Isotopic Character of Nitrous Oxide Emitted from Streams

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Supporting Information

ABSTRACT: Global models have indicated agriculturally impacted rivers and streams may be important sources of the greenhouse gas nitrous oxide (N\textsubscript{2}O). However, there is significant uncertainty in N\textsubscript{2}O budgets. Isotopic characterization can be used to help constrain N\textsubscript{2}O budgets. We present the first published measurements of the isotopic character of N\textsubscript{2}O emitted from low (2–4) order streams. Isotopic character of N\textsubscript{2}O varied seasonally, among streams, and over diel periods. On an annual basis, δ\textsuperscript{18}O of emitted N\textsubscript{2}O (+47.4 to +51.4‰; relative to VSMOW) was higher than previously reported for larger rivers, but δ\textsuperscript{15}N of emitted N\textsubscript{2}O (−16.2 to +2.4‰ among streams; relative to atmospheric N\textsubscript{2}O) was similar to that of past studies. On an annual basis, all streams emitted N\textsubscript{2}O with lower δ\textsuperscript{15}N than tropospheric N\textsubscript{2}O. Given these streams have elevated nitrate concentrations which are associated with enhanced N\textsubscript{2}O fluxes, this supports the hypothesis that streams are contributing to the accumulation of 15N-depleted N\textsubscript{2}O in the troposphere.

INTRODUCTION

Concentrations of the biogenic greenhouse gas nitrous oxide (N\textsubscript{2}O) are increasing in the atmosphere. Agricultural nitrogen (N) fertilization is the dominant cause of anthropogenic N\textsubscript{2}O emissions. The contribution of N\textsubscript{2}O to warming is modest in global terms (<10% of that of CO\textsubscript{2}). However, projected increases in fertilizer use\textsuperscript{2} suggest that atmospheric concentrations of N\textsubscript{2}O will continue to grow. Beyond its contribution to climate change, N\textsubscript{2}O is also the largest current emission contributing to stratospheric ozone depletion.\textsuperscript{3}

Soils are a major site of both natural and anthropogenic N\textsubscript{2}O emissions. IPCC budget methods suggest that a large amount of anthropogenic N applied to agricultural soils is leached to aquatic ecosystems where it can lead to N\textsubscript{2}O production.\textsuperscript{4} Surface waters are frequently supersaturated with N\textsubscript{2}O, indicating they function as net sources.\textsuperscript{5} The strength of anthropogenic emissions from streams and rivers has been estimated at more than 1 Tg of N\textsubscript{2}O—N y\textsuperscript{−1},\textsuperscript{6} or the equivalent of 298 Tg CO\textsubscript{2} y\textsuperscript{−1} over a 100-year time span. However, uncertainty in emissions budgets is high.\textsuperscript{4}

Isotopic characterization of N\textsubscript{2}O has contributed to our understanding of global N\textsubscript{2}O dynamics by helping to constrain global N\textsubscript{2}O budgets.\textsuperscript{7} Global isotope mass balances have helped characterize the importance of stratospheric N\textsubscript{2}O loss, associated transport of enriched stratospheric N\textsubscript{2}O to the troposphere,\textsuperscript{7} and confirmed the increased strength of an isotopically depleted anthropogenic N\textsubscript{2}O source, largely from agricultural soils,\textsuperscript{8} but possibly derived in part from streams and rivers. Low order streams are thought to be important sites of N cycling,\textsuperscript{9} suggesting they could be hotspots of N\textsubscript{2}O emissions and important contributors to global N\textsubscript{2}O budgets. Although emissions from streams and rivers are reported as agricultural emissions,\textsuperscript{9} if N\textsubscript{2}O emitted from fluvial systems has a signature distinct from that of agricultural soils or if observed rates of N\textsubscript{2}O fluxes were large, this could suggest that global isotopic N\textsubscript{2}O budgets require reassessment to separately account for river and stream emissions.

The isotopic character of N\textsubscript{2}O emitted from soils and aquatic ecosystems is governed by fractionation associated with biological processing (nitrification, denitrification, N\textsubscript{2}O consumption and/or transport, physical fractionation due to gas exchange in aquatic ecosystems, physical fractionation due to gas exchange\textsuperscript{10} can lead to differences between the character of dissolved and emitted N\textsubscript{2}O. In this paper we assess the isotopic character of N\textsubscript{2}O emitted from a series of low order (second—fourth order) streams, and contrast the character of emissions to those of larger rivers, soils, and oceans.
Table 1. Range of $\delta^{15}$N, $\delta^{18}$O of Emitted N$_2$O, N$_2$O Flux, Percent Saturation, and Concentrations of Nitrate (NO$_3^-$) and Ammonium (NH$_4^+$)\textsuperscript{a}

<table>
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<tr>
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<th>$\delta^{15}$N–N$_2$O (%)</th>
<th>$\delta^{18}$O–N$_2$O (%)</th>
<th>N$_2$O (%) saturation</th>
<th>N$_2$O flux (µmol m$^{-2}$ h$^{-1}$)</th>
<th>[NO$_3^-$] (µM)</th>
<th>[NH$_4^+$] (µM)</th>
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\textsuperscript{a} The number of samples used to estimate isotopic character of emissions is in brackets.

Materials and Methods

Our study streams are located in southern Ontario, Canada (Figure S1) in an area of mixed land use with low urban development (<3% of catchment area), moderate wetland coverage (9–25%), and significant agricultural land use (51–76% in annual and forage crops, idle lands, hay, pasture, and marginal lands).\textsuperscript{11} Streams were sampled from March 2007 to October 2008 excluding periods of ice cover, when gas exchange is expected to be negligible. In Jackson Creek (JC), Layton Creek (LC), and the Black River (BR), we typically sampled a single site per stream. In Mariposa Brook (MB) we sampled two sites (upper site designated as MB-U, lower is MB-L) to help characterize spatial variation. The distance between the two sampling sites (7 km) was sufficiently long that upstream gases had minimal effect on downstream gases (<5%, based on first-order loss equations; see SI). MB receives tile drain inputs which may affect N$_2$O emissions. None of the other streams are known to have tile drains affecting sampling sites, and none of the streams received direct inflows of animal waste or sewage. MB, JC, and BR were fourth order (Strahler) streams at our sampling sites, and LC was a second order stream. Mean discharge was <300 L s$^{-1}$ in MB, BR, and LC (over the period where we calculated annual emissions, see Table S1). In JC our ability to characterize emissions was limited to two dates, when mean discharge was 1200 L s$^{-1}$. Annual discharge from these streams is approximately 100 times lower than the Bang Nara, Grand, or Tama Rivers, where the isotopic character of N$_2$O has been previously reported (Table S2). For clarity, we refer to the current study systems (MB, BR, LC, and JC) as streams, and the past studies from higher discharge ecosystems as rivers.

Samples for nutrient analysis were filtered (preashed, B-pure leached glass-fiber filters). Nitrate concentrations were measured using the red azo dye method after reduction to nitrite.\textsuperscript{12} Ammonium concentrations were measured using the indophenol blue method, in a buffered alkaline solution.\textsuperscript{12} Samples for determination of N$_2$O concentrations were obtained in 125-mL serum bottles, sealed with a prebaked (at 60 °C for 24 h) Vacutainer rubber stopper, and immediately preserved with 0.4% (v/v) saturated mercuric chloride. Samples were analyzed following headspace equilibration using a Varian CP-3800 gas chromatograph equipped with an electron capture detector. Analytical error, estimated from triplicate equilibrations, was 4.3% (coefficient of variation). N$_2$O flux was calculated by multiplying the piston velocity (k, in units of m h$^{-1}$) by the difference between measured and equilibrium N$_2$O concentrations. Piston velocity was estimated using oxygen\textsuperscript{13} and tracer-based methods,\textsuperscript{14} normalized to stream temperature and N$_2$O. Results were extrapolated to different flow conditions by calibrating empirical models using direct measurements (see SI).\textsuperscript{15} Annual estimates of the character of emitted N$_2$O are based on daytime sampling and are reported only for BR, LC, and two sites on MB (see SI). Reported N chemistry and N$_2$O data (Table 1) reflect dates and locations for which we estimate the isotopic character of N$_2$O flux. In addition to routine (daytime) sampling, we performed diel sampling twice at three locations (LC, BR, MB-L) and once at MB-U. Diel sampling was performed under low discharge conditions (Table S1).

Samples for isotopic analysis of dissolved N$_2$O were obtained in 500-mL media bottles (sealed with black lyophilization stoppers) or 125-mL serum bottles (as for N$_2$O concentration
analyses) and immediately preserved with a saturated mercuric chloride solution (final concentration 0.4% v/v). Samples for isotope analysis were prepared by purging the sample with ultra-high purity helium, while using liquid N₂ to trap N₂O following the removal of water vapor (using magnesium perchlorate and a nafion dryer) and CO₂ (chemical removal via Carbosorb). Cryogenic trapping was performed in pre-evacuated (~ 0.1 mbar) round-bottomed vials packed with Pyrex wool and glass beads, and sealed with thick (13 mm) blue butyl rubber stoppers (Bellco) and crimp rings (Chromatographic Specialties). Samples were overpressurized with ultrahigh purity helium, and 4–10 nmol of N₂O was injected into a GV Trace Gas preconcentrator system (which chromatographically separated N₂O from any residual CO₂), coupled to a GV Isotope mass spectrometer.16

All isotope ratios are reported in delta notation (δ) in units of parts per thousand (‰) relative to 15N/14N of atmospheric N₂ and 18O/16O of Vienna Standard Mean Ocean Water (VSMOW) (see SI). There are currently no internationally recognized reference materials for isotopic analysis of N₂O. However, there is little variation in the isotopic character of tropospheric N₂O throughout the northern hemisphere.17 Based on this, internal standards were calibrated using samples of tropospheric N₂O obtained at the University of Waterloo, assuming tropospheric N₂O had δ15N of 6.72‰ and δ18O of 44.62‰.17 Tropospheric N₂O at the University of Waterloo showed high reproducibility over months (standard deviation δ15N = 0.2‰, δ18O = 0.5‰). Two internal standards were used (δ15N = +2.8‰, δ18O = +4.0‰, and δ15N = −92.6‰, δ18O = +20.5‰) and 15% of our samples were analyzed in duplicate or triplicate. Mean error (deviation from the mean) was ±0.34‰ for δ15N and ±0.66‰ for δ18O. Isotope analyses were performed in the University of Waterloo Environmental Isotope Laboratory.

Isotopic ratios of dissolved N₂O differ from emitted N₂O due to the effects of kinetic fractionation during invasion and evasion. The isotopic ratio of emitted N₂O was calculated using equations described in the SI. Estimates of the δ15N and δ18O of N₂O emitted from our study streams are subject to error associated with measurement of dissolved N₂O concentrations and isotopic ratios. We estimated error in the isotopic character of emitted N₂O by bounding the data using our measured error terms (concentration: ±4.3%, δ15N = −5.7%–5.8% and δ18O = −0.66‰) and determining the maximum error calculated (multiplicative error resulting from error in analysis of concentrations and isotopes). Reported data are restricted to sites and dates where this maximum error did not affect the calculated mean emissions character by more than ±5‰ for δ15N or δ18O. We then calculated mean annual (flux and time-weighted) isotopic composition of emitted N₂O (see SI). Although sampling was generally performed at least monthly, there are gaps in the time series when low concentrations precluded isotopic analysis, or the error term exceeded 5%. Notably, for this reason, we can report the isotopic character of N₂O emitted from JC for only two dates. Annual estimates of isotopic character reflect our period of most frequent measurements (April or May 2007–2008) and are not reported for JC.

We determined whether the character of emitted N₂O (annual mean δ15N and δ18O) differed between this current study which focused on low order streams (LC, BR, mean of 2 sites on MB), and the mean value for rivers (based on three rivers: Bang Nara, Grand, Tama; δ15N/N₂O: −8.4‰, δ18O/N₂O: ±36.6‰).16 We extended the analysis to determine whether the δ18O/N₂O across our study streams differed from the maximum annual value previously reported (δ18O/N₂O: +41.1‰ in the Tama River17). Using one-sample (two-tailed) t-tests, we also determined whether δ15N and δ18O of N₂O emitted from fluvial systems differed from tropospheric values.17 This analysis used annual means from our study streams (LC, BR, and mean value of two sites for MB) and reported values for rivers (Bang Nara, Grand, Tama16).

We used 2-way analysis of variance (ANOVA) to assess whether δ15N and δ18O varied spatially and seasonally (between summer months of June–July–August and other months). Full ANOVA tables are presented in the SI. We first tested for differences between MB-U and MB-D, then combined data for the two MB sites and tested for differences among streams and seasons. We determined which pairs of streams differed using Bonferroni-corrected multiple pairwise contrasts. One outlier was apparent for both δ15N and δ18O and was excluded from analyses (absolute value of studentized residual >3.5). We used correlation analysis to determine relationships between % saturation and character of emitted N₂O, as well as δ15N and δ18O of emitted N₂O. Finally, we used paired t-tests to contrast daytime (nearest solar noon) and nighttime (farthest from solar noon) samples across all sampling efforts. All statistical analyses were performed in Systat 13.0. A level of significance of 0.05 was used in all analyses and data were tested for adherence to the assumptions of each analysis.

**RESULTS AND DISCUSSION**

Although oceans were one of the first frontiers for isotopic characterization of N₂O, and research on the character of N₂O emitted from soils has expanded rapidly, work in fresh water is only starting. Data are available from only three rivers, two of which are temperate rivers with significant sewage inflows (Grand River,16 Tama River16) while the third is a tropical river in a forested, swamp catchment (Bang Nara River).16,18 Our data almost uniformly indicate that stream-emitted N₂O has lower δ15N (range: −22.6 to +10.7‰, Table 1, Figures 1–3) than tropospheric N₂O (δ15N: +6.72‰).17 This is consistent with past studies of rivers.5,16 The annual mean δ15N of N₂O emissions from the 6 fluvial systems where δ15N of N₂O has now been reported is lower than tropospheric N₂O (one sample t-test: p = 0.009, df = 5). Emitted δ15N/N₂O did not vary between our study streams and the mean of past reports for rivers (one sample t-test, p = 0.3, df = 2). Although the systems with sewage inputs (Tama and Grand Rivers) might be expected to differ in N₂O character based on potential differences in N cycling and N substrates (see SI), δ15N of N₂O emissions from sewage-influenced systems was similar to that of systems that did not have direct inflows (Figure 3).

All of our study streams (BR, LC, JC, MB) have elevated NO₃− concentrations (Table 1; estimated predevelopment concentration <11 μM²). N enrichment of fluvial ecosystems is a widespread phenomenon occurring in many regions of the globe.6 Based on the observation that fluvial systems emit 15N-depleted N₂O relative to tropospheric N₂O, and the widely accepted view that N enrichment is contributing to increased N₂O fluxes,16 we suggest that the N-enrichment of streams is contributing to the observed accumulation of 15N-depleted N₂O in the troposphere. On a global scale, the relative importance of fluvial emissions remains highly uncertain. However, current
IPCC emissions factors for fluvial ecosystems are lower than proposed in early models.6,21 Interestingly, δ18O emitted N2O from our study streams is distinct from past studies of fluvial systems (annual time and flux-weighted range: +47.4 to +51.5% among streams; Figure 3; one sample t-test against annual mean of past studies: p = 0.01, df = 2; one sample t-test against maximum of past studies p = 0.03, df = 2). Although error in our estimation of gas transfer (Table S1) may contribute to error in estimation of the character of annual emissions (see SI), this does not affect daily or instantaneous data. Our finding of a distinct δ18O-N2O is supported in seasonal and diel data (Figures 1 and 2). Across all measurements (across streams, dates, and including diel sampling) emitted δ18O-N2O was less than 41.1% (the highest

Figure 1. Seasonal variation in δ15N (plots a–d) and δ18O (plots e–h) of emitted N2O (daytime sampling, 2007–2008). Data from LC (white triangles) are plotted on the same panels as data from JC (gray circles). Error bars show error in isotopic character of emitted N2O based on analytical error terms.

Figure 2. Diel variation in δ15N (plots a–g) and δ18O (plots h–n) of emitted N2O. Black line over plots indicates period between sunset and sunrise. Gray lines indicate time and flux-weighted character N2O. Error bars show error in isotopic character of emitted N2O based on analytical error terms.
annual estimate in past studies, see Figure 3) in fewer than 10% of cases. There are a number of reasons δ\textsubscript{15}N—N\textsubscript{2}O could vary between streams and rivers. If denitrification rates are higher in small streams due to their morphology,\textsuperscript{a} δ\textsubscript{18}O—N\textsubscript{2}O may be more \textsuperscript{18}O-enriched than in systems where pelagic N cycling processes are more important (see SI). Small streams may also receive significant N\textsubscript{2}O inputs from ground waters and tile drains\textsuperscript{b} which can have a high δ\textsubscript{18}O—N\textsubscript{2}O.\textsuperscript{c} O-exchange processes are another key determinant of δ\textsubscript{18}O—N\textsubscript{2}O (see SI; refs \textsuperscript{24,25}). Although δ\textsubscript{18}O of stream-emitted N\textsubscript{2}O differs from the few available measurements of rivers, these values lie within the bounds of past values reported for soils.\textsuperscript{32,27} The sole exception is LC, where emitted N\textsubscript{2}O is more enriched than most soil emissions, but within the range of values observed in oceans.\textsuperscript{28} Across all fluvial systems studied to date, the δ\textsubscript{15}N of emitted N\textsubscript{2}O did not differ from tropospheric N\textsubscript{2}O (one sample t-test: \textit{p} = 0.5, \textit{df} = 5).

MB sites did not differ significantly in δ\textsubscript{15}N or δ\textsubscript{18}O—N\textsubscript{2}O (Figures 1 and 3; 2-way ANOVA, Tables S3, S4), despite their spatial separation, but did show evidence of seasonal changes in δ\textsubscript{18}O and δ\textsubscript{15}N (2-way ANOVA, Tables S3, S4, \textit{p} < 0.01). The δ\textsubscript{15}N of emitted N\textsubscript{2}O varied across streams and seasons (2-way ANOVA, \textit{p} < 0.001, Table S5) and δ\textsubscript{15}N—N\textsubscript{2}O emissions differed among all pairwise combinations of streams (LC, BR, MB; Bonferroni, \textit{p} < 0.05). The BR emitted the most \textsuperscript{15}N-depleted N\textsubscript{2}O (Figure 1). LC emitted N\textsubscript{2}O with the highest δ\textsubscript{15}N (Figures 1 and 3). N\textsubscript{2}O emitted from JC on the two occasions on which it could be characterized was within the range of the other study streams (Figure 1). δ\textsubscript{18}O also varied among streams (2-way ANOVA, Table S6, \textit{p} = 0.049), but only BR and LC showed significant differences in pairwise contrasts (Bonferroni, \textit{p} = 0.046).

Emitted N\textsubscript{2}O generally had higher δ\textsubscript{15}N and δ\textsubscript{18}O in summer (June—September) than other seasons (Figure 1), although this trend was statistically significant for δ\textsubscript{15}N (2-way ANOVA, Table S5, \textit{p} = 0.046) and not for δ\textsubscript{18}O (2-way ANOVA, Table S6, \textit{p} = 0.06). Similar patterns observed in the Grand River, Ontario, were thought to be due at least in part to greater kinetic isotope effects associated with lower N\textsubscript{2}O production rates in cooler weather.\textsuperscript{16} Another possible factor is the increase in N\textsubscript{2}O reduction with temperature\textsuperscript{29} which could result in isotopic enrichment of N\textsubscript{2}O in summer. Changes in composition of the end members may also contribute. For example, δ\textsubscript{18}O of H\textsubscript{2}O was likely highest during dry summer months.\textsuperscript{30} δ\textsubscript{18}O—O\textsubscript{2} is expected to undergo the largest degree of diel variation in summer months when aquatic productivity is highest (see SI). N\textsubscript{2}O percent saturation was not related to δ\textsubscript{15}N or δ\textsubscript{18}O of emitted N\textsubscript{2}O within a stream (seasonal data). δ15N and δ\textsubscript{18}O of emitted N\textsubscript{2}O were correlated (BR: \textit{r} = +0.66, \textit{p} = 0.02; LC: \textit{r} = +0.63, \textit{p} = 0.03; MB: \textit{r} = +0.83, \textit{p} < 0.0001). This may reflect fractionation associated with denitrification (Table S7, S8) or gas exchange.

Major diel changes in N cycling processes have been shown in some systems,\textsuperscript{31} which could contribute to changes in the character of emitted N\textsubscript{2}O. The extent of day—night variation in the isotopic character of emitted N\textsubscript{2}O actually nears the extent of seasonal variation in some streams (Figures 1 and 2); however, consistent day—night patterns were not apparent (Figure 2; also see SI). Contrasting pairs of samples closest and farthest from solar noon showed no significant difference for either isotope (paired t-test: δ\textsubscript{15}N \textit{p} = 0.9, δ\textsubscript{18}O \textit{p} = 0.83, \textit{df} = 6). This is probably due, at least in part, to variable rates of gas transfer among streams. Streams with slower gas exchange will show a longer lag period before a change in the character of newly produced N\textsubscript{2}O (e.g., due to a change in pathway or substrate composition) significantly affects the character of emitted N\textsubscript{2}O. While the average gas transfer coefficient was 5.3 per day across streams during diel sampling, the upstream site in MB had much lower rates of gas exchange (1.5 per day), which helps explain the relatively stable isotopic composition at this site. The large magnitude of variation in δ\textsubscript{18}O of emitted N\textsubscript{2}O suggests changes were occurring either in the processes (nitrification vs denitrification), the importance of O-exchange reactions, or δ\textsubscript{15}O of the NO\textsubscript{3}— end member. δ\textsubscript{18}O—H\textsubscript{2}O is expected to be relatively stable during diel sampling, and clear relationships with δ15N—O\textsubscript{2} were not apparent (see SI).

Diel variation in the isotopic character of emitted N\textsubscript{2}O has been investigated in two past studies. The first was a study of N\textsubscript{2}O emissions from a urine-amended soil. Changes in the character of emitted N\textsubscript{2}O occurred over short time periods, but did not appear to be related to day—night cycles.\textsuperscript{32} The second study addressed diel changes in the eutrophic Grand River. Emissions of N\textsubscript{2}O from the Grand River during the daytime had higher δ\textsubscript{15}N—N\textsubscript{2}O than at night. This was likely due to diel changes in the isotopic composition of the substrates as well as switching of the major N\textsubscript{2}O production pathways.\textsuperscript{16} In conclusion, streams and rivers, like soils, tend to emit N\textsubscript{2}O with δ\textsubscript{15}N that is lower than tropospheric N\textsubscript{2}O. Our results suggest not only that increased N\textsubscript{2}O emissions from N-enriched streams are contributing to the accumulation of \textsuperscript{15}N-depleted N\textsubscript{2}O in the atmosphere, but also indicate that the exclusion of fluvial sources from global isotope mass balances\textsuperscript{27} could lead to the overestimation of soil sources because of their similarity in δ\textsubscript{15}N, and the exclusion of fluvial systems from past work. Relatively small fluxes from our study streams in the context of emissions from agricultural soils in the catchment (stream emissions estimated at 0.2—12% of emissions from agricultural soils + streams\textsuperscript{31}) indicate the error may be small compared to the uncertainty in emissions budgets.\textsuperscript{14} However, relatively few long-term estimates of N\textsubscript{2}O flux exist for fluvial systems, and accumulating evidence suggests that sewage and tile-drain
impacted systems may have high emissions. A more complete assessment of the role of fluvial systems in global N$_2$O budgets requires additional measurements across a broad spectrum of ecosystems and should include isotopic characterization.

**ASSOCIATED CONTENT**

Supporting Information. Additional information on study sites and stream discharge, calculations (isotopic notation, bulk N$_2$O flux calculations, equations for calculation of isotopic character of N$_2$O flux, first order loss equation), statistics (ANOVA tables), and the processes affecting the isotopic signature of dissolved and emitted N$_2$O (Figure S1; Tables S1–S9). This information is available free of charge via the Internet at http://pubs.acs.org.

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