PRODUCTION, ISOTOPIC COMPOSITION, AND ATMOSPHERIC FATE OF BIOLOGICALLY PRODUCED NITROUS OXIDE

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Abstract The anthropogenic production of greenhouse gases and their consequent effects on global climate have garnered international attention for years. A remaining challenge facing scientists is to unambiguously quantify both sources and sinks of targeted gases. Microbiological metabolism accounts for the largest source of nitrous oxide (N₂O), mostly due to global conversion of land for agriculture and massive usage of nitrogen-based fertilizers. A most powerful method for characterizing the sources of N₂O lies in its multi-isotope signature. This review summarizes mechanisms that lead to biological N₂O production and how discriminate placement of ¹⁵N into molecules of N₂O occurs. Through direct measurements and atmospheric modeling, we can now place a constraint on the isotopic composition of biological sources of N₂O and trace its fate in the atmosphere. This powerful interdisciplinary combination of biology and atmospheric chemistry is rapidly advancing the closure of the global N₂O budget.

INTRODUCTION

Nitrous oxide (N₂O) is a potent greenhouse gas and the major source of stratospheric reactive nitrogen species that participate in the catalytic destruction of ozone. It has an atmospheric residence time of 118 ± 25 years (Minschwaner et al. 1998, Olsen et al. 2001). The infrared radiative forcing of N₂O is about 206 times that of CO₂ on a per molecule basis at present atmospheric conditions (IPCC 2001). This is illustrated in Figure 1, which shows the blackbody radiation of an atmosphere at 273 K and the infrared bands of N₂O. The fundamental bands are 𝜈₂, 𝜈₁, and 𝜈₃ at 589, 1285, and 2224 cm⁻¹, respectively; the most important for greenhouse warming of the atmosphere is the 𝜈₁ band at 7.78 µm (Goody & Yung 1989).
Figure 1  

(a) Radiance due to a blackbody at 273 K as a function of wavenumber. The units are in W/(m² cm⁻¹ sr), where sr is steradian.  

(b) Absorption coefficient for N₂O in the infrared. The strongest absorption bands are ν₂, ν₁, and ν₃ at 589, 1285 and 2224 cm⁻¹, respectively. The others are overtone or combination bands. Data taken from the HITRAN (high-resolution transmission molecular absorption database), as described in Goody & Yung (1989).

There has been a steady increase in atmospheric N₂O since the Industrial Revolution, as shown in Figure 2 (IPCC 2001). The early data are deduced from gases trapped in air bubbles in polar ice. Over the recent few decades, the concentration of N₂O has been accumulating in the atmosphere (see inset of Figure 2) at a rate of 0.6 ppb · year⁻¹, adding to the current concentration of about 314 ppb (parts per billion by volume) (IPCC 2001). The radiative forcing, approximately on a linear scale for small perturbations, is plotted on the right axis. There has been an increase of about 0.15 Wm⁻² since the Industrial Revolution. For comparison, the radiative forcing due to CO₂ over the same period is about 1.4 Wm⁻². Although many sources of N₂O have been identified, including agricultural soils, oceans, animal waste, biomass burning, fuel combustion, and industry, the combined strength of these sources calculated in 1994 only matched two thirds of the known sinks, which are removal and accumulation in the atmosphere (IPCC 2001). Upon re-evaluation of
the strength of each source, it was found that the application of nitrogen-based fertilizers to agricultural soils accounted for a much larger source of $\text{N}_2\text{O}$ than previously thought (Mosier & Kroeze 1998, Nevison & Holland 1997). By 1998, the balance between the total sources and total sinks of $\text{N}_2\text{O}$ added up to a budget of $16 \text{Tg N} \cdot \text{year}^{-1}$ (IPCC 2001). However, large uncertainty remains as to the actual strength of individual sources of $\text{N}_2\text{O}$ (Table 1).

Increased fertilizer usage over the past several years has stimulated the rates of microbial metabolism, especially those processes involved in the nitrogen cycle (Figure 3). Nitrification and denitrification are the primary metabolic activities leading to $\text{N}_2\text{O}$ production. However, several physiological and ecological feedbacks can greatly influence the rates of these metabolic pathways. One of the purposes of this review is to examine the diversity of enzymatic, organismal, community, and environmental factors that are simultaneously involved in producing $\text{N}_2\text{O}$. Once we achieve an understanding of the complexity of $\text{N}_2\text{O}$ production by microbial communities, the next goal is to quantify how much $\text{N}_2\text{O}$ is produced by nitrification and denitrification under a particular set of environmental conditions.

To accomplish this feat, many researchers are attempting to track discrete sources of $\text{N}_2\text{O}$ accumulating in the atmosphere by using differential isotopic signatures of $^{15}\text{N}^{15}\text{N}^{18}\text{O}$ as produced by specific microbial communities. This concept necessarily relies on enzymatic, organismal, community, and environmental factors that influence the isotopic ratios and intramolecular site-preference of $^{15}\text{N}$. Here, we review current research on organisms and enzymes within the microbial community.
Table 1: Sources of N₂O (in Tg N year⁻¹)

<table>
<thead>
<tr>
<th>Source</th>
<th>1994ᵃ</th>
<th>Range</th>
<th>1990ᵇ</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropogenic sources</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agricultural soils</td>
<td>4.2</td>
<td>0.6–14.8</td>
<td>3.6</td>
<td>2.8–5.7</td>
</tr>
<tr>
<td>Biomass burning</td>
<td>0.5</td>
<td>0.2–1.0</td>
<td>0.5</td>
<td>0.2–0.8</td>
</tr>
<tr>
<td>Industrial sources</td>
<td>1.3</td>
<td>0.7–1.8</td>
<td>0.7</td>
<td>0.2–1.1</td>
</tr>
<tr>
<td>Cattle and feedlots</td>
<td>2.1</td>
<td>0.6–3.1</td>
<td>1.0</td>
<td>0.2–2.0</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>8.1</td>
<td>2.1–20.7</td>
<td>4.1</td>
<td>1.3–7.7</td>
</tr>
<tr>
<td><strong>Natural sources</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocean</td>
<td>3.0</td>
<td>1.0–5.0</td>
<td>3.6</td>
<td>2.8–5.7</td>
</tr>
<tr>
<td>Atmosphere (NH₃ oxidation)</td>
<td>0.6</td>
<td>0.3–1.2</td>
<td>0.6</td>
<td>0.3–1.2</td>
</tr>
<tr>
<td>Tropical soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet forest</td>
<td>3.0</td>
<td>2.2–3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry savannas</td>
<td>1.0</td>
<td>0.5–2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forests</td>
<td>1.0</td>
<td>0.1–2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grasslands</td>
<td>1.0</td>
<td>0.5–2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>All soils</strong></td>
<td></td>
<td></td>
<td>6.6</td>
<td>3.3–9.9</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>9.6</td>
<td>4.6–15.9</td>
<td>10.8</td>
<td>6.4–16.8</td>
</tr>
<tr>
<td><strong>Total sources</strong></td>
<td>17.7</td>
<td>6.7–36.6</td>
<td>14.9</td>
<td>7.7–24.5</td>
</tr>
<tr>
<td><strong>Imbalance (trend)</strong></td>
<td>3.9</td>
<td>3.1–4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total sinks (stratospheric)</strong></td>
<td>12.3</td>
<td>9–16</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Implied total sources</strong></td>
<td>16.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ᵃ(Kroeze 1999, Mosier et al. 1998).
ᵇ(Olivier et al. 1998).
	nitrogen cycle, how isotopic site-preference and fractionation occur, measurements of N₂O isotopologue and isotopomer abundances using spectroscopic techniques, and fate of N₂O isotopologues and isotopomers in the atmosphere (definitions of terms in IUPAC 1997). There are three important isotopologues of N₂O, ¹⁵NNO, N₂¹⁸O and N₂¹⁷O, and two isotopomers, ¹⁵NNO and N¹⁵NO.

The isotopologues and isotopomers of N₂O will greatly advance our understanding of the budgets of these gases, primarily because atmospheric budgets of trace gas species must balance for both the species as a whole and also for individual isotopic analogs. Because most sources and sinks have characteristic fractionation processes, the different isotopic species provide valuable additional constraints in determining the budgets of these gases. The expansion of the database to include isotopic composition obviously increases the information content of the system under study, but it comes with a challenge. The basic physical, chemical, biological, and atmospheric processes must be well understood before isotopic information can be used to place useful constraints on sources and sinks of N₂O.
Figure 3  Microbial nitrogen cycle.

A most powerful method for characterizing the sources of N$_2$O (and other biogenic trace gases as well) lies in its multi-isotope signature, as demonstrated in the seminal work by Kim & Craig (1993). The measurements made in this study for $\delta^{15}$N versus $\delta^{18}$O in N$_2$O are summarized in Figure 1 of their paper [an updated version is reproduced here as Figure 4; $\delta = (R/R_{\text{standard}} - 1) \times 1000$, where $R$ = isotopic ratio (Craig 1961)]. The reference isotopic fractionations of atmospheric N$_2$ and O$_2$ are taken to be zero. Average tropospheric N$_2$O has $\delta^{15}$N = 7.0 and $\delta^{18}$O = 20.7 per mil. In the stratosphere, $\delta^{15}$N and $\delta^{18}$O appear to be enriched by as much as 15 and 12 per mil, respectively, relative to tropospheric N$_2$O. These startling results for stratospheric N$_2$O were confirmed and extended by Rahn & Wahlen (1997). Referring to Figure 4, the significant results of Kim & Craig (1993) are (a) the major land biospheric sources of N$_2$O are light in both $^{15}$N and $^{18}$O, relative to tropospheric N$_2$O; (b) stratospheric N$_2$O is enriched in both $^{15}$N and $^{18}$O, relative to tropospheric N$_2$O; and (c) there is a large “back flux” of $^{15}$N-enriched N$_2$O from the stratosphere to the troposphere in order to account for the isotopic composition of tropospheric N$_2$O. A large part of the recent work on N$_2$O consists of understanding the underlying mechanism of isotopic fractionation in the atmosphere implied by Kim & Craig (1993) (Johnson et al. 2001, Yung & Miller 1997).

MICROBIAL PATHWAYS THAT PRODUCE N$_2$O

Microbial Nitrogen Transformations and Intramolecular Site Preference of $^{15}$N

The requirement for nitrogen in biological molecules necessitates a diversity of mechanisms for assimilating nitrogen into the living cell. The majority of assimilatory mechanisms in bacteria, except the direct fixation of N$_2$, require the uptake of either ammonia (NH$_3$) or nitrate (NO$_3^-$). In addition to assimilation, nitrogenous
Figure 4  A plot of $\delta^{15}$N versus $\delta^{18}$O with highlighted fields representing the range of data found in surface oceans (gray field) and terrestrial systems (textured field). Symbols representing the work of various authors are defined in the legend. The oceanic results shown from Kim & Craig (1990) are the measured values in the top 1000 m of the subtropical Pacific Ocean. The symbols representing Yoshinari et al. (1997) are the near-surface averages of their data from the Indian Ocean and Arabian Sea. Also from the Arabian Sea is the near-surface average of Naqvi et al. (1998). The subtropical North Pacific end-member value proposed by Dore et al. (1998) is also shown. In the terrestrial field, the open circles associated with the data of Pérez et al. (2000) and Casciotti et al. (1997) are proportional to the relative magnitude of the flux for each of the points. Terrestrial data of Kim & Craig (1993) have no concurrent flux results. The open square is the commonly reported average value of tropospheric N$_2$O. The $\delta^{15}$N and $\delta^{18}$O values are referenced to atmospheric N$_2$ and O$_2$, respectively. Taken from Rahn & Wahlen (2000).

compounds are also extremely important for energy generation due to the spectrum of oxidation states from $-3$ to $+5$ (Table 2). Microbial enzymes have evolved that specialize in shuttling electrons to or from these various molecules, and several are described in Table 3.

Both assimilatory and dissimilatory processes are important for the fractionation and final placement of isotopes within N$_2$O molecules, although dissimilatory processes have the largest effects. The enzymes in dissimilatory pathways process a large flux of molecules relative to assimilatory pathways, and isotopic discrimination is based predominantly on catalytic rate and number of enzymatic steps (Cleland et al. 1977). In general, enzymes prefer to transform molecules with a smaller molecular weight, such that the end products within a nitrogen transformation pathway will almost always be isotopically lighter than molecules
TABLE 2 Oxidation state of nitrogenous compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical formula</th>
<th>Oxidation state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>NH₃</td>
<td>−3</td>
</tr>
<tr>
<td>Hydrazine</td>
<td>N₂H₄</td>
<td>−2</td>
</tr>
<tr>
<td>Dimine</td>
<td>N₂H₂</td>
<td>−1</td>
</tr>
<tr>
<td>Hydroxylamine</td>
<td>NH₂OH</td>
<td>−1</td>
</tr>
<tr>
<td>Dinitrogen</td>
<td>N₂</td>
<td>0</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>N₂O</td>
<td>+1</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>NO</td>
<td>+2</td>
</tr>
<tr>
<td>Nitrite</td>
<td>NO₂⁻</td>
<td>+3</td>
</tr>
<tr>
<td>Nitrogen dioxide</td>
<td>NO₂</td>
<td>+4</td>
</tr>
<tr>
<td>Nitrate</td>
<td>NO₃⁻</td>
<td>+5</td>
</tr>
</tbody>
</table>

in prior steps. However, as is evident in Figure 3, there are pathways that contribute to the formation of N₂O and those that consume it. These pathways are in constant competition depending on environmental conditions and can lead to a range of ¹⁵N values based on the number and type of enzymatic steps involved. Additionally, some enzymes will allow for the exchange of substrate molecules at the active-site of the enzyme before the conversion of substrate to product is complete. This process will lead to less isotopic discrimination of nitrogen due to the slowed rate of catalysis, as occurs in the process of nitrogen fixation (Beaumont et al. 2000, Hoering & Ford 1960).

The complexity of enzymatic possibilities, processivity of reactions (i.e., rate of exchange), and competition between pathways makes the task of quantifying individual microbial contributions to the budget and isotopic fractionation of N₂O very difficult. The following descriptions explain, in basic terms, the pathways that are found in broad groups of microorganisms, how discrete enzymatic pathways might fractionate ¹⁵N leading to a traceable isotopic signature in the N₂O pool, and how environmental factors influence the activities of microbial groups leading to bias in the composition of N₂O isotopologues and isotopomers.

**Nitrification**

The process of nitrification includes the oxidation of ammonia to nitrate via nitrite (Figure 3). Several groups of microorganisms are involved in nitrification—some use it for their central metabolism, whereas others perform parts of the process fortuitously through co-metabolism (Prosser 1986). The first four groups of bacteria listed in Table 3 all participate in nitrification to varying degrees.

The best characterized microbial pathway for the oxidation of ammonia to nitrite (NO₂⁻) is performed by chemolithotrophic bacteria, i.e., they consume only inorganic substrates to generate energy, and in the case of ammonia oxidizers,
TABLE 3  Bacteria in the nitrogen cycle and their enzymes

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Process</th>
<th>Main enzyme</th>
<th>Metabolism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemolithotrophic</td>
<td>Ammonia-oxidizers</td>
<td>AMO</td>
<td>dissim.</td>
<td>Wood 1986</td>
</tr>
<tr>
<td>Ammonia-oxidizers</td>
<td>NH₃ oxidation to NO₂⁻ via NH₂OH</td>
<td>HAO</td>
<td>dissim.</td>
<td>Wood 1986</td>
</tr>
<tr>
<td></td>
<td>NH₂OH oxidation to NO</td>
<td>HAO</td>
<td>dissim.</td>
<td>Wood 1986</td>
</tr>
<tr>
<td></td>
<td>and N₂O</td>
<td>HAO or nonenzyme</td>
<td>dissim.</td>
<td>Hooper &amp; Terry 1979</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻ reduction to NO</td>
<td>NIR</td>
<td>dissim.</td>
<td>Hooper 1968</td>
</tr>
<tr>
<td></td>
<td>NO reduction to N₂O</td>
<td>NOR</td>
<td>dissim.</td>
<td>Remde &amp; Conrad 1990</td>
</tr>
<tr>
<td></td>
<td>NO₂⁻ reduction to N₂</td>
<td>?</td>
<td>dissim.</td>
<td>Bock 1995</td>
</tr>
<tr>
<td>Methane oxidizers</td>
<td>NH₃ oxidation to NO₂⁻ via NH₂OH</td>
<td>MMO</td>
<td>fortuitous</td>
<td>Dalton 1977, Zahn et al. 1994</td>
</tr>
<tr>
<td></td>
<td>NH₂OH oxidation to NO</td>
<td>P-460</td>
<td>fortuitous</td>
<td>Zahn et al. 1994</td>
</tr>
<tr>
<td></td>
<td>and N₂O</td>
<td>?</td>
<td>dissim.</td>
<td>Knowles &amp; Topp 1988</td>
</tr>
<tr>
<td></td>
<td>NO₂⁻ reduction to NO₂⁻</td>
<td>?</td>
<td>dissim.</td>
<td>Uncharacterized</td>
</tr>
<tr>
<td></td>
<td>NO₂⁻ reduction to NH₃</td>
<td>?</td>
<td>dissim.</td>
<td>Uncharacterized</td>
</tr>
<tr>
<td></td>
<td>N₂ fixation to NH₃</td>
<td>nitrogenase</td>
<td>dissim.</td>
<td>Auman et al. 2001</td>
</tr>
<tr>
<td></td>
<td>NO₂⁻ reduction to NO</td>
<td>?</td>
<td>dissim.</td>
<td>Ye &amp; Thomas 2001</td>
</tr>
<tr>
<td></td>
<td>NO reduction to N₂O</td>
<td>NOR</td>
<td>dissim.</td>
<td>Ye &amp; Thomas 2001</td>
</tr>
<tr>
<td>Heterotrophic</td>
<td>ammonia-oxidizers</td>
<td>NH₃ oxidation to NO₂⁻ via NH₂OH</td>
<td>AMO</td>
<td>dissim.</td>
</tr>
<tr>
<td>Nitrite oxidizers</td>
<td>NO₃⁻ oxidation to NO₂⁻</td>
<td>nitrite oxidase</td>
<td>dissim.</td>
<td>Prosser 1989</td>
</tr>
<tr>
<td></td>
<td>NO₂⁻ reduction to NO₂⁻</td>
<td>nitrite oxidase</td>
<td>dissim.</td>
<td>Freitag et al. 1987</td>
</tr>
<tr>
<td></td>
<td>NH₃, and N₂O</td>
<td>?</td>
<td>dissim.</td>
<td>Freitag &amp; Bock 1990</td>
</tr>
<tr>
<td></td>
<td>NO oxidation to NO₂⁻</td>
<td>?</td>
<td>dissim.</td>
<td>Freitag &amp; Bock 1990</td>
</tr>
<tr>
<td></td>
<td>and NO₃⁻</td>
<td>?</td>
<td>dissim.</td>
<td>Freitag &amp; Bock 1990</td>
</tr>
<tr>
<td>Dissimilatory denitrifiers</td>
<td>NO₃⁻ reduction to NO₂⁻</td>
<td>NAR, NAP</td>
<td>dissim.</td>
<td>Richardson &amp; Watmough 1999</td>
</tr>
<tr>
<td></td>
<td>NO₂⁻ reduction to NO</td>
<td>NIR</td>
<td>dissim.</td>
<td>Richardson &amp; Watmough 1999</td>
</tr>
<tr>
<td></td>
<td>NO reduction to N₂O</td>
<td>NOR</td>
<td>dissim.</td>
<td>Richardson &amp; Watmough 1999</td>
</tr>
<tr>
<td></td>
<td>NO oxidation to N₂O</td>
<td>NOS</td>
<td>dissim.</td>
<td>Richardson &amp; Watmough 1999</td>
</tr>
<tr>
<td></td>
<td>N₂O reduction to N₂</td>
<td>N₂OR</td>
<td>dissim.</td>
<td>Rasmussen et al. 2000</td>
</tr>
<tr>
<td></td>
<td>NO oxidation to NO₃⁻</td>
<td>?</td>
<td>dissim.</td>
<td>Baumgartner et al. 1996</td>
</tr>
<tr>
<td></td>
<td>NO₂⁻ reduction to NH₃</td>
<td>NAR</td>
<td>dissim.</td>
<td>Nakano et al. 1998</td>
</tr>
<tr>
<td></td>
<td>via NO₃⁻</td>
<td>NAS</td>
<td>dissim.</td>
<td>Lin &amp; Stewart 1998</td>
</tr>
<tr>
<td>Assimilatory denitrifiers</td>
<td>NO₂⁻ reduction to NO₂⁻</td>
<td>NAS</td>
<td>assim.</td>
<td>Brittain et al. 1992</td>
</tr>
<tr>
<td></td>
<td>NO₂⁻ reduction to NH₃</td>
<td>sirohaem NIR, hexahaem NIR</td>
<td>assim.</td>
<td>Kobayashi &amp; Shoun 1995</td>
</tr>
<tr>
<td>Fungal denitrifiers</td>
<td>NO₃⁻ reduction to NO</td>
<td>Cu-NIR</td>
<td>?</td>
<td>Kizawa et al. 1991</td>
</tr>
<tr>
<td></td>
<td>NO reduction to N₂O</td>
<td>P450nor</td>
<td>?</td>
<td>Kizawa et al. 1991</td>
</tr>
<tr>
<td>Nitrogen fixers</td>
<td>N₂ to NH₃</td>
<td>nitrogenase</td>
<td>assim.</td>
<td>Postgate 1998</td>
</tr>
<tr>
<td>Anammox</td>
<td>NH₃ and NO₂⁻ to N₂ via NH₂O and NH₂OH</td>
<td>nitrite reductase hydrazine hydrolase hydrazine oxidase</td>
<td>dissim.</td>
<td>Jetten et al. 2001</td>
</tr>
</tbody>
</table>

Enzyme acronyms from cited references.
Assim. = assimilatory, dissim. = dissimilatory, fortuitous = co-metabolic.
they fix inorganic carbon. Ammonia-oxidizing bacteria require molecular oxygen to perform their metabolism and produce hydroxylamine (NH$_2$OH) as a metabolic intermediate (Wood 1986). Ammonia oxidation by these bacteria is highly stimulated by the addition of nitrogen-based fertilizers to agricultural soils, and the NO$_2$ they produce stimulates other processes in the nitrogen cycle, including the production of N$_2$O (Conrad 1996). Ammonia oxidizers are also capable of producing N$_2$O under microaerophilic conditions by directly reducing NO$_2^-$, presumably through the activities of denitrifying enzymes (Goreau et al. 1980, Hooper 1968). This process, known as aerobic or lithotrophic denitrification, has been observed in only a small group of ammonia-oxidizing species (Casciotti & Ward 2001, Colliver & Stephenson 2000). However, the enzymes involved, the phylogenetic breadth, and metabolic purpose remain unknown.

Methane-oxidizing bacteria, or methanotrophs, are closely related evolutionarily to the ammonia oxidizers (Holmes et al. 1995). These bacteria oxidize ammonia to NO$_2^-$ through a co-metabolic process, and thus do not gain energy from it (Dalton 1977). They also reduce NO$_2^-$ to N$_2$O similarly to ammonia oxidizers, but this metabolism is even less well characterized for the methanotrophs than for the ammonia oxidizers (Knowles & Topp 1988, Yoshinari 1984). The contribution of methanotrophs to the N$_2$O budget is currently unknown, but may be significant (Mandernack et al. 2000). As shown in Table 3, methanotrophs also process nitrogen through other metabolic processes, including the consumption of NO, which is the substrate for N$_2$O production (Krämer et al. 1990).

Heterotrophic ammonia-oxidizers, bacteria that consume organic carbon as their primary metabolism, also oxidize ammonia to NO$_2^-$, but like the methanotrophs, they do not gain energy from it (Killham 1986, Verstraete 1975). It is thought that these bacteria could oxidize as much ammonia as the chemolithotrophic ammonia-oxidizers provided that enough organic carbon is available for energy production (Kuenen & Robertson 1994). Additionally, many heterotrophic nitrifiers reduce NO$_2^-$ to N$_2$O under microaerophilic conditions using standard denitrifying enzymes (Table 3) (Castignetti et al. 1984). Therefore, the chemolithotrophic ammonia-oxidizers, methanotrophs, and heterotrophic ammonia-oxidizers all occupy the same environmental niche and are all extremely important for N$_2$O production in aerobic zones (Lipschultz et al. 1981). In addition to the reduction of NO$_2^-$ to NO and N$_2$O, the oxidation of NH$_2$OH, a metabolic intermediate of ammonia oxidation, also produces N$_2$O (Hooper & Terry 1979). However, because NH$_2$OH is rapidly converted to NO$_2^-$ in the environment, the amount of N$_2$O from NH$_2$OH oxidation is presumably insignificant compared to the amount produced through reduction of NO$_2^-$ and NO by denitrifying enzymes (Whittaker et al. 2000).

The other half of nitrification, NO$_2^-$ oxidation to NO$_3^-$, is performed by a separate group of bacteria known as nitrite oxidizers (Bock & Koops 1991). The NO$_3^-$ produced by this group of bacteria provides much of the substrate for denitrification, a process discussed in the next section, which largely controls the rate of N$_2$O production in anaerobic environments. The nitrite oxidizers have been highly understudied, but they appear to play an additional role in the direct production
of N\textsubscript{2}O (Freitag et al. 1987), and/or in the consumption of NO (Freitag & Bock 1990).

**Denitrification**

The process of denitrification, the reduction of NO\textsubscript{3} to N\textsubscript{2} via NO\textsubscript{2}, NO, and N\textsubscript{2}O, is performed by a very diverse group of microorganisms, including archaea and some fungi (Kizawa et al. 1991, Zumft 1992). The enzymatic diversity and ubiquity of this pathway among microbes argue for its central role in energy generation, nitrogen assimilation, and competition in most ecosystems. Denitrifying bacteria can utilize a number of food sources from organic carbon to Fe(II) (Straub et al. 1996) and use the nitrogenous molecules as electron acceptors to complete the task of energy generation. Because denitrification is made up of a sequence of reductive events, this process is thought to occur predominantly in anaerobic environments. The potential for N\textsubscript{2}O emission from denitrification increases in water-saturated environments that contain large concentrations of organic carbon and NO\textsubscript{3} (Baumgartner & Conrad 1992). High concentrations of NO\textsubscript{3} inhibit the reduction of N\textsubscript{2}Ot oN\textsubscript{2} and stimulate the reduction of NO to N\textsubscript{2}O causing the escape of N\textsubscript{2}O from soils to the atmosphere (Gaskell et al. 1981).

There has been a long-standing debate as to whether nitrifying or denitrifying processes contribute the most N\textsubscript{2}O from agricultural environments. It appears that the extent of isotopic fractionation from these processes is quite different due to energy conservation and the consumption of N\textsubscript{2}O during denitrification (Pérez et al. 2001). As discussed below, this argument will necessitate a closer look at the carbon to nitrogen ratio of soils, available oxygen, temperature, water, and pH.

**Other Processes in the Nitrogen Cycle that Influence N\textsubscript{2}O Production**

Obviously, the direct production of N\textsubscript{2}O by microorganisms will have the greatest influence on isotopic fractionation. However, because all aspects of the nitrogen cycle influence the availability of substrates for N\textsubscript{2}O production or consumption, indirect processes must also be considered when quantifying the roles of individual microbial groups that participate in N\textsubscript{2}O production. In environments that are not heavily influenced by agriculture or industry, N\textsubscript{2} fixation provides much of the ammonia that is subsequently oxidized by the three main groups of ammonia oxidizers discussed above. N\textsubscript{2} fixation provides a ready nitrogen source for assimilation by many types of organisms in natural systems, especially plants, fungi, and bacteria (Postgate 1998). The influence of naturally occurring N\textsubscript{2} fixation on the nitrogen cycle has recently been eclipsed by the wide-spread production and application of ammonia-based fertilizers and atmospheric deposition of ammonia from automobile exhaust and industrial processes (Howarth et al. 1996).

Bacteria that are capable of anaerobically oxidizing ammonia, a process known as anammox, also participate in the nitrogen cycle by transforming NH\textsubscript{3} and NO\textsubscript{2} to N\textsubscript{2} via hydrazine and hydroxylamine intermediates (Jetten et al. 2001). It is
currently unknown whether this process is globally relevant, but significant rates have been described in marine sediments from continental shelves (Thamdrup & Dalsgaard 2002). Thus, under the right conditions, anammox could compete with denitrification in anaerobic environments by removing substrates that are necessary for producing N₂O.

ENZYME STRUCTURE AS A CAUSE FOR SITE PREFERENCE OF ¹⁵N IN N₂O

Differences Between Nitrification and Denitrification

As described above, there are many groups of microorganisms that perform a variety of processes leading to either production or consumption of N₂O in the environment. Our eventual goal is to close the global budget for N₂O by understanding the relative strength of nitrifying versus denitrifying processes. One possibility for quantifying the relative contribution of microbial groups is to find differences in the composition of N₂O isotopomers and isotopologues that they each produce. In other words, is the placement of ¹⁵N and extent of ¹⁵N fractionation in N₂O molecules different for each microbial process? Several studies have shown that N₂O produced by nitrification is much more depleted in ¹⁵N than that produced by denitrification (−45‰ to −66‰ for nitrification versus −13‰ to −28‰ for denitrification) (Barford et al. 1999, Pérez et al. 2001, Webster & Hopkins 1996, Yoshida 1988). This observation makes sense due to the conservation of energy during denitrification and the consumption of N₂O, processes that decrease the extent of isotopic fractionation.

A study by Yoshida & Toyoda suggested that we could discriminate among N₂O molecules produced by nitrification, denitrification, and bulk industrial emissions (automobile and factory) by looking at the intramolecular site-preference for ¹⁵N within the N₂O molecule (Yoshida & Toyoda 2000). This study proposed that in the N⁰N⁰O molecule, N₂O produced by microbes will be more fractionated at the N⁰ position than at the N⁰ position based on the formation and cleaving of the N⁰–O bond during ammonia oxidation and N₂O reduction, respectively. Following this logic, environments dominated by nitrification should result in a relatively low ratio of ¹⁴N¹⁵NO:¹⁵N¹⁴NO, whereas denitrification-dominated environments would have a more equalized ratio due to the preferential reduction of ¹⁵N¹⁴NO. Although hypothetically sound, the exact opposite effect has been measured for N₂O emitted from fertilized agricultural soils dominated by nitrification, which showed enrichment of ¹⁵N at the central N position (Pérez et al. 2001). This observation is supported by the mechanism of the N₂O-producing enzyme as discussed below. Industrial processes were found to cause insignificant fractionation of ¹⁵N because they are not catalyzed enzymatically, and can thus be easily distinguished from biological N₂O production (Yoshida & Toyoda 2000).

Few studies have investigated the potential for site discrimination of ¹⁵N within N₂O by distinct species of nitrifying versus denitrifying bacteria (Barford et al.
1999; Garber & Hollocher 1982a,b; Webster & Hopkins 1996; Weeg-Aerssens et al. 1988; Yoshida 1988). Furthermore, these studies used model organisms that are not necessarily reflective of the types of bacteria that are most active in the environment (Kowalchuk et al. 1997). In addition to direct studies of model organisms, another way to approach this question is by analyzing differences in enzyme structure among different bacterial groups to predict how $^{15}$N site-preference might occur. Both nitrifiers and denitrifiers directly produce N$_2$O through the presumed activity of at least two enzymes: nitrite reductase, which reduces NO$_3^-$ to NO, and nitric oxide reductase, which reduces NO to N$_2$O (Richardson & Watmough 1999). We will limit our discussion to these two groups of enzymes because they are directly related to N$_2$O production.

Nitrite Reductase

Nitrite reductase enzymes are fairly widespread throughout the bacterial kingdom due to the high usage of denitrification as an energy-generating process and for nitrogen assimilation (Brittain et al. 1992). There are two known types of dissimilatory nitrite reductases that reduce NO$_3^-$ to NO, and one known assimilatory enzyme that reduces NO$_3^-$ to NH$_3$ (Richardson & Watmough 1999). Of the dissimilatory enzymes, the cd$_1$-cytochrome nitrite reductase is more highly represented in the denitrifying bacteria, whereas the copper nitrite reductase is present in more taxonomically diverse species (Coyne et al. 1989, Hochstein & Tomlinson 1988). Both types of nitrite reductase have been found in the genome of the methanotroph Methylomonas sp. strain 16a, the only dual occurrence known, but only the copper enzyme has been found in the chemolithotrophic ammonia-oxidizers (Casciotti & Ward 2001, Ye & Thomas 2001). Based on physiological studies, it remains inconclusive as to whether the nitrite reductase in ammonia oxidizers is actually responsible for dissimilatory nitrite reduction; however, studies are currently underway to resolve this issue (Beaumont et al. 2002).

Figure 5 compares the catalytic mechanism of NO$_3^-$ reduction by the cd$_1$-cytochrome enzyme (Figure 5a) and the copper enzyme (Figure 5b) (Cutruzzolà 1999). Although these mechanisms are largely similar, the intermediate steps may be different enough in their relative rate of catalysis to cause differences in isotopic fractionation. NO is a highly toxic product, it inhibits nitrite reductase, and it is shuttled almost instantaneously to nitric oxide reductase upon formation (Averill 1996). In fact, there was a long-standing debate as to whether NO was an independent product from nitrite reductase because it is transformed to N$_2$O so rapidly under physiological conditions (Zumft 1993). Thus, the rate of NO formation by nitrite reductase and the rate of NO transfer to nitric oxide reductase will largely govern the fractionation of isotopes within N$_2$O molecules.

Nitric Oxide Reductase

Nitric oxide reductase (NOR) is likely the pivotal enzyme that determines the relative fractionation and site preference of $^{15}$N within the N$_2$O molecule. It is
Figure 5  Catalytic mechanism of nitrite reductase—cytochrome cd$_{1}$ (a) versus copper (b) active-site. Redrawn from Cutruzzola (1999).

also the least well-characterized enzyme within the microbial nitrogen cycle. This enzyme is responsible for bringing two NO molecules together and forming the N=N bond (Hendriks et al. 2000). There are three known classes of NOR enzymes: P-450nor, cNOR, and qNOR. P-450nor is found in denitrifying fungi, cNOR is the most common enzyme found in denitrifying bacteria, and qNOR is most often found in non-denitrifying pathogenic bacteria (Hendriks et al. 2000). Studies of P-450nor, the best-characterized enzyme in this family, have indicated that each NO molecule binds separately to the enzyme before the formation of the N=N bond (Shiro et al. 1995). Sequential binding could hypothetically lead to a preference for initial $^{15}$NO binding followed by a slower, less-discriminatory binding of the second NO molecule (Figure 6b). Once the second NO binds, this leads to the loss of the O atom from the first NO molecule and release of N$_2$O (Shiro et al. 1995). This model would lead to greater fractionation at the N$^\beta$ position than the N$^\alpha$ position (in N$^\beta$N$^\alpha$O), contradicting the assumption proffered by Yoshida & Toyoda and leading to a higher emission of $^{14}$N$^{15}$NO than $^{15}$N$^{14}$NO from the microbial environment.

Figure 6  Simultaneous (a) versus sequential (b) binding of NO to NOR. Formation of predominant N$_2$O isotopologue(s) indicated for each mechanism: Simultaneous binding leads to equivalent fractionation at N$^\alpha$ and N$^\beta$; sequential binding leads to greater fractionation at N$^\beta$. 
The catalytic mechanism of cNOR remains unclear. There are two possible mechanisms for NO binding and reduction to N\textsubscript{2}O; one requires sequential binding of the two NO molecules similarly to that of the P-450nor (Zhao et al. 1995), whereas the other suggests a simultaneous binding of the two NO molecules (Figure 6) (Averill 1996). As illustrated in Figure 6, sequential binding would result in accumulation of $^{14}$N at the N$^6$ position, assuming a slower rate of binding by the second NO molecule (and thus with less bias toward the lighter molecule) and loss of the O atom by the first NO molecule to bind. Simultaneous binding would likely result in limited site-preference due to equal positioning of the molecules for loss of the O atom and release of N\textsubscript{2}O from the enzyme. Currently, there is scant evidence for sequential binding of NO molecules, but the finding of intramolecular site-preference for $^{15}$N in N\textsubscript{2}O emitted from fertilized soils suggests that this mechanism does occur (Pérez et al. 2001).

The qNOR enzyme, normally associated with detoxification of NO by pathogenic bacteria, has recently been identified in the genome of *Methylomonas* sp. strain 16a (Ye & Thomas 2001). It is unknown whether qNOR or cNOR is the dominant enzyme in other methanotrophs or chemolithotrophic ammonia-oxidizers, as very little information is available. However, the dominance of qNOR in these bacteria would indicate that their denitrification pathway is present for detoxification rather than for energy generation. Detoxification would likely lead to a greater extent of isotopic fractionation because energy would not have to be conserved at each step in the enzymatic pathway, as is the case for denitrification. Differences in the catalytic mechanism of cNOR and qNOR are unknown, but the different physiological role of these two enzymes in denitrifiers versus nitrifiers may be pivotal in generating isotopic site-preferences.

Obviously, much more work is required to understand how the structure and function of different NOR enzymes within nitrifiers versus denitrifiers may influence the fractionation and site preference of $^{15}$N within N\textsubscript{2}O molecules. However, if the general enzymatic mechanisms are equivalent among organisms, there would be little difference in enrichment of $^{15}$N at either the central or terminal N positions. In this case, only the kinetics and consumption of N\textsubscript{2}O as influenced by environmental conditions would discriminate between the pathways.

**INFLUENCE OF THE ENVIRONMENT ON NITROGEN TRANSFORMING PATHWAYS**

The status of the environment plays an extremely important role along with organism and enzymatic diversity in both the production and amount of isotopic fractionation of N\textsubscript{2}O. Among the dominating environmental factors that influence nitrification and denitrification are oxygen availability, which is determined largely by soil moisture; organic carbon versus nitrogen availability; temperature; and pH.

So far, we have divided N\textsubscript{2}O production by oxygen availability—nitrification dominates the process in aerobic environments and denitrification dominates in...
GLOBAL BUDGET OF NITROUS OXIDE

anaerobic environments. However, there appears to be a continuum in the classes of organisms that lead to N$_2$O production in particular regions. For example, heterotrophic ammonia-oxidizers express denitrifying enzymes with high activity under aerobic conditions (Anderson et al. 1993, Bell & Ferguson 1991). Denitrification activity in chemolithotrophic ammonia-oxidizers is highest at reduced O$_2$ tensions (Goreau et al. 1980). The expression of denitrifying enzymes in denitrifying bacteria is only detectable in anaerobically grown cells (Coyne et al. 1990). Based on O$_2$ availability alone, we now have a situation where different organisms actively denitrify in different regions and, perhaps, at different times. For example, in dryer soils, chemolithotrophic ammonia-oxidizers appear to produce more NO than N$_2$O, whereas in wet soils, the bulk of N$_2$O is produced by denitrifiers (Davidson 1993). This situation has been tested by measuring changes in the isotopic composition of emitted N$_2$O from dry versus wet soils, an indication that the N$_2$O isotopologue pool changes depending on the strength of nitrifying or denitrifying activities (P´erez et al. 2001).

In addition to O$_2$ availability, the activities of microbes are also controlled by the availability of nutrients for energy generation. In nitrifying communities, the metabolic activity and population of chemolithotrophic versus heterotrophic ammonia-oxidizers are highly controlled by the carbon to nitrogen ratio (C/N). Chemolithotrophic metabolism dominates in low C/N environments, whereas high C/N stimulates heterotrophic ammonia oxidation (Kuenen & Robertson 1994). Thus, N$_2$O production in the aerobic to suboxic environment may be controlled by carbon and nitrogen as well as by oxygen availability due to the growth of one population over another. In denitrifying environments, organic carbon and nitrate availability determine whether denitrifiers produce mostly N$_2$ or N$_2$O. To further complicate matters, some intermediates in the nitrogen cycle, such as NO$_2^-$ and NO, are highly inhibitory to nitrogen-transforming enzymes, which will influence the population composition and metabolic activity of the community (Averill 1996, Stein & Arp 1998). Thus, a buildup of NO$_2^-$ from ammonia oxidation, which occurs in newly fertilized agricultural fields, may stimulate N$_2$O production by nitrifiers while inhibiting N$_2$O production by denitrifiers.

Temperature plays an important but poorly understood role in regulating the flux of N$_2$O from the environment. In soils, increasing temperatures generally increase the rates of biological activity (Radmer & Kok 1979). This is potentially an extremely important effect because as the mean temperature of Earth increases due to global warming, more greenhouse gases will be released, possibly causing a run-away greenhouse effect (Crutzen 1983). Few studies have shown whether rates of denitrification change in different climatic regions or with imposed changes in temperature, and contradictory results have been described (Powlson et al. 1988, Saad & Conrad 1993). More work is necessary to determine whether temperature is a strong factor in predicting N$_2$O emissions from the environment.

The pH of the environment may also have an effect on the production of N$_2$O because NO$_2^-$ chemically decomposes to NO$_2$ and NO under acidic conditions (Reuss & Smith 1965). The activity of chemolithotrophic ammonia-oxidizers
produces large amounts of acidity, which will influence the pH of their immediate surroundings (Wood 1986). Studies on the effects of pH on the release of N₂O from soils have indicated that multiple complex interactions that are difficult to interpret occur between organisms and enzyme populations (Nägele & Conrad 1990). No simple correlations have been found between pH changes and their effects on N₂O production.

Combinations of environmental, population, and enzymatic effects make quantification of N₂O production by specific bacterial groups very complicated in real-world systems. We have provided a brief overview of how interactions among all of these factors work together to produce a pool of N₂O that is likely a combination of isotopic signatures with bias toward either nitrifying or denitrifying processes. Environments that are heavily influenced by a few parameters, such as recently fertilized or irrigated agricultural fields, often have a very strong bias from either nitrifying or denitrifying processes that can be discerned by relative quantities of specific N₂O isotopomers and isotopologues (Pérez et al. 2001).

The current challenge for quantifying the global budget of N₂O is to correlate the biological sources described above with sinks from chemical reactions in the atmosphere. The remaining portion of this review discusses the predominant sink of N₂O in the atmosphere, i.e., preferential photolysis of N₂O isotopomers. Additionally, we present how direct measurements of the isotopic composition of N₂O from both the biosphere and the stratosphere are furthering the goal of closing the N₂O budget.

**FATE OF N₂O IN THE ATMOSPHERE AND ISOTOPIC FRACTIONATION**

Once N₂O is produced and released into the environment, it is removed from the atmosphere by photodissociation, which accounts for 90% of the total N₂O sink,

\[ \text{N}_2\text{O} + h\nu \rightarrow \text{N}_2 + \text{O}(^{1}\text{D}). \]  

(1)

The rest is lost via the photo-oxidation reaction with O(^{1}\text{D}), an excited state of the O atom derived from photolysis of O₃,

\[ \text{N}_2\text{O} + \text{O}(^{1}\text{D}) \rightarrow 2\text{NO} \]  

\[ \rightarrow \text{N}_2 + \text{O}_2 \]  

(2a)

(2b)

Both Reactions 1 and 2 primarily take place in the stratosphere, resulting in a mean lifetime of over 100 years for N₂O. Reaction 2a is the major source of odd nitrogen (NOₓ) to the stratosphere and plays a fundamental role in regulating the ozone layer (e.g., Logan et al. 1978, Wennberg et al. 1994). Figure 7 shows the distribution of N₂O in the atmosphere for four representative months computed by the Caltech/JPL two-dimensional (2-D) model of the terrestrial atmosphere (Yung & Miller 1997). The surface concentration was set to 300 ppb. N₂O is well mixed
Figure 7  Mixing ratio of N$_2$O in the atmosphere computed by the Caltech/JPL 2-D model of the terrestrial atmosphere: (a) January, (b) April, (c) July, and (d) October. The units are ppb. The surface mixing of N$_2$O is 300 ppb in the model. The pressure (P) in hPa refers to the left axis. The altitude (Z) is defined by $Z = 6.948712 \text{ km} \times \log_{10}(1000/P)$ and can be regarded as an approximate altitude.

in the troposphere because of its long lifetime. The decrease in mixing ratio in the stratosphere is the result of the aforementioned reactions, and is borne out by measurements (e.g., Gunson et al. 1990). The bulge in the contours in the tropics is due to the upwelling of tropospheric air that is rich in N$_2$O. The low mixing ratios in the polar stratosphere are due to the downwelling of upper stratospheric air that is depleted in N$_2$O. The exchange of air between the troposphere and the stratosphere is known as the Brewer-Dobson circulation (Holton et al. 1995). This circulation is strongest in the winter hemisphere, and its effects on N$_2$O are evident in the north (south) polar stratosphere in January (July). The model results shown in Figure 7 are consistent with measurements obtained by the Upper Atmosphere Research Satellite (UARS) (Kumer et al. 1993). Version 9 of the UARS data for N$_2$O is currently available at the Goddard DAAC for the period October 25, 1991 to May 5, 1993 from 20 to 50 km. For details, refer to the website http://www.lmsal.com/9120/CLAES/.

Following the discovery of the N$_2$O isotopic anomaly by Kim & Craig (1993), Johnston et al. (1995) attempted to verify the suggestion that stratospheric
chemistry results in N₂O isotopic fractionation. Yung & Miller (1997) proposed a wavelength-dependent mechanism for the photolytic fractionation of N₂O based on subtle shifts in the zero point energy with isotopic substitution. For example, substituting ¹⁸O for ¹⁶O results in a calculated zero point energy blue shift for the heavier isotopologue of \(-27.5 \text{ cm}^{-1}\). Analogous to determining the kinetic fractionation for a chemical reaction, the photolytic fractionation factor will be equal to the ratio of the heavy-to-light photolysis rates and thus to the ratio of the cross sections. For N₂O, the theoretical enrichment factors can be calculated analytically as a function of wavelength using the spectral function of Selwyn et al. (1977) and the Yung & Miller (1997) model was able to match, at least qualitatively, the observed enrichments of both ¹⁵N and ¹⁸O.

Photolysis experiments investigating the validity of the Yung & Miller (1997) theory were performed by several different research teams (Rahn et al. 1998, Röckmann et al. 2000, Turatti et al. 2000, Zhang et al. 2000). The data are clearly consistent with a simple Rayleigh fractionation model, and the trend of the heavy isotope enrichment with wavelength was consistent with that predicted by Yung & Miller (1997). It was observed that enrichments of ¹⁵N to ¹⁸O yields ratios were slightly greater than unity at wavelengths close to the effective, or mean, stratospheric photolysis wavelength of 205 nm. This is nearly identical to the ratio of the enrichments observed in Rahn & Wahlen (1997) and close to that predicted in Figure 2 of Yung & Miller (1997); it supports the hypothesis that photolysis is the principal mechanism responsible for the observed stratospheric enrichments and that the standard model of stratospheric N₂O chemistry is essentially complete. Turatti et al. (2000) and Zhang et al. (2000) observed that the fractionation rate for the ¹⁴N¹⁵N¹⁶O isotopomer was greater than the rate for the ¹⁵N¹⁴N¹⁶O, as predicted by the Yung & Miller (1997) hypothesis. However, the magnitudes of the experimentally determined enrichments were all found to be much greater than that predicted by YM97 Yung & Miller (1997). For example, the ¹⁸O fractionation at 207.6 nm was found to be \(-46.0\) per mil, more than twice that predicted by Yung & Miller (1997).

Theoretical efforts to extend photodestruction-induced isotopic fractionation to include other aspects of photolysis have been done by Johnson et al. (2001) and Blake et al. (2002). Factors other than ZPE that affect isotopic fractionation include the contribution of vibrationally excited N₂O in the ground electronic state, the transition dipole moment, and the shape of the ground-state wavefunction. Because the electronically excited state of N₂O is bent, vibrational excitation of N₂O in the ground electronic state leads to better Franck-Condon overlap and a larger contribution to the overall absorption profile. This would lead to a temperature dependence on isotopomer fractionation. Changes in the vibrational wavefunction causes the absorption profile to narrow, especially for N₂O in the 200–215-nm region, the critical region for photolysis in the stratosphere. Johnson et al. (2001), using time-dependent Hermite propagation, can reproduce the laboratory results of enrichment for all of the isotopomers with the exception of the ¹⁵N¹⁴N¹⁶O isotopomer. Blake et al. (2002) have taken a simpler, semi-empirical approach to
the theoretical calculation of enrichment factors, and the method has been able to reproduce stratospheric observations of N₂O fractionation in the stratosphere. Figure 8 shows a comparison for the absorption cross-section for the most abundant isotopologue $^{14}$N$^{14}$N$^{16}$O (solid line) and the minor isotopologue $^{14}$N$^{15}$N$^{16}$O (dashed line). The heavier isotopomer has a slightly smaller cross-section, resulting in less destruction by photolysis and its enrichment relative to the main isotopologue.

Although Reaction 2 accounts for only 10% of the overall destruction of N₂O in the atmosphere (Reaction 1 is the primary sink), in the lower stratosphere (below 22 km) it is actually more important than photolysis. The fractionation in this reaction has recently been measured by Kaiser & Brenninkmeijer (2002), and can be expressed as:

$$
\alpha = \frac{k_5 \text{ (isotopologue)}}{k_5 \text{ (standard)}}.
$$

The laboratory measurements give $\alpha_{556} = -1$, $\alpha_{546} = -1$, and $\alpha_{448} = -1$ as $-8.87$, $-2.22$, and $-12.38$ per mil, respectively, where pqrs is a shorthand for an isotopologues of N₂O, $^{1}$r$^{14}$N$^{16}$O.

The isotopic fractionation due to Reactions 1–3 were incorporated in a recent study using the Caltech/JPL 2-D model (Morgan et al. 2002). The results for January are shown in Figure 9a–d for $\delta^{18}$O, $\delta^{17}$O, $\delta^{15}$N$^\alpha$, and $\delta^{15}$N$^\beta$, respectively. There is little fractionation in the troposphere. The bulk of the isotopic fractionation takes place in the stratosphere and the $\delta$ values can reach as high as 100 per mil in the upper stratosphere. Model results above 40 km must be taken with caution, as the mixing ratio of N₂O rapidly approaches zero (see Figure 7). Note that the destruction of N₂O in the stratosphere results in the enrichment of the heavier
isotopologues. Therefore, the stratosphere is a source of isotopically heavy N\textsubscript{2}O, as first pointed out in the insightful analysis of Kim & Craig (1993). The globally and seasonally averaged fractionation due to atmospheric chemistry for $\delta$\textsuperscript{18}O, $\delta$\textsuperscript{17}O, $\delta$\textsuperscript{15}N\textsubscript{α}, and $\delta$\textsuperscript{15}N\textsubscript{β} are 14.5, 6.4, 20.0, and 9.8, respectively. If we do not distinguish between $\delta$\textsuperscript{15}N\textsubscript{α} and $\delta$\textsuperscript{15}N\textsubscript{β} (as in a mass spectrometer), we can define a mean $\delta$\textsuperscript{15}N = ($\delta$\textsuperscript{15}N\textsubscript{α} + $\delta$\textsuperscript{15}N\textsubscript{β})/2 = 14.9. The quantitative determination of these fractionation values will now allow an estimation of the mean isotopic composition of the sources of N\textsubscript{2}O.

MEASURING N\textsubscript{2}O ISOTOPOLOGUES AND ISOTOPOMERS TO QUANTIFY INDIVIDUAL SOURCES AND SINKS OF N\textsubscript{2}O

Given the large uncertainties in estimates for sources of N\textsubscript{2}O, as shown in Table 1, it is natural to ask whether the isotopic composition of the sources can pose useful constraints. As pointed out earlier, the combination of quantifying the isotopic composition of atmospheric N\textsubscript{2}O along with the modeling of isotopic fractionation due to reactions in the atmosphere allows us to estimate the isotopic composition of the source. The results are summarized in Figure 10, in which the mean atmospheric
isotopic composition is given by the square and the mean source is given by the dark dot. The source must be lighter than the atmosphere because of the enrichment of the heavy isotopes in the destruction processes in the stratosphere. Thus, the best estimate for the average isotopic composition of N$_2$O is given by $\delta^{18}\text{O} = 7$ per mil and $\delta^{15}\text{N} = -7.5$ per mil. Referring to Figure 4, the above results imply that the ocean could not be a major source of N$_2$O. Additional information is available if we distinguish between $\delta^{15}\text{N}^\alpha$ and $\delta^{15}\text{N}^\beta$. Figure 11 summarizes recent measurements of $\delta^{15}\text{N}^\alpha$ and $\delta^{15}\text{N}^\beta$.

![Graph showing isotopic composition of N$_2$O](image)

**Figure 10** Isotopic composition of N$_2$O: Square = average of atmospheric composition, dot = average of all sources.

![Graph showing isotopic composition of positional N$_2$O](image)

**Figure 11** Isotopic composition of positional N$_2$O in the troposphere, stratosphere, soil, and ocean. • from Yoshinari et al. 1997; ◦, × from Yoshida & Toyoda 2000; and + from Pérez et al. 2001.
Figure 12 Isotopic composition of positional N₂O:
Square = average of atmospheric composition, dot = average of all sources.

The average atmospheric and the implied source composition are shown in Figure 12. The characteristic values of $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ should provide powerful further constraints on the sources. For example, the average source must have a small positive $\delta^{15}N^{\alpha}$ but a large negative $\delta^{15}N^{\beta}$. Isotopic measurements of N₂O from fertilized agricultural soils are consistent with this constraint (Pérez et al. 2001). In a perceptive analysis, Rahn & Wahlen (2000) pointed out that the isotopic budget evolved with time, implying that N₂O is generated from a biological source. Their simple model predicted a decrease in $\delta^{18}O$ and $\delta^{15}N$ of about 1.6 and 1.8 per mil, respectively, since the Industrial Revolution. Preliminary results from ice cores appear to support this simple model (T. Röckmann, unpublished data).

CONCLUDING REMARKS

Judging from the current datasets for global sources of N₂O (Table 1), biological processes contribute by far the most N₂O to the atmosphere relative to purely industrial sources. Additions of nitrogen to the environment, especially in agricultural and wet soils, greatly stimulate the microbial nitrogen cycle leading to large-scale production and emission of nitrogen oxide intermediates (based on gas production per unit area). This review has presented the current understanding of how microorganisms produce N₂O and the various biological and environmental factors that control nitrogen cycling processes. We are now closer than ever to understanding how different microorganisms produce different ratios of N₂O isotopologues and isotopomers. It is also evident that further study must be accomplished from enzymological to landscape ecology scales to fully appreciate and quantify discrete sources of N₂O.
A most-powerful method for characterizing the sources of N₂O lies in its multi-isotope signature. It is now known that (a) the major land biospheric sources of N₂O are light in both ¹⁵N and ¹⁸O, relative to tropospheric N₂O; (b) stratospheric loss of N₂O results in enriching both ¹⁵N and ¹⁸O; and (c) there is a large “back flux” of heavy N₂O from the stratosphere to the troposphere. Therefore, if we could compute (c) accurately in an atmospheric model, we could have a constraint on the isotopic composition of the biological source. By assigning production of specific isotopomers and isotopologes of N₂O to different microbial groups, and by quantifying the rates of photolysis of specific N₂O pools, we are determining the conditions for N₂O production in the biosphere and tracing its fate in the atmosphere.

The combination of knowledge from both biological and atmospheric sciences is rapidly leading to a tightened global budget for N₂O. The information from this collaboration will greatly improve international efforts to stem the continued accumulation N₂O to the atmosphere by expanding and encompassing the scientific view from molecular to global levels.

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