From the Ground Up: Global Nitrous Oxide Sources are Constrained by Stable Isotope Values

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Abstract

Rising concentrations of nitrous oxide (N₂O) in the atmosphere are causing widespread concern because this trace gas plays a key role in the destruction of stratospheric ozone and it is a strong greenhouse gas. The successful mitigation of N₂O emissions requires a solid understanding of the relative importance of all N₂O sources and sinks. Stable isotope ratio measurements (δ¹⁵N-N₂O and δ¹⁸O-N₂O), including the intramolecular distribution of ¹⁵N (site preference), are one way to track different sources if they are isotopically distinct. ‘Top-down’ isotope mass-balance studies have had limited success balancing the global N₂O budget thus far because the isotopic signatures of soil, freshwater, and marine sources are poorly constrained and a comprehensive analysis of global N₂O stable isotope measurements has not been done. Here we used a robust analysis of all available in situ measurements to define key global N₂O sources. We showed that the marine source is isotopically distinct from soil and freshwater N₂O (the continental source). Further, the global average source (sum of all natural and anthropogenic sources) is largely controlled by soils and freshwaters. These findings substantiate past modelling studies that relied on several assumptions about the global N₂O cycle. Finally, a two-box-model and a Bayesian isotope mixing model revealed marine and continental N₂O sources have relative contributions of 24–26% and 74–76% to the total, respectively. Further, the Bayesian modeling exercise indicated the N₂O flux from freshwaters may be much larger than currently thought.

Introduction

Since the advent of the Haber-Bosch process one century ago, humans have vastly perturbed the global nitrogen (N) cycle. Current anthropogenic activities contribute 51% of the total N fixed worldwide (210 of 413 Tg N yr⁻¹) [1]. One negative consequence of this is an increase in atmospheric nitrous oxide (N₂O) [2], a long-lived trace gas that contributes to climate warming and the destruction of stratospheric ozone [3]. The current concentration of N₂O in the
troposphere is 325 parts per billion (ppb) [4]. Future concentrations of atmospheric N₂O are difficult to predict, yet this information is an essential input parameter for global climate change models. Further, both the prediction and mitigation of N₂O concentrations depend on an accurate understanding of the emissions from key N₂O sources.

Most emissions of N₂O (natural and anthropogenic) occur from terrestrial, freshwater, and marine environments, where N compounds are processed by nitrifying and denitrifying microorganisms. These processes account for ~89% of the total annual N₂O emissions, or almost 16 Teragrams (Tg = 10¹² g) N/year [5]. However, scientists’ best estimates of the N₂O budget are still highly uncertain. The most recent Intergovernmental Panel on Climate Change Assessment Report (IPCC-AR5) reveals wide ranges in the relative uncertainty of many individual N₂O sources. In addition, the uncertainty on the annual cumulative emissions of N₂O for 2006 from natural soils, oceans, rivers, estuaries, coastal zones, and agriculture combined ranged between 6.9–26.1 Tg N [5].

The clear separation and accounting of individual N₂O sources remains challenging, but is essential if we are to make meaningful reductions in emissions. Measurements of stable isotope ratios (δ¹⁵N-N₂O and δ¹⁸O-N₂O) and the intramolecular site preference (SP) of¹⁵N are one way to track sources if they are isotopically distinct. Several accounts of the global N₂O budget have used ‘top-down’ isotope mass-balance models to estimate the strength and isotopic composition of anthropogenic and natural N₂O sources [2,6–11]. In this approach, changes in atmospheric N₂O over time are modelled by comparing our modern-day atmosphere (a mixture of post-industrial, anthropogenic N₂O and natural N₂O) to relic air trapped in glacial firn and ice. All these studies have assumed that soils are the main source of post-industrial N₂O because its calculated isotopic composition was most similar to a limited body of published soil N₂O measurements. Yet we do not have a clear synthesis of the isotopic character of individual N₂O sources. For example, freshwaters and estuaries may contribute up to 25% of anthropogenic N₂O emissions [5], but prior to 2009 there was only one publication reporting freshwater δ¹⁵N-N₂O and δ¹⁸O-N₂O values [12] (S1 Dataset). In reality, there is extreme variation in the measured values of δ¹⁵N-N₂O and δ¹⁸O-N₂O (Fig. 1), and no systematic examination of individual sources has occurred.

In this paper, we use a ‘bottom-up’ approach to define key N₂O sources and demonstrate that their global average δ¹⁵N and δ¹⁸O values are isotopically unique. Further we use these in situ N₂O isotope data to substantiate what ‘top-down’ global atmospheric models have predicted; soils, and not marine or freshwater ecosystems, are the main source of rising atmospheric N₂O levels.

Methods

We mined 1920 data points from 52 studies that measured in situ δ¹⁵N-N₂O and δ¹⁸O-N₂O in atmospheric, terrestrial and marine systems from 1987 to present [2,10–60]. If the published data was not tabulated, we used the software ‘g3data’ (http://www.frantz.fi/software/g3data.php) to extract data from figures [61]. The accuracy of our method was tested by plotting a subset of data from Well et al. [51], re-extracting it, and then comparing it to the original values. The mean (min/max) difference (%) was 0.06 (0.00/0.13) for δ¹⁵N and 0.02 (0.00/0.07) for δ¹⁸O. This represents a worst-case accuracy of our ability to extract data from figures because the test data had an unusually wide range (~80 to +120% for δ¹⁵N, and 0 to +120% for δ¹⁸O; n = 53) and all other published graphs had much smaller scales. Values of δ¹⁸O-N₂O reported vs. atmospheric O₂ were converted to δ¹⁸O-N₂O vs. Vienna Standard Mean Ocean Water (VSMOW) according to Kim and Craig [19].
Twenty-seven studies also measured the intramolecular distribution of $^{15}$N in the linear NNO molecule (780 data points) and these data are provided in the supplementary datasets (S1 Dataset and S2 Dataset). This difference between the central ($\delta^{15}$N$^\alpha$) and terminal ($\delta^{15}$N$^\beta$) $^{15}$N enrichment is often expressed as the site preference (SP). This parameter is thought to be a unique indicator of the microbial pathway that produces N$_2$O and not to be affected by variations in the isotopic ratios of substrates. Recently, this idea has been called into question by Yang et al. [62], who showed that SP can vary depending on the growth conditions of microbial cultures. Regardless, if the SP of different global sources is unique it can be used in conjunction with traditional measures of $\delta^{15}$N-N$_2$O and $\delta^{18}$O-N$_2$O values to separate sources in three-dimensional isotope space.

To this compendium of published data we added 1367 new in situ $\delta^{15}$N-N$_2$O and $\delta^{18}$O-N$_2$O data from 16 sites across Ontario and New Brunswick, Canada (S1 Dataset). Soil pore gas and static flux chambers were sampled at four Ontario sites. Urban wastewater treatment plants, streams, rivers, and agricultural drainage tile outlets were sampled across four watersheds in Ontario and New Brunswick. Groundwaters were sampled from numerous domestic
and monitoring wells (some multi-level) distributed across nine research sites in Ontario and New Brunswick.

Liquid samples were stripped of N₂O using an off-line purge-and-trap system described in Baulch et al. [35]. With the exception of samples from one location (ERS) that were analyzed at UC Davis-SIF, all analyses occurred at the University of Waterloo on an IsoPrime isotope ratio mass spectrometer (IRMS) with a TraceGas pre-concentrator with an analytical precision of 0.2‰ ($\delta^{15}$N-N₂O) and 0.4‰ ($\delta^{18}$O-N₂O). All samples were analyzed alongside an internal N₂O isotope standard that was previously calibrated at the University of Waterloo against local tropospheric air (assumed to be equal to 6.72‰ for $\delta^{15}$N and 44.62‰ for $\delta^{18}$O [17]). This internal standard gas was also submitted to UC Davis-SIF for isotopic analysis, and the standard deviation of 8 replicates (at varying concentrations including ambient) was 0.34‰ (for $\delta^{15}$N) and 0.77‰ (for $\delta^{18}$O). The absolute difference in the assigned value of this internal standard gas (blind inter-lab comparison) was 0.29‰ (for $\delta^{15}$N) and 0.81‰ (for $\delta^{18}$O). Given there are no internationally-recognized standardization methods or materials for N₂O isotope analysis, these inter-laboratory results are in good agreement with one another. All values are reported here in units of per mill (‰) relative to air-N₂ and VSMOW for $\delta^{15}$N and $\delta^{18}$O, respectively.

All data were categorized as either Antarctic, freshwater, groundwater, marine, soil, stratosphere, troposphere, or urban wastewater, and a bivariate ellipse-based metric [63] was used to analyze and describe individual N₂O reservoirs (Table 1). This circular statistical analysis is an improvement over other techniques that qualitatively summarize isotope data with a polygon or a freeform shape e.g., [6,15,46,47]. There is often a high degree of covariance between $\delta^{15}$N-N₂O and $\delta^{18}$O-N₂O and this statistical technique provides an accurate description of the central tendency of the data. By definition, the standard ellipse contains ~40% of the data, is centered on the mean and has standard deviations of the bivariate data as semi-axes (Fig. 2) [63,64]. The data and an R file that contains the code to perform the statistical analyses and create the figures shown here are found at https://github.com/jjvenk/Global-N2O-Ellipses.

Table 1. Summary statistics of global $\delta^{15}$N-N₂O and $\delta^{18}$O-N₂O values as standard ellipses*.

| Category          | n   | Sample-size-corrected ellipse area (% air N₂ x % VSMOW) | Mean $\delta^{15}$N ± 1σ (%) | Mean $\delta^{18}$O ± 1σ (%) | Correlation (r) | Semi-major axis | Semi-minor axis | Slope of ellipse | Theta (θ, rads) |
|-------------------|-----|------------------------------------------------------|-------------------------------|-------------------------------|-----------------|-----------------|-----------------|-----------------|----------------|
| Stratosphere      | 298 | 44                                                   | 20.31 ± 20.79                 | 56.39 ± 18.44                 | 0.9994          | 27.8            | 0.5             | 0.89            | 0.73           |
| Troposphere       | 225 | 0.47                                                 | 6.55 ± 0.47                   | 44.40 ± 0.34                  | 0.3758          | 0.5             | 0.3             | 0.46            | 0.43           |
| Soil              | 884 | 296                                                  | −14.85 ± 12.01                | 31.23 ± 9.89                  | 0.6083          | 14.0            | 6.7             | 0.73            | 0.63           |
| Freshwater        | 738 | 203                                                  | −4.65 ± 9.84                  | 41.77 ± 8.79                  | 0.6656          | 12.1            | 5.4             | 0.85            | 0.70           |
| Marine            | 495 | 92                                                   | 6.63 ± 3.50                   | 47.35 ± 9.54                  | 0.4866          | 9.7             | 3.0             | 5.05            | 1.38           |
| Groundwater       | 530 | 768                                                  | −13.97 ± 15.46                | 45.34 ± 17.74                 | 0.4552          | 20.2            | 12.1            | 1.35            | 0.93           |
| Antarctic         | 35  | 3086                                                 | −40.84 ± 30.75                | 29.03 ± 31.82                 | 0.2256          | 34.7            | 27.9            | 1.16            | 0.86           |
| Urban Wastewater  | 92  | 545                                                  | −11.56 ± 12.70                | 31.51 ± 14.14                 | 0.2922          | 15.4            | 11.2            | 1.43            | 0.96           |

*A visual description of the standard ellipse is found in Fig. 2.

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Results and Discussion

The data are highly non-uniform within and between categories (Figs. 1 and 3), and even within individual field sites (S1 Dataset). Historically, this has made it challenging to define an ‘isotopic signature’ for a given environment. Multiple factors cause this variability: (1) N\(_2\)O is produced by nitrification and denitrification, and the isotopic composition of N and oxygen (O) endmembers can vary widely [26,31,32,54]; (2) the apparent fractionation of \(^{15}\)N/\(^{14}\)N and \(^{18}\)O/\(^{16}\)O during N transformations is not constant, nor is it easily predicted; and (3) oxygen exchange between N\(_2\)O precursors and water imparts a large control on \(\delta^{18}\)O-N\(_2\)O values during N\(_2\)O formation. While the exact mechanisms are not fully understood, it appears that greater amounts of exchange occur in unsaturated environments than in saturated ones [66–68]. Additionally, the reduction of N\(_2\)O to N\(_2\) in anaerobic environments causes enrichment of \(^{15}\)N and \(^{18}\)O isotopes in the remaining N\(_2\)O pool, which displaces \(\delta^{15}\)N-N\(_2\)O and \(\delta^{18}\)O-N\(_2\)O values away from their original source values [26,51]. An initial analysis of all the data compiled in this study shows there is no clear separation of sources because each is described by an ellipse that overlaps at least one other source category (Figs. 1 and 3).

A similar comparison of all the published SP data (excluding Antarctic and groundwater categories) shows poor isotopic separation of sources (Fig. 4). It is difficult to determine how much of this variability is real, and how much is due to standardization issues and differences.
in measurement techniques. A recent inter-laboratory assessment of the methods used to determine nitrogen isotopomers revealed poor SP reproducibility [69]. Eleven laboratories employing either IRMS or laser spectroscopy techniques analyzed a single N₂O target gas and the resulting standard deviation for SP was 4.24‰. Further, the inter-lab variation in the mean SP value was high, spanning a range of 11.62‰ [69]. This may help explain why there are two distinct groupings of SP data in each of the troposphere [2,17] and the stratosphere [29,45,58].
Reaching an international consensus on standardization methods for the measurement and reporting of nitrogen isotopomers should vastly improve the utility of this data in source-apportionment studies at all scales. Finally, we note the reproducibility of $\delta^{15}$N-N$_2$O and $\delta^{18}$O-N$_2$O measurements in this recent round-robin test was much better than for SP, which gives us confidence in our ability to use these data here to make useful comparisons. The standard deviation (and range) of the N$_2$O target gas in the inter-lab comparison was 1.37‰ (1.89‰) for $\delta^{15}$N-N$_2$O and 1.00‰ (3.47‰) for $\delta^{18}$O-N$_2$O [69].

Much of the low concentration data from soils and surface waters are highly influenced by mixing with tropospheric N$_2$O. This is evident by the high density of soil, freshwater and marine data that lies near the tropospheric N$_2$O value (Fig. 3). In contrast, groundwater N$_2$O, which does not mix with the atmosphere following recharge, is unaffected by this mixing process. Other processes such as substrate enrichment and N$_2$O consumption control the isotopic composition of groundwater N$_2$O, which displays extreme variability even within the same location (Fig. 1) [50,51]. Only 15 studies reported flux-weighted average $\delta^{15}$N-N$_2$O, SP, and/or $\delta^{18}$O-N$_2$O values, or provided enough information for us to calculate these values (2 freshwater studies, 2 marine studies, 10 soil studies, and 1 urban wastewater study; Fig. 5; S2 Dataset). The available flux-weighted data from soil and freshwater environments shows much overlap among this combined continental source, but the flux-weighted marine source appears to be unique. Importantly, there are very few flux-weighted data from all sources so robust conclusions cannot be made at this time. Additionally, these data were not weighted equally across studies so conclusions drawn from this analysis can be misleading. Only some of the values are time-weighted, and the sample size used to calculate the flux-weighted average varies from 3 to $\sim$ 50 (S2 Dataset). Emissions of N$_2$O from soils (and potentially freshwaters and oceans) are inherently episodic, so future estimates of the flux-weighted average should attempt to include multiple measurements made over long timescales (months to years and encompassing seasonal differences) whenever possible.
The results of our analyses shown in Figs. 1 and 3 are not flux-weighted, nor are all the categories important atmospheric sources. For example, the most recent IPCC assessment reports that human excreta (all forms of treated/untreated sewage) contributes between 0.1–0.3 Tg N yr\(^{-1}\) as N\(_2\)O, or only \(\ast 1.1\%\) of all natural and anthropogenic sources [5]. Of this, N\(_2\)O emissions from urban wastewater treatment plants constitutes a very small fraction. To address this, we analyzed subsets of the data from important atmospheric sources (freshwaters, oceans, and soils) that were not strongly influenced by mixing with tropospheric N\(_2\)O, and thereby make an important contribution to the flux-weighted average source value (Table 2; Fig. 6). To do this we filtered the data to include: (i) all reports of emitted N\(_2\)O, regardless of the strength of the flux (two freshwater studies [13,46], two marine studies [16,43] and several soil studies) (see S2 Dataset); (ii) isotope data in the soil profile that had concentrations of N\(_2\)O > 650 ppb v/v (or 200% ambient); and (iii) isotope data in freshwater and near-surface marine environments (depths > 100 m) with dissolved N\(_2\)O concentrations > 200% saturation with respect to atmospheric N\(_2\)O [70].

Most of the freshwater and soil data were retained (71% and 90%, respectively), and the ellipses of these data subsets are similar to the ellipses for all data in these categories (Table 1). Although the median \(\delta^{15}\)N and \(\delta^{18}\)O values of freshwater and soil N\(_2\)O are significantly different (\(p < 0.001\), Mann-Whitney test), their ellipses intersect one another at the 1\(\sigma\) level (Fig. 6–top panel), and we conclude that these sources are not isotopically distinct at the global scale.
In order to further delineate freshwater and soil N₂O more stable isotope measurements from freshwaters are needed; especially from non-temperate environments because the current data coverage from these systems is lacking. Measurements of SP may prove to be a useful means of separating these sources because the ellipses that describe SP vs. δ¹⁵N_bulk for freshwater and soils do not overlap (Fig. 6—middle panel).

Of the 495 published marine values compiled here, only 62 originated from the top 100 m of the ocean and were >200% saturation. Relative to continental N₂O sources, the δ¹⁵N and δ¹⁸O values of near-surface oceanic N₂O sources are poorly constrained. We found no reports of N₂O isotope values from estuaries, which could represent an important fraction of the marine source but should be similar to marine or freshwater values. Tropical systems including reservoirs are also poorly studied. Future campaigns to more fully characterize δ¹⁵N and δ¹⁸O values of N₂O emissions from aquatic environments, especially those impacted by anthropogenic N sources, are needed.

Although N₂O generated from fossil fuel and biomass combustion may contribute ~8% of the total source (or ~20% of the anthropogenic source [5]), its isotopic composition is largely unknown. A lab-scale investigation of coal combustion revealed δ¹⁵N-N₂O and δ¹⁸O-N₂O values that were both enriched (unstaged combustion) and slightly depleted (air-staged combustion) relative to tropospheric N₂O [71], indicating coal-derived N₂O isotope values might be similar to marine sources but are dependent upon combustion conditions. A controlled study of gasoline-powered automobile exhaust concluded the average δ¹⁵N-N₂O and δ¹⁸O-N₂O values are similar to freshwater sources (−4.9 ± 8.2‰ and +43.5 ± 13.9‰, respectively) [72]. Finally, N₂O derived from biomass burning appears to closely resemble the δ¹⁵N and δ¹⁸O values of its endmembers; biomass-N and atmospheric O₂, respectively [73]. We recognize the need to further investigate these potentially important sources and evaluate how they might affect the global N₂O isotope budget.

After analyzing the filtered δ¹⁵N-N₂O and δ¹⁸O-N₂O data, the ‘bottom-up’ global N₂O sources defined here were compared to estimates derived from ‘top-down’ atmospheric models (Fig. 7). Modelled estimates of the average anthropogenic and natural source fall within (or very close to) the soil ellipse and along a mixing line between soil and tropospheric N₂O. All but two of the modelled estimates fall outside the freshwater ellipse, indicating the bulk of the combined anthropogenic and natural sources are not from freshwaters. If freshwaters were a major source of atmospheric N₂O, a mixing line between freshwater and tropospheric N₂O would be much closer to the anthropogenic and natural source values. It is not, and therefore

Table 2. Summary statistics of filtered δ¹⁵N-N₂O and δ¹⁸O-N₂O data. These values show a subset of data that were not strongly influenced by mixing with tropospheric N₂O, and thereby make an important contribution to the flux-weighted average source value (see Fig. 6).

| Category | n  | Sample-size-corrected ellipse area (% air N₂ x 1‰ VSMOW) | Mean δ¹⁵N ± 1σ (%) | Mean δ¹⁸O ± 1σ (%) | Correlation (r) | Semi-major axis | Semi-minor axis | Slope of ellipse | Theta (θ, rads) |
|----------|----|--------------------------------------------------------|--------------------|-------------------|-----------------|----------------|---------------|----------------|----------------|
| Soil     | 794 | 288                                                   | −16.66 ± 11.24     | 30.05 ± 9.63      | 0.5341          | 13.0           | 7.0           | 0.75           | 0.64           |
| Freshwater | 527 | 215                                                   | −7.78 ± 9.72       | 40.75 ± 9.63      | 0.6821          | 12.5           | 5.5           | 0.99           | 0.78           |
| Marine   | 62  | 22                                                    | 5.14 ± 1.93        | 44.76 ± 6.93      | 0.0435          | 3.6            | 1.9           | 30.87          | 1.54           |
| Continental* | 1321 | 299                                                  | −13.11 ± 11.51     | 34.32 ± 10.96     | 0.6577          | 14.5           | 6.6           | 0.93           | 0.75           |

*The continental source, operationally defined here as Soil + Freshwater, is used along with the Marine source in our box-model calculations.

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we confirm what ‘top-down’ approaches have previously inferred: soil is the main source of N₂O to the atmosphere.

We used the newly constrained δ¹⁵N-N₂O and δ¹⁸O-N₂O values for freshwater and soil (the combined continental source) and the ocean source to model their relative contributions to the total annual flux of N₂O. We began with a box-model approach similar to that presented in [8,9,11,29]. We adapted the following isotope mass-balance equation for atmospheric N₂O from Park et al. [29]:

\[
\text{Burden} \times \frac{\partial \delta_{\text{Trop}}}{\partial t} = \sum \text{Sources}(\delta_{\text{Sources}} - \delta_{\text{Trop}}) - (\varepsilon \times L)
\]

where, \(\text{Burden}\) = Present-day burden of N₂O in the troposphere [1553 (± 21.742) Tg N] as reported by Stocker et al. [5]. This estimate is for year 2011, and is updated from data provided in Prather et al. [74], who report an uncertainty of 1.4% for year 2010.

\(\frac{\partial \delta_{\text{Trop}}}{\partial t}\) = Desseasonalized, linear trend in archived samples of tropospheric N₂O measured by Park et al. [2]. The linear trends for δ¹⁵N-N₂O and δ¹⁸O-N₂O are −0.035‰ yr⁻¹ (± 0.002) and −0.022‰ yr⁻¹ (± 0.004), respectively.

Σ Source = Annual N₂O emissions from all sources (17.9 Tg N yr⁻¹) [5]. For our calculations we applied an uncertainty of 25% to this parameter.

δSources = Flux-weighted δ¹⁵N-N₂O or δ¹⁸O-N₂O value (‰) of the average modern source (all natural and anthropogenic sources).

δTrop = δ¹⁵N-N₂O or δ¹⁸O-N₂O value (‰) of the modern troposphere (provided in Table 1).

\(\varepsilon\) = Apparent enrichment factor (‰) for N₂O destruction processes in the stratosphere. These values are taken from Table 3 in Park et al. [29], and are −14.9‰ (± 0.5) for ¹⁵N and −13.5‰ (± 0.5) for ¹⁸O. Note, the ratio of enrichment factors provided by Park et al. [29] (¹⁸O: ¹⁵N = 0.906) is very close to the slope of the regression line of the stratospheric N₂O data shown in Fig. 1 (δ¹⁸O-N₂O:δ¹⁵N-N₂O = 0.886).

\(L\) = Photochemical loss rate of N₂O in the stratosphere (14.3 Tg N yr⁻¹) [5]. Following [29], we applied an uncertainty of 25% in our calculations.

The term (− \(\varepsilon \times L\)) is a very close approximation of the ‘Net Isotope Flux’ (‰ Tg N yr⁻¹) as defined in [29], and is the net annual flux of N₂O isotopologues from the stratosphere to the troposphere.

Equation 1 can be rearranged to solve for δSources (Eq. 2), and all the known quantities provided above can be substituted into Eq. 2 to derive a flux-weighted, average modern source value (δSources) for δ¹⁵N-N₂O and δ¹⁸O-N₂O (‰).

\[
\delta_{\text{Sources}} = \left(\frac{\text{Burden} \times \delta_{\text{Trop}}}{\text{Sources}}\right) + (\varepsilon \times L) + \left(\frac{\sum \text{Sources} \times \delta_{\text{Trop}}}{\sum \text{Sources}}\right)
\]

Accordingly, we derive an average modern source value (± propagated standard deviation) for δ¹⁵N-N₂O and δ¹⁸O-N₂O of −8.4‰ (± 4.0) and +31.7‰ (± 13.9), respectively.
Fig 7. A comparison of bottom-up measurements to top-down estimates of N₂O sources. Previous top-down studies have used a variety of modelling approaches to apportion the global N₂O budget into different sources, identified here by colour. Ishijima et al. [10] measured N₂O in firn air and calculated the isotopic composition of the anthropogenic source for two time periods: 1952–1970 and 1970–2001 that differed markedly in δ¹⁵N. Park et al. [2] constrained the pre-industrial, natural N₂O source from δ¹⁵N-N₂O and δ¹⁸O-N₂O measurements in firn air and then calculated the current anthropogenic N₂O source using recent archived air samples. Rahn and Wahlen [6] evaluated a depleted ocean scenario [DOS, originally proposed by Kim and Craig [20]], and an enriched ocean scenario [EOS, originally proposed by Kim and Craig [19]] to calculate corresponding terrestrial N₂O sources. Röckmann et al. [9] measured N₂O in firn air and modelled the pre-industrial (natural) source, the modern global average source (pink circle with black outline), and the anthropogenic source under the IPCC3 (higher value) and IPCC2 (lower value) scenarios. Sowers et al. [11] measured firm air and gas trapped in an ice core to calculate a range of values for the isotopic composition of the average anthropogenic N₂O source. Toyoda et al. [7] estimated the δ¹⁵N and δ¹⁸O value of the oceanic N₂O source using 'Keeling Plots' of detailed water column data. Toyoda et al. [8] monitored the isotopic ratio of tropospheric N₂O in the northern hemisphere on a monthly basis from 2000–2011, and then used a box-model to estimate the current anthropogenic source. For reference, we show the Continental N₂O source, which is used along with the Marine source in our box-model calculations, and is operationally defined as Soil + Freshwater.
If we were to assume that all N$_2$O fluxes ($F$) originate only from marine and continental sources, then:

$$\sum \text{Sources} \approx F_{\text{Ocean}} + F_{\text{Cont}} \approx 17.9 \text{ Tg N yr}^{-1} \quad (3)$$

and the flux-weighted modern source value is approximated by:

$$\delta_{\text{Sources}} \approx (\delta_{\text{Cont}} \times F_{\text{Cont}}) + (\delta_{\text{Ocean}} \times F_{\text{Ocean}}) \quad \sum \text{Sources} \quad (4)$$

where the $\delta$ value of the continental and ocean sources are given in Table 2.

Combining Eq. 2 with Eq. 4 yields:

$$(\delta_{\text{Cont}} \times F_{\text{Cont}}) + (\delta_{\text{Ocean}} \times F_{\text{Ocean}}) \approx \left( \text{Burden} \times \frac{\partial \delta_{\text{Trop}}}{\partial t} \right) + (\varepsilon \times L) + (\sum \text{Sources} \times \delta_{\text{Trop}}) \quad (5)$$

Given the assumption that $F_{\text{Cont}} = \Sigma \text{Sources} - F_{\text{Ocean}}$ (Eq. 3), we can approximate $F_{\text{Ocean}}$ by:

$$F_{\text{Ocean}} \approx \frac{\left( \text{Burden} \times \frac{\partial \delta_{\text{Trop}}}{\partial t} \right) + (\varepsilon \times L) + (\sum \text{Sources} \times \delta_{\text{Trop}} - \delta_{\text{Cont}})}{\delta_{\text{Ocean}} - \delta_{\text{Cont}}} \quad (6)$$

Accordingly, using N isotope ratios we derive a value for $F_{\text{Ocean}}$ of $\sim 4.6 \pm 12.6$ Tg N yr$^{-1}$, which is $\sim 26\%$ of all sources (17.9 Tg N yr$^{-1}$) [5]. The $F_{\text{Cont}}$ is found by difference, and is approximately equal to 13.3 (± 13.4) Tg N yr$^{-1}$, or 74\% of all natural and anthropogenic N$_2$O sources. The largest source of uncertainty in the N isotope mass-balance lies in the $\delta^{15}$N value of the continental source ($1\sigma = 11.5\%$), followed by $\Sigma \text{Sources}$ and $L$, which have a relative uncertainty of 25\% in our model.

The most recent N$_2$O budget estimates the combined soil, freshwater, and ocean flux to be $\sim 15.7$ Tg N yr$^{-1}$, or 87.7\% of the total source [5]. Our approach assumes the $\Sigma \text{Sources} = 17.9$ Tg N yr$^{-1}$ (as reported in IPCC-AR5) because other terms in the mass-balance (e.g., N$_2$O burden and loss rate) are based on a budget that includes all known sources. As such, we ignore the contributions from smaller sources such as human sewage, fossil fuels, industry, biomass combustion, and chemical production processes in the atmosphere, which have a combined annual flux of $\sim 2.2$ Tg N yr$^{-1}$ [5]. Despite this, our result for $F_{\text{Ocean}}$ is similar to the estimate provided in IPCC-AR5, which shows oceans contribute 21\% of the annual N$_2$O budget [5].

The O isotope mass-balance fails to derive a positive ocean flux ($F_{\text{Ocean}} = -4.5 \pm 19.9$ Tg N yr$^{-1}$). This is because the $\delta^{18}$O separation between the troposphere and the continental source is smaller than it is for $\delta^{15}$N, and the term $\Sigma \text{Sources} (\delta_{\text{Trop}} - \delta_{\text{Cont}})$ is too small to make the numerator in Eq. 6 a net positive number. However, decreasing the loss rate ($L$) by 4 Tg N

### Table 3. MixSIAR model output summary.

| Category | Mean Contribution | Standard Deviation ($\sigma$) | Confidence Interval |
|----------|-------------------|-----------------------------|---------------------|
|          |                   | 2.5%  | 5%  | 25%  | 50%  | 75%  | 95%  | 97.5% |
| 2-isotope ($\delta^{15}$N$_{bulk}$, $\delta^{18}$O) mixing model | Soil | 0.34 | 0.23 | 0.02 | 0.03 | 0.15 | 0.31 | 0.51 | 0.77 | 0.83 |
|          | Freshwater | 0.24 | 0.16 | 0.01 | 0.02 | 0.11 | 0.22 | 0.35 | 0.51 | 0.57 |
|          | Marine | 0.42 | 0.21 | 0.03 | 0.07 | 0.26 | 0.43 | 0.58 | 0.77 | 0.83 |
| 3-isotope ($\delta^{15}$N$_{bulk}$, $\delta^{18}$O, SP) mixing model | Soil | 0.33 | 0.23 | 0.02 | 0.03 | 0.15 | 0.31 | 0.49 | 0.72 | 0.78 |
|          | Freshwater | 0.24 | 0.16 | 0.01 | 0.02 | 0.11 | 0.21 | 0.35 | 0.53 | 0.59 |
|          | Marine | 0.43 | 0.19 | 0.07 | 0.13 | 0.30 | 0.42 | 0.55 | 0.75 | 0.82 |

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yr\(^{-1}\) and increasing \(\Sigma\) Sources by the same amount yields a positive ocean flux = 4.6 Tg N yr\(^{-1}\). Therefore, if the uncertainty of these parameters is reduced in the future we may find that the O isotope budget balances.

Finally, we used a stable isotope Bayesian mixing model (MixSIAR) \[75\] to determine the proportions of soil, freshwater, and marine N\(_2\)O that best predicted the average modern source (anthropogenic plus natural) (Eq. 7).

\[
\delta_{\text{sources}} = (\delta_{\text{Soil}} \times F_{\text{Soil}}) + (\delta_{\text{Freshwater}} \times F_{\text{Freshwater}}) + (\delta_{\text{Ocean}} \times F_{\text{Ocean}})
\]  

where, \(F_{\text{Soil}} + F_{\text{Freshwater}} + F_{\text{Ocean}} = 1\).

MixSIAR, which is a front-end interface of the model SIAR (Stable Isotope Analysis in R) \[76\], is an ecological mixing model traditionally used to describe food web and predator-prey relationships. Values of \(\delta^{15}\)N\(_{\text{bulk-N}_2\text{O}}\), \(\delta^{15}\)N\(_{\alpha-N}_2\text{O}\), \(\delta^{15}\)N\(_{\beta-N}_2\text{O}\), and \(\delta^{18}\)O-N\(_2\text{O}\) for the average modern source (the mixture) were taken from Röckmann et al. \[9\]. These estimates are almost identical to the ones calculated in our 2-box-model (above), and Röckmann et al. \[9\] provided \(^{15}\)N isotopomers, which allowed us to use 3 variables in our model runs (\(\delta^{15}\)N\(_{\text{bulk}}, \delta^{18}\)O, and SP). A series of Markov Chain Monte Carlo simulations, using values for soil, freshwater, and marine N\(_2\)O (filtered, raw data compiled in this study), were done to find mixing solutions that best fit the average modern source (Table 3). Gelman-Rubin and Geweke diagnostic tests indicated a chain length of 300,000, burn in of 200,000, thinning of 50 (2-isotope) or 100 (3-isotope), and 3 chains were appropriate.

Model runs using only the \(\delta^{15}\)N-N\(_2\text{O}\) and \(\delta^{18}\)O-N\(_2\text{O}\) data (2-isotope mixing model, \(n = 1383\) data pairs) produced results very similar to the model runs that also included SP data (3-isotope mixing model, \(n = 235\) data triads). Overall, this Bayesian modeling exercise predicted the soil, freshwater, and ocean contributions (± 1\(\sigma\)) to the average modern N\(_2\)O source were 0.43 (0.20), 0.34 (0.22), and 0.24 (0.16), respectively (Table 3). Unlike the box-model, this approach does not place \textit{a priori} bounds on the data, and is not constrained by terms such as the stratospheric N\(_2\)O loss rate (\(L\)), which have large uncertainty. However, this method also ignores the contributions of several small sources, which have a combined contribution of ~ 12.3\% to the total budget presented in IPCC-AR5 \[5\].

Both SIAR and the box-model predict the ocean flux to be 24\% and 26\% of the total, respectively, which closely confirms the scientific community’s best estimate of the ocean flux as presented in IPCC-AR5 (21\% of the total source). Further, the SIAR model output shows that freshwaters may contribute much more N\(_2\)O than previously thought. The current N\(_2\)O budget estimates the combined flux from rivers, estuaries, and coastal zones is 0.6 Tg N yr\(^{-1}\), or just 3\% of the total source. While we acknowledge that there is \(\delta^{15}\)N-\(\delta^{18}\)O overlap in the soil and freshwater source (Fig. 6–top and bottom panels), these sources appear to be unique in \(\delta^{15}\)N-SP space (Fig. 6–middle panel). Therefore, we suggest there is a great need to quantify N\(_2\)O fluxes from freshwaters, estuaries, and coastal zones, which have received considerably less attention than soil and off-shore marine environments.

### Supporting Information

**S1 Dataset.** A comma-delimited text file with all the data collected and analyzed in this study. In addition to \(\delta^{15}\)N-N\(_2\text{O}\), SP and \(\delta^{18}\)O-N\(_2\text{O}\) values, we provide a reference citation, the category, a brief site description, and the criteria used to filter the data subsets (S1_Dataset.csv).

(CSV)
S2 Dataset. A comma-delimited text file with weighted average $\delta^{15}$N-$\text{N}_2\text{O}$, SP and $\delta^{18}$O-$\text{N}_2\text{O}$ values from select freshwater, soil and urban wastewater studies (S2_Dataset.csv). (CSV)

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Author Contributions
Conceived and designed the experiments: DMS JJV SLS JS. Performed the experiments: DMS JJV. Analyzed the data: DMS JJV. Wrote the paper: DMS JJV SLS JS.

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