	23.9 ± 1.7	9.7 ± 0.6	18.3 ± 1.7	14.4 ± 1.9	10.7 ± 0.7
25% USGS-34, 75% USGS-35	36.8	36.8	42.6	14.2	34.2	34.8	15.7
25% USGS-34, 75% USGS-35	36.3	36.1	42.5	13.6	33.4	34.7	15.0
25% USGS-34, 75% USGS-35	34.2	(31.7) <sup>d</sup>	(36.3) <sup>d</sup>	(12.5) <sup>d</sup>	(28.6) <sup>d</sup>	(27.9) <sup>d</sup>	(13.8) <sup>d</sup>
average	35.8 ± 1.4	36.4 ± 0.5	42.6 ± 0.1	13.9 ± 0.4	33.8 ± 0.5	34.7 ± -0.1	15.3 ± 0.5
average SD		0.8 ± 0.4	0.9 ± 0.6	0.5 ± 0.1	0.9 ± 0.4	0.9 ± 0.7	0.5 ± 0.1

<sup>a</sup> Relative to O<sub>2</sub> working reference tank; prior to scale normalization, blank and exchange correction. <sup>b</sup> Relative to VSMOW; after scale normalization, blank and exchange correction. <sup>c</sup> Calculated using eq 1. <sup>d</sup> Outlier, excluded from calculation of averages.



**Figure 3.** (a)  $\delta^{18}\text{O}(\text{measured})$  vs  $\delta^{18}\text{O}(\text{expected})$  of mixtures of N<sub>2</sub>O-discharge-3 (most depleted sample) and N<sub>2</sub>O-normal (most enriched sample). The  $\delta^{18}\text{O}(\text{measured})$  values are based on O<sub>2</sub> derived from thermal decomposition of N<sub>2</sub>O. The  $\delta^{18}\text{O}(\text{expected})$  values are based on direct N<sub>2</sub>O isotope analysis. A linear fit gives  $\delta^{18}\text{O}(\text{measured}) = (1.091 \pm 0.001)\delta^{18}\text{O}(\text{expected}) + 0.089 \pm 0.087\text{‰}$  ( $r^2 = 0.9999$ ). (b) Residual difference between normalized  $\delta^{18}\text{O}$  and expected  $\delta^{18}\text{O}$  values, calculated with scale normalization parameter  $\lambda = 1.097$  (eq 2).

As demonstrated for mixtures of N<sub>2</sub>O (Figure 3a), we have also verified the linearity of the  $\Delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  scales with mixtures of the nitrate reference materials USGS-34 and USGS-35 (Figure 4). Using the normalized and blank and exchange corrected  $\Delta^{17}\text{O}(\text{NO}_3^-)$  and  $\delta^{18}\text{O}(\text{NO}_3^-)$  values of USGS-35 versus USGS-34, we compute expected  $\Delta^{17}\text{O}(\text{NO}_3^-)$  and  $\delta^{18}\text{O}(\text{NO}_3^-)$  values for mixtures of USGS-34 and USGS-35 in ratios of 3:1, 1:1, and 1:3, as

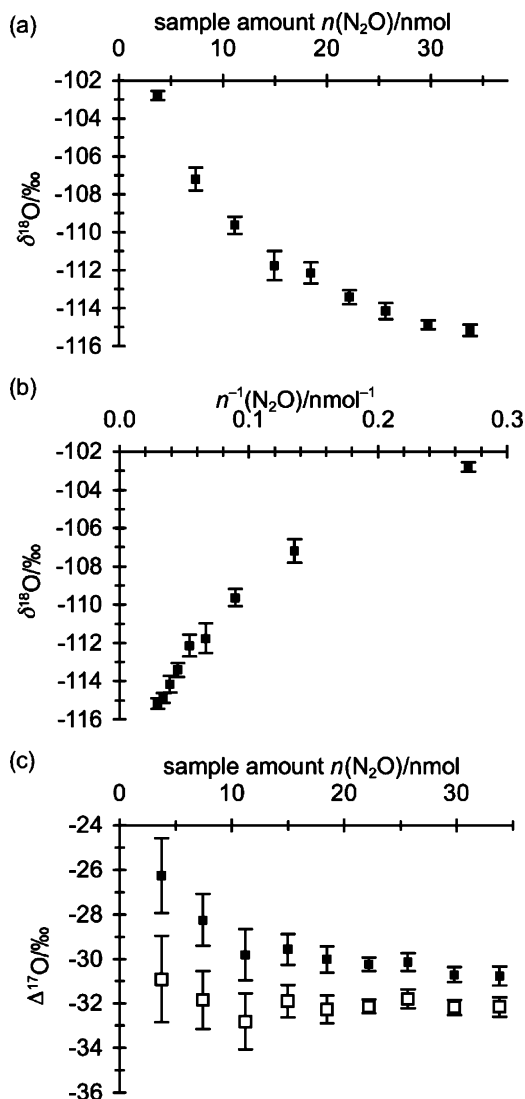


**Figure 4.** Scale linearity for mixtures of USGS-34 (bottom left end member) and USGS-35 (top-right end member). Three mixtures of USGS-34 and USGS-35 in ratios of 1:3, 1:1, and 3:1 were prepared. The normalized measurements are compared to the expected results based on the mixing ratios and the normalized results for USGS-34 and USGS-35.

examples of unknown samples (Table 2). After data reduction, the measured results for the unknown samples agree within the errors with the results expected for them (Figure 4), thus establishing the linearity of the  $\Delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  scales and the suitability of the chosen normalization procedure.

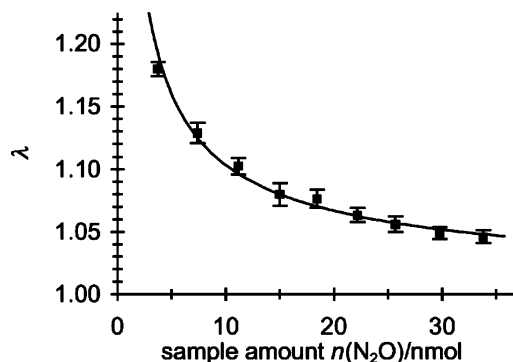
**Sample Size Dependence of  $\Delta^{17}\text{O}$  and  $\delta^{18}\text{O}$ .** The origin of the compressed  $\delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  scales for the gold furnace method is unclear. For example, there is no significant O<sub>2</sub> blank associated with the analysis (O<sub>2</sub> peak below detection when running a pseudosample without N<sub>2</sub>O).

In order to elucidate this scale compression, we measured the difference in the oxygen triple isotope composition of N<sub>2</sub>O-discharge-3 and N<sub>2</sub>O-normal as a function of sample size (Figure



**Figure 5.** (a) Measured difference in  $\delta^{18}\text{O}(\text{O}_2)$  between  $\text{N}_2\text{O}$ -discharge-3 and  $\text{N}_2\text{O}$  normal as a function of sample size. Error bars indicate the standard deviation of three individual measurements. (b) Keeling plot of  $\delta^{18}\text{O}(\text{O}_2)$  difference as a function of inverse sample size. (c) Closed symbols, raw (not normalized)  $\Delta^{17}\text{O}(\text{O}_2)$  difference; open symbols, normalized  $\Delta^{17}\text{O}(\text{O}_2)$  difference.

5a). As expected, the uncertainty of the measured  $\delta^{17}\text{O}(\text{O}_2)$  and  $\delta^{18}\text{O}(\text{O}_2)$  decreases with increasing sample size, due to counting statistics. The difference between  $\text{N}_2\text{O}$ -discharge-3 and  $\text{N}_2\text{O}$ -normal also increases in magnitude for larger sample sizes, but even for the largest sample size investigated, it is smaller than the reference value derived from  $\delta^{18}\text{O}(\text{N}_2\text{O})$  isotope analysis. This discrepancy is unlikely to be due to isotope exchange with or dilution by an unidentified  $\text{O}_2$  reservoir, as this should lead to a linear array in a Keeling plot of inverse sample amounts ( $n^{-1}$ ) and  $\delta^{18}\text{O}(\text{O}_2)$  values. However, a Keeling plot (Figure 5b) actually shows a curved relationship between  $n^{-1}$  and  $\delta^{18}\text{O}(\text{O}_2)$ . An isotope exchange mechanism is also likely to lead to a memory effect, which we showed to be absent at the beginning of Results and Discussion. A possible explanation might be a blank size that decreases with increasing with sample size. However, this appears to be a rather nonphysical explanation, because, if at all, one would expect to see an increase of the blank size with increasing sample size, e.g., if the sample somehow liberated some blank material.



**Figure 6.** Scale normalization parameter  $\lambda$  derived from the results shown in Figure 5a. A power law fit gives  $\lambda = 1 + (0.44 \pm 0.03) [n(\text{N}_2\text{O})/\text{nmol}]^{-0.63 \pm 0.03}$  with  $r^2 = 0.99$ .

A sample size dependence is also found for  $\Delta^{17}\text{O}(\text{O}_2)$  (Figure 5c), but normalization of the  $\delta^{17}\text{O}(\text{O}_2)$  and  $\delta^{18}\text{O}(\text{O}_2)$  values with the  $\delta^{18}\text{O}$  scale normalization parameter found for the appropriate sample size corrects the  $\Delta^{17}\text{O}(\text{O}_2)$  values toward a uniform value of  $-32.0 \pm 0.5\%$ , on average.

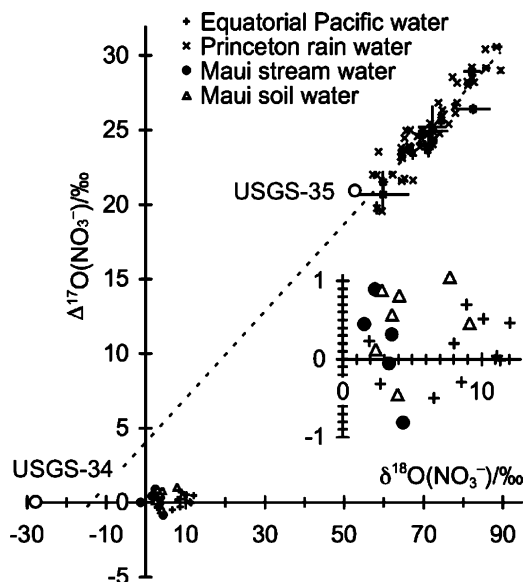
Although we cannot explain the origin of the isotope scale compression, it can be described by an empirical fit of the scale normalization parameter  $\lambda$  as a function of sample size (Figure 6). To a good degree of approximation, the sample size dependence of  $\lambda$  can be described by a power law:  $\lambda = 1 + (0.44 \pm 0.03) [n(\text{N}_2\text{O})/\text{nmol}]^{-0.63 \pm 0.03}$  with  $r^2 = 0.99$ . This allows a scale normalization of any measurement results, for which the sample sizes are not in agreement with the sample size of the reference material.

#### Application to Rain, Stream, Sea, and Soil Water Samples.

As a first application of the new method to environmental samples, we analyzed aqueous nitrate samples from (a) rainwater collected in Princeton, NJ, (b) stream and soil waters from a series of Hawaiian montane rainforests,<sup>27</sup> and (c) ocean water samples from the eastern Equatorial Pacific. Only the rainwater samples show a significant oxygen isotope anomaly (Figure 7). This is due to particulate and gaseous contributions of atmospheric nitrate. The range encountered here ( $19.5\% < \Delta^{17}\text{O}(\text{NO}_3^-) < 30.6\%$ ) is very similar to what Michalski et al.<sup>9</sup> found for atmospheric nitrate aerosol collected in La Jolla, CA ( $17.0\% < \Delta^{17}\text{O}(\text{NO}_3^-) < 29.7\%$ , rescaled according to eq 1 with  $\beta = 0.5279$ ). The  $\delta^{18}\text{O}(\text{NO}_3^-)$  values are also very similar, specifically  $57.2\% < \delta^{18}\text{O}(\text{NO}_3^-) < 89.5\%$  for Princeton rainwater and  $59.3\% < \delta^{18}\text{O}(\text{NO}_3^-) < 88.4\%$  for La Jolla aerosol.

However, these analyses also revealed an important complication, at least for environmental samples. The  $\delta^{18}\text{O}$  values of the Princeton rainwater samples measured by the  $\text{N}_2\text{O}$  decomposition method are on average  $2.3 \pm 4.0\%$  higher than the  $\delta^{18}\text{O}$  values measured on  $\text{N}_2\text{O}$ , with a range from  $-5.5$  to  $9.9\%$ . For the Hawaiian stream and soil samples and the equatorial Pacific surface waters, the mean differences are  $2.0 \pm 3.3$  and  $-0.1 \pm 2.7\%$ , respectively. The average difference does not deviate significantly from zero; however, the range of variability is of concern. In contrast, the average difference for the nine USGS-34/USGS-35 mixtures shown above (Table 2) was  $2.1 \pm 1.3\%$ . Unlike the other comparisons, which were based on measure-

(27) Houlton, B. Z.; Sigman, D. M.; Hedin, L. O. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 8745–8750.



**Figure 7.**  $\Delta^{17}\text{O}(\text{NO}_3^-)$  and  $\delta^{18}\text{O}(\text{NO}_3^-)$  values of environmental nitrate samples (relative to VSMOW). The inset gives an enlarged view of the values near the origin. Only the rainwater samples (collected in Princeton, NJ,  $40^\circ 21' \text{N}$ ,  $74^\circ 40' \text{W}$ ,  $n = 60$ ) show significant oxygen isotope anomalies ( $\Delta^{17}\text{O} > 2$  standard deviations). The relationship between  $\Delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  for these samples can be described by the following relationship:  $\Delta^{17}\text{O}(\text{NO}_3^-) = (0.29 \pm 0.02)\delta^{18}\text{O}(\text{NO}_3^-) + 4 \pm 1\text{‰}$  with  $r^2 = 0.86$ , resulting in a  $\delta^{18}\text{O}(\text{NO}_3^-)$  axis intersection at  $-14 \pm 4\text{‰}$ . The stream and soil water samples were collected on mountain locations of the subtropical Hawaiian island of Maui.<sup>27</sup> The Equatorial Pacific samples were collected at  $95^\circ \text{W}$  between  $-3^\circ \text{S}$  and  $-0.5^\circ \text{S}$ , mostly from the upper 10 m of the mixed layer, except for two samples from 800 and 1000 m depth, characterized by low  $\delta^{18}\text{O}(\text{NO}_3^-)$  values of  $\sim 2\text{‰}$ .

ments made more than a year apart, the  $\text{O}_2$  and  $\text{N}_2\text{O}$  analyses for this test were performed within a day.

The origin of these discrepancies is not understood. It can be partially attributed to the uncertainties of the  $\text{O}_2$  and  $\text{N}_2\text{O}$  methods (0.9 and 0.3‰, respectively) plus calibration errors, which introduce an uncertainty of similar magnitude. A possible explanation is that natural samples or their interaction with the denitrifiers and bacterial medium release an unknown set of reactive and volatile compounds that interfere at the heated gold reactor. This may be compared to early findings from the method development that a trap for volatile organics was required to prevent  $\text{O}_2$ -consuming reactions at gold reactor; however, in contrast to these early findings, we do not observe a corresponding decrease of the  $\text{O}_2$  peak size for the natural samples analyzed here. Future work should focus on this issue.

Despite the discrepancies in the  $\delta^{18}\text{O}$  values between the two methods, we expect the  $\Delta^{17}\text{O}$  values not to be affected significantly, as the discrepancies are most likely caused by mass-dependent isotope effects due to sample treatment (supported by the fact that the  $\Delta^{17}\text{O}$  values are zero for samples we expect them to be zero, such as for USGS-34 or the seawater and Hawaiian forest samples). Even if the responsible mass-dependent isotope effect had a three-isotope exponent of 0.5 rather than 0.5279, a  $\delta^{18}\text{O}$  change of 5‰ would affect the  $\Delta^{17}\text{O}$  value by less than 0.15‰, which is negligible compared to the measurement precision of  $\sim 0.5\text{‰}$ .

The  $\Delta^{17}\text{O}$  anomaly of nitrate is unique to atmospheric sources and can therefore be used to distinguish atmospheric inputs from other nitrate sources in an ecosystem. The absence of a significant  $\Delta^{17}\text{O}$  anomaly in the stream and soil waters from the Hawaiian forests and deep and surface waters from the equatorial Pacific (Figure 7) suggests a minor contribution of atmospheric nitrate inputs in these environments, in line with independent estimates of their nitrogen budgets.<sup>28,29</sup> Note that the  $^{17}\text{O}$  anomaly does not constrain the importance of atmospheric nitrate relative to other external nitrogen inputs to a given environment. It rather informs us about the importance of atmospheric nitrate in relation to other processes generating nitrate. Any reactions that replace the oxygen atoms on nitrate, such as the sequence of nitrate assimilation, remineralization, and nitrification, will remove the  $^{17}\text{O}$  anomaly from nitrate that originated from the atmosphere.

The online triple oxygen isotope analysis method used here required only tens of nanomoles of nitrate, whereas offline methods would have required  $\sim 10 \mu\text{mol}$  of nitrate.<sup>8</sup> At ambient nitrate concentrations of the order of  $1 \mu\text{mol L}^{-1}$ , this corresponds to a sample size difference of tens of milliliters instead of liters. The online method thus opens up the route to nitrate isotope analyses for samples with much lower concentrations and limited sample availability, e.g., polar firn and ice samples.

## CONCLUSIONS

The gas chromatography/isotope ratio mass spectrometry method for triple oxygen isotope analyses of nitrate presented here (dubbed “ $\text{N}_2\text{O}$  decomposition method”) has several advantages over existing off-line methods:<sup>8</sup> (a) required sample sizes are 100–1000 times smaller; (b) analysis of samples with high salt concentrations (e.g., seawater) is possible; (c) method is suitable for straightforward automation.

The precision for  $\Delta^{17}\text{O}$  of the online gold furnace method is  $\sim 0.5\text{‰}$ , compared to the precision of 0.1‰ achieved by Michalski et al.<sup>8</sup> However, the online method has room for improvement: We identified VOC removal during the  $\text{N}_2\text{O}$  purification as critical for the overall precision of the method. At present, these compounds are removed by a periodically reactivated purge trap. A better purification can possibly be achieved by gas chromatography and cryofocusing of the  $\text{N}_2\text{O}$  sample, prior to thermal decomposition in the gold furnace. The VOC problem can also be addressed by chemical conversion of nitrate to  $\text{N}_2\text{O}$ , as achieved, for example, by the azide method,<sup>30</sup> although the latter requires a larger isotope exchange correction.

Note that the present method can also be used for  $\text{N}_2^{17}\text{O}$  analysis.<sup>31</sup> The achievable precision for  $\Delta^{17}\text{O}$  of  $\text{N}_2\text{O}$  is better than for nitrate ( $\sim 0.3\text{‰}$ ) because the VOC removal step is not required.

The lowest  $\text{N}_2\text{O}$  sample size that can be analyzed without significant loss of precision is  $\sim 15 \text{ nmol}$  of  $\text{N}_2\text{O}$  (Figure 4), corresponding to 30 nmol of nitrate. However, even at  $\text{N}_2\text{O}$  sample sizes as low as 3.5 nmol of  $\text{N}_2\text{O}$  (or 7 nmol of nitrate), we were

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still able to achieve results that would be suitable for most questions addressed by  $^{17}\text{O}$  analysis, albeit with greater uncertainties. A low sample size requirement is essential if samples from environments with low nitrate concentrations and limited availability, such as polar ice, are to be analyzed.

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#### **SUPPORTING INFORMATION AVAILABLE**

Detailed description of the isotope data reduction and scale normalization procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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