Identifying the agricultural imprint on the global N\textsubscript{2}O budget using stable isotopes


Abstract. Agricultural soils are the most important anthropogenic source of nitrous oxide to the atmosphere. We observed large shifts with time in the emission rate (from 170 to 16 ng N cm\textsuperscript{-2} h\textsuperscript{-1}) and in $^{15}$N of N\textsubscript{2}O emitted (from -46% to +5% relative to atmospheric N\textsubscript{2}O) from a urea-fertilized and irrigated agricultural field in Mexico. We calculated overall instantaneous enrichment factors for the sampling period, which suggest that the microbial N\textsubscript{2}O production shifts from nitrification (week 1) to denitrification (week 2). Isotopic signatures of N\textsubscript{2}O emissions were not always in accord with other proxies (such as NO/N\textsubscript{2}O emission ratio or water-filled pore space) used to estimate the relative importance of nitrification and denitrification as sources of N\textsubscript{2}O. These observations strongly suggest that the soil surface emissions integrate processes occurring at different depths in the soil and a decoupling of NO and N\textsubscript{2}O production in this system. Further clues as to the source of N\textsubscript{2}O come from the positional dependence of $^{15}$N in the emitted N\textsubscript{2}O reported here for the first time in soil emissions. Enrichment at the central N position increased relative to the terminal N position by 9.3% during the first 4 days after irrigation, implying that nitrification preferentially enriches the central N position compared to denitrification. The overall $\delta^{15}$N signature measured for N\textsubscript{2}O emitted from N-fertilized agricultural systems is more depleted than observed $\delta^{15}$N values for N\textsubscript{2}O emitted from more N-limited forest soils. Assuming that one half of the total agricultural N\textsubscript{2}O emissions associated with the global increase in soil-nitrogen fertilizer use have an isotopic composition comparable to those of the agricultural fields reported here, we predict a decline in the isotopic signature of tropospheric N\textsubscript{2}O during this century of as much as 3% for $^{15}$N. Although many uncertainties remain, we suggest that measurements of $\delta^{15}$N-N\textsubscript{2}O in firm air will provide constraints on how the N\textsubscript{2}O budget has changed during the past century.

1. Introduction

Nitrous oxide (N\textsubscript{2}O) is a greenhouse gas primarily produced by bacteria in soils and oceans during the processes of nitrification and denitrification. The principal global N\textsubscript{2}O sources are tropical rain forest soils, agricultural fields, and oceans, whereas the major sink is stratospheric destruction [Khali and Rasmussen, 1992]. Most of the observed increase of N\textsubscript{2}O in the troposphere (~0.25% per year) has been attributed to increased N\textsubscript{2}O emissions associated with the expansion of agriculture since ~1900 [Kroeze et al., 1999; Machida and Nakazawa, 1995; Minami, 1987]. Attempts to balance the global N\textsubscript{2}O budget have been hampered by the limited number of emission studies coupled with the high spatial and temporal variability associated with N\textsubscript{2}O fluxes [Cicerone, 1989; Prather et al., 1995].

Recent publications suggest that the use of stable isotopes of N and O in atmospheric N\textsubscript{2}O and its sources may better constrain the global N\textsubscript{2}O budget [Chiff and Thiemens, 1997; Dore et al., 1998; Kim and Craig, 1993; Naqvi et al., 1998; Rahn and Wahlen, 1997; Yoshinari et al., 1997; Yung and Miller, 1997]. A very simplified interpretation of the global isotopic budget for N\textsubscript{2}O assumes that “light” (or $^{14}$N-depleted) N\textsubscript{2}O from sources such as soils and the ocean surface are balanced by “heavy” (or $^{15}$N-enriched) N\textsubscript{2}O that mixes down from the stratosphere [Kim and Craig, 1993]. However, significant uncertainties remain in estimating the global isotopic signature of both oceanic and soil sources because of the paucity of measurements. Isotopic signatures fall victim to the same problem that is notorious for flux measurements: large spatial and temporal variations. Most previous studies have determined isotope signatures from measurements made at a single place or time [Dore et al., 1998; Kim and Craig, 1993; Naqvi et al., 1998; Pérez et al., 2000; Yoshinari et al., 1997]. In this paper we examine the causes of short-term temporal variation in isotopic signatures within an agricultural system and explore the implications of these spatial and temporal variations for the use of isotopes as a tool to identify the relative contributions of different microbial pathways for N\textsubscript{2}O production and the changing importance of agriculture in the global N\textsubscript{2}O budget over time.

2. Field Study and Site Characteristics

Field studies were performed following experimental fertilization and irrigation of an agricultural field in the Yaqui Val-
Table 1. Soil Physical and Chemical Properties at Different Soil Depths During the Experiment

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>pH H₂O</th>
<th>CEC (NH₄Ac) cmol.kg⁻¹</th>
<th>Clay %</th>
<th>Silt %</th>
<th>Sand %</th>
<th>Soil Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15</td>
<td>8.5</td>
<td>41.10</td>
<td>48.5</td>
<td>20.3</td>
<td>31.2</td>
<td>clay</td>
</tr>
<tr>
<td>15-30</td>
<td>8.6</td>
<td>41.82</td>
<td>51.2</td>
<td>15.0</td>
<td>33.8</td>
<td>clay</td>
</tr>
<tr>
<td>30-60</td>
<td>8.3</td>
<td>44.76</td>
<td>48.7</td>
<td>17.5</td>
<td>33.8</td>
<td>clay</td>
</tr>
<tr>
<td>60-90</td>
<td>8.0</td>
<td>51.01</td>
<td>44.9</td>
<td>28.9</td>
<td>26.2</td>
<td>clay</td>
</tr>
</tbody>
</table>

CEC is the cation exchange capacity of the soil.

ley of Sonora, Mexico, during November 1998. This area is part of the Sonora desert and has a long history of agricultural use. The Yaqui Valley area (40 m above sea level) has 225,000 ha of cultivated and irrigated land located from 26°45'N to 27°33'N and 109°30'W to 110°37'W. The mean annual precipitation is 292 mm with highest precipitation during late summer (J. I. Ortiz-Monasterio, personal communication, 1998). The soils in the Yaqui Valley are classified as typical calcisols (U.S. system). They are a combination of coarse sandy clay and montmorillonitic clay. Soil properties are given in Table 1. The Yaqui Valley has been the location of a number of studies on genetic progress in wheat grain yield and quality and nitrogen use efficiency under different nitrogen fertilization rates as well as the effect of nitrogen management on greenhouse emissions and nitrogen leaching [Graham et al., 1997; Matson et al., 1998; Ortiz-Monasterio et al., 1997a,b; Riley et al., 2001].

The typical sequence of events associated with wheat agriculture in this region begins with the burning of plant residues (when present) from the previous crop in October. In November, a first fertilizer application of 150 to 190 kg N ha⁻¹ is applied as urea (broadcast) or anhydrous ammonia (injected). The fertilizer is incorporated with a disk before the formation of beds where planting will take place. A few days after bed formation, the field is furrow-irrigated and the soils are left to drain for a period of 2 to 4 weeks, after which planting takes place. A second, smaller fertilizer application (63 to 100 kg N ha⁻¹) occurs with the first so-called "riego de auxilio" (postplanting irrigation) 6 weeks after planting. This last procedure completes the total annual fertilizer application of ~250 kg N ha⁻¹. The crop is irrigated for 5 to five times more before the fields are harvested in April-May. Previous studies of gaseous N loss from these fields [Watson et al., 1998; Panek et al., 2000; Riley et al., 2001] show that the largest losses of N₂O for the entire planting cycle occur during the irrigation period following the first fertilization. We selected this time period for our study. Overall losses of fertilizer nitrogen from fertilization to harvest (as N₂, N₂O, NO, NH₃ volatilization, and NO₃ leaching) can be as high as 28% [Matson et al., 1998].

3. Fertilization and Sampling Procedure

3.1. Fertilization Experiment

The study site was at Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) experimental fields and focused on changes following first fertilizer application. The field was fertilized with 150 kg N ha⁻¹ (15 g N m⁻²) as broadcast urea on November 3, 1998, and incorporated to ~20 cm to make beds that were ~50 cm wide. Furrows were ~15 cm wide each and were spaced 80 cm apart (Figure 1). Irrigation took place on November 5.

3.2. Soil Measurements

32.1. Soil water content and inorganic nitrogen concentrations. During the 2-week period of the experiment starting from the day of irrigation, we sampled soils each day to measure the water content, the ¹⁸O composition of soil H₂O, total soil carbon and nitrogen content, the N H₂ and NO₃ concep-
Figure 2. Parameters measured during a 15-day period following urea fertilization and irrigation: (a) δ¹⁵N in N₂O; (b) N₂O and NO emissions; (c) δ¹⁵N in NH₄⁺ and NO₃⁻; (d) NH₄⁺, NO₃⁻; total nitrogen abundance, and an estimate of initial urea-derived NH₄⁺ concentration (assuming that all urea applied was hydrolyzed and converted to NH₄⁺); (e) water content (0-5 cm); (f) δ¹⁸O in N₂O and H₂O; and (g) δ¹⁵N¹⁴NO - δ¹⁵N¹⁴NO.
traction in soils, and the isotopic composition of these nitrogenous species. Integrated soil samples were taken across the beds (4 cm long x 50 cm wide x 5 cm depth) so as to average the total nitrogen content per bed (see Figure 1). A previous study has shown that N concentrations vary significantly across the beds (J. I. Ortiz-Monasterio, personal communication, 1998). Soil samples were collected at the same time each day (1030 to 1130 local time). Soil temperatures were measured using a thermocouple at 3 cm depth.

Approximately 200 grams of homogenized soil were weighed and oven dried at 105°C for 48 hours. After that time the samples were weighed again and gravimetric water content was determined by weight loss. Bulk density values (0-15 cm) were obtained previously by collecting soil sample cores from 5 to 10 cm depth (J. I. Ortiz-Monasterio, personal communication, 1998). The values used are 1.1 g cm⁻³ (beds) and 1.21 g cm⁻³ (furrows). We extracted soluble N on the day of soil collection. An aliquot of 10 g of soil was added to 100 mL of 2 M KCl, shaken for 1 min, and left at room temperature to equilibrate for 24 hours [Matson et al., 1996]. The solution was filtered with a KCl prewashed Whatman 42 filter and stored at 4°C prior to analysis for NH₄⁺ and NO₃⁻ at the University of California Irvine (UCI). Concentrations of NH₄⁺ and NO₃⁻ were determined using the salicylate–hypochlorite and modified Griess–Illosvay methods, respectively [Mulvaney, 1996]. Reported NO₃⁻ concentrations are the sum of NO₃⁻ + NO₂⁻. Both ions were measured using a spectrophotometer (HACH DR/2010).

3.2. Total carbon and nitrogen content. Soil samples taken every day from 0-5 cm depth were dried at 60°C for 4 hours. Samples were sieved and milled, and total carbon and nitrogen content was determined by combustion using a Fisons 5200 elemental analyzer. Nitrogen content analyzed this way is the sum of organic and inorganic N. Measurements are reported in mg N kg⁻¹ dry soil.

3.2.3. The δ¹⁵N measurements in NO₃⁻ and NH₄⁺ and δ¹⁸O-H₂O from soil water. Natural abundance δ¹⁵N in the inorganic nitrogen pool (NH₄⁺ and NO₃⁻) was analyzed at R. Mulvaney's laboratory (University of Illinois, Urbana) using methods described elsewhere [Mulvaney, 1997]. Water soil extraction and δ¹⁸O and isotopic composition were determined by conventional methods [Allison et al., 1983; Socki et al., 1992].

3.3. Trace Gas Emissions and Isotopic Measurements

3.3.1. NO emissions. NO was measured using a dynamic chamber method [Davidson et al., 1993, Davidson et al., 1991]. A lid was placed on a PVC ring (25-cm diameter, 10-cm height) that was previously inserted 2 cm into the ground. A mixture of CO₂ free and dry ambient air (~680 mL min⁻¹) and soil chamber air (~100 mL min⁻¹) was passed through a CrO₂ converter that oxidized NO to NO₂. NO₂ was detected by chemiluminescence using a Scintrex LMA detector (Scintrex, Inc., Ontario, Canada) and using luminol solution as an oxidizer. Calibration curves were made each day prior to sampling NO soil emissions by diluting a NO standard (115.2 ppb NO in N₂, Scott Specialty gases) with different amounts of ambient air. In each case the calibration curve was corrected for background NO concentration present in the ambient air.

3.3.2. N₂O emissions and δ¹⁵N and δ¹⁸O-N₂O. N₂O emissions were determined by collection of four samples with 20 mL syringes at 10-min intervals after chamber closure and measured by electron capture detector (ECD) gas chromatography. The natural abundance δ¹⁵N and δ¹⁸O were collected after syringe sampling by circulation of air from the chamber through a trapping system. The N₂O was trapped using a molecular sieve 5Å trap, then transported to UCl for purification of N₂O and measurements of N₂O isotopes. A more detailed description of these methods is given elsewhere [Pérez et al., 2000].

3.3.3. The δ¹⁵N positioning of N₂O isotopomers. We determined the changes in δ¹⁵N positioning of N₂O isotopomers by Fourier transform infrared (FTIR) spectrometry [Estler et al., 2001] for the first four samples taken in this experiment. The technique required a relatively large amount of pure N₂O (7 µmol); only the high emissions of N₂O early in the experiment permitted collection of sufficient N₂O. The site position preference of nitrogen isotopomers in the N₂O molecules is expressed as the difference between the site-specific delta values for the two isotopic isotopomers (δ¹⁵N^1⁵N⁰ - δ¹⁵N^¹⁵N⁰) after Yoshiida and Toyoda [2000].

3.3.4. Isotope units. Isotopic data are reported as δ values, where δ= ([Rsample/Rstandard] -1 ) 1000, and Rsample and Rstandard are R =¹⁵N/¹⁴N or ¹⁸O/¹⁶O for sample and standard, respectively. Delta values are reported as deviations from δ¹⁵N of atmospheric N₂ and δ¹⁸O of atmospheric O₂. The conversion for the δ¹⁸Osm standard to SWMO standard is δ¹⁸Osm = [3× +δ¹⁸Osm SWMO/1.0235] [Kim and Craig, 1990].

4. Results

4.1. The δ¹⁵N in Emitted N₂O

During week 1 (starting from the day of irrigation) when N₂O emissions were the highest (Figure 2b), both δ¹⁵N and δ¹⁸O values were very light (depleted in the heavy isotope) (Figures 2a and 2b). The first two measurements immediately following irrigation showed heavier δ¹⁵N values (the average δ¹⁵N=N₂O value from two different chambers was -41.42 ± 0.93%) compared to the N₂O emitted 2 days after irrigation (-46.6%). Through the course of the experiment (from days 3 to 14 after irrigation) the N₂O emissions decreased and δ¹⁵N-N₂O increased, as did the δ¹⁵N signature of NH₄⁺.

4.2. N₂O and NO Fluxes

The instantaneous N₂O emissions, measured at the same time we collected samples for stable isotope analysis, ranged from 246 to 1.7 ng N cm⁻² h⁻¹. N₂O emissions were the highest the first 4 days after irrigation and then progressively decreased to very small values from day 5 to day 14 after irrigation (Figure 2b). NO instantaneous emissions were bimodal with a smaller peak during the first week (105 to 168 ng N cm⁻² h⁻¹ on the 3rd day after irrigation), which decreased at the end of week 1. During week 2 after irrigation, NO emissions increased again to the highest observed values during the middle of the 2nd week (209 to 283 ng N cm⁻² h⁻¹ on the 9th day after irrigation), and then diminished by the end of the 2nd week (Figure 2b).

4.3. Concentrations and Natural Abundance δ¹⁵N in Inorganic Nitrogen (NH₄⁺, NO₃⁻) and Total Carbon and Nitrogen Content

The δ¹⁵N values of NH₄⁺ extracted from 0- to 5-cm soil become enriched during the 1st week following irrigation (Fig-
This increase is due to the preference by the soil bacterial population for the lighter ($^4\text{N}$) isotope as the nitrogen pool is consumed [Nadelhoff and Fry, 1994]. The $^4\text{N}$-NH$_4^+$ values increase linearly with time ($r^2 = 0.89$), while NH$_4^+$ concentrations drop exponentially during the first 7 days after irrigation (from 255 to $\approx$16 mg N kg$^{-1}$ dry soil, Figure 2d). Both isotope and concentration values plateau during 2nd week, suggesting that a steady state condition with NH$_4^+$ production matching NH$_4^+$ loss rates has been reached.

The amount of KCl-extractable nitrate increased from 281 to 500 mg N kg$^{-1}$ dry soil (Figure 2d). The increase in NO$_3^-$ matches the decline in NH$_4^+$, and the $^4\text{N}$ signature of NO$_3^-$ is depleted compared to $^4\text{N}$-NH$_4^+$, suggesting that most of the NO$_3^-$ is produced from NH$_4^+$ via nitrification. The isotopic signature of $^{15}\text{N}$ in NO$_3^-$ during 1st week of the experiment is not available. During the 2nd week the $^{15}\text{N}$-NO$_3^-$ reached a maximum of 10.2‰ and then progressively decreased with time.

The total nitrogen content (organic plus inorganic) from 0-5 cm (Figure 2d) derives mostly from applied fertilizer N because these soils have a low organic matter content (organic C was $9.03 \pm 0.3$ g C kg$^{-1}$ soil, n=15). During the time we sampled, the sum of extractable N (NH$_4^+$ + NO$_3^-$) averaged 87% of the total N (organic + inorganic). No change in organic C content was observed during the experiment, although C inputs to the soil were zero. This indicates that organic matter decomposition is not a significant source of available nitrogen in these soils. We attribute the total N, NO$_3^-$ concentration and $^{15}\text{N}$-NO$_3^-$ decrease during the 2nd week after irrigation to NO$_3^-$ leaching to deeper layers in the soil. Riley et al. [2001] found leaching of NO$_3^-$ and NO$_2^-$ from the surface to 1 to 5 m depth accounted for between 5% and 28% of the applied nitrogen after a similar fertilization/irrigation procedure at this site.

4.4. Water Content and $^{18}\text{O}$ of Emitted N$_2$O and Soil H$_2$O

Water-filled pore space (WFPS) decreased from 0.86 to 0.46 in 15 days (Figure 2e). The $^{18}\text{O}$-H$_2$O values increased from 26 to 82%. During the same time, $^{18}\text{O}$-NO$_3^-$ increased from 3% to 9% (Figure 2f). The overall magnitude of the $^{18}\text{O}$-NO$_3^-$ increase was not as great as that observed for $^{15}\text{N}$-N$_2$O (Figure 2a).

4.5. The $^{15}\text{N}$ Positioning of N$_2$O Isotopomer

Changes in the $^{15}\text{N}/^{14}\text{N}$ ratio of N$_2$O emitted from the day of irrigation until 4 days later were accompanied by a significant change in the relative positional $^{15}\text{N}$ values in the N$_2$O molecules as shown in Figure 2g. Isotopomer site preference shifted 9.3% over the first 4 days after irrigation, in the sense that the N$_2$O molecules were heavier by 9.3% in the central $^{15}\text{N}$ relative to the terminal N$_2$O on the 4th day compared to the day of irrigation. The actual site preference ranged from +4.9% to +14.2% relative to the N$_2$O reference gas Standard Nitrous Oxide Working-gas (SNOW) [Rahn & Wahlen, 1997]. If we assume that SNOW has an absolute site preference close to zero, the absolute site preferences are within the range recently published by Yoshiida and Toyoda [2000] for soil and oceanic N$_2$O sources, -0.5% to +15%.

5. Discussion

5.1. Differentiation Between Nitrification and Denitrification as Sources of N$_2$O Using Stable Isotopes

Spatial and temporal variability in the $^{15}\text{N}$ of N$_2$O emitted from soils is caused by variations in substrate availability, the isotopic content of substrate, and shifts in microbial processes controlling N$_2$O production and consumption [Pérez et al., 2000]. The $^{15}\text{N}$ signature of N$_2$O emitted from the Yee Valley agricultural field (Figure 2a) showed dramatic shifts over time, ranging from highly depleted values (-46‰) during the 1st week when N$_2$O emissions were the highest, to enriched values (+5‰) at the end of the 2nd week when emissions were low.

N$_2$O is produced as a reaction byproduct or intermediate during nitrification (NH$_4^+$ $\rightarrow$ N$_2$O) and denitrification (NO$_3^-$ $\rightarrow$ N$_2$). Both processes produce N$_2$O molecules with distinct isotope signatures. The difference between $^{15}\text{N}$ of emitted N$_2$O and the substrate NH$_4^+$ or NO$_3^-$ is expressed as an enrichment factor $\varepsilon$, where $\varepsilon = 1000(\alpha - 1)$ and $\alpha$ is the isotopic fractionation factor of the reaction $R$(product)/$R$(substrate) = ($^{15}\text{N}/^{14}\text{N}_{\text{sample}}$)/($^{15}\text{N}/^{14}\text{N}_{\text{standard}}$) (published enrichment factors for nitrification (NH$_4^+$ $\rightarrow$ N$_2$O), $\varepsilon$ is from 45 to 66‰ [Ueda et al., 1999; Yoshiida, 1988]. N$_2$O produced via denitrification by soil denitrifiers has two characteristic enrichment factors reflecting the role of N$_2$O as an intermediate in this process: $\varepsilon$ of $-13$‰ to $-28$‰ for the N$_2$O to N$_2$ step [Barford et al., 1999; Wada and Ueda, 1996 and references therein] and $\varepsilon$ of $-13$‰ to $-27$‰ for the N$_2$O to N$_2$ step [Barford et al., 1999; Wada and Ueda, 1996]. Therefore, if the substrates (NH$_4^+$ or NO$_3^-$) have a $^{15}$N isotopic signature equal to 0‰, we expect to see differences in the isotopic signature of emitted N$_2$O, with nitrification producing N$_2$O that is more depleted in $^{15}$N (-45‰ to -66‰) and denitrification producing less $^{15}$N depleted values (-13‰ to -28‰).

With our data it is impossible for us to estimate quantitatively the relative contribution of nitrification versus denitrification during the sampling period because of the lack of information on the amount of N$_2$O reduced to N$_2$ (which would further enrich the $^{15}$N values of unconsumed N$_2$O; see Pérez et al. [2000] for discussion). However, we can qualitatively es-
timate which process predominates by comparing observed instantaneous enrichment factors, which integrate the influence of the whole bacterial community, with published values for the different processes that generate N₂O. We used measured δ¹⁵N values for NH₄⁺ and NO₃⁻ substrates (Figure 2c) and emitted N₂O (Figure 2a) to calculate instantaneous enrichment factors (e = δ¹⁵Nproducer/δ¹⁵Nsubstrate) [Goericke et al., 1994] for N₂O each day assuming that the entire N₂O production was either via nitrification (e_overall-nit = δ¹⁵N-N₂Oemitted − δ¹⁵N-NH₄⁺) or denitrification (e_overall-denit = δ¹⁵N-N₂Oemitted − δ¹⁵N-NO₃⁻) (Figure 3). We calculated e_overall-denit values for the 2nd week of the experiment only, because δ¹⁵N-NO₃⁻ values were not available during the 1st week.

During the 1st week following irrigation, the calculated e_overall-nit Values generally were within the range of published enrichment factors for nitrification (bottom shaded area in Figure 3). An important exception occurred on the day of irrigation, when the highest N₂O emission rates observed were associated with δ¹⁵N-N₂O values that were several per mil more enriched (+42%) than those measured on the subsequent days (-46 to -42%) (Figure 2a). This enrichment may have been caused by addition of some N₂O produced via denitrification. During the 2nd week, when N₂O fluxes decreased and much of the NH₄⁺ had been converted to NO₃⁻ (Figure 2d), instantaneous e_overall-nit values fell above the range of published e nit Values. In contrast, instantaneous e_overall-denit values were within the range of e denit in the literature during most of the 2nd week. Despite the fact that we cannot calculate values for e_overall-denit during the 1st week, these results suggest that most of the N₂O emission in the 1st week was derived from nitrification of abundant ammonium derived from the hydrolysis of urea. Once most of the NH₄⁺ was converted to NO₃⁻ (Figure 2d), denitrification increased in importance and overall N₂O emissions decreased. Enrichment factors on the final day of the experiment did not correspond to published ranges of e for either nitrification or denitrification; either N₂O was being produced outside of the range of published fractionation factors or another mechanism was controlling the isotopic signature of N₂O at that time.

5.2. Differentiation Between Nitrification and Denitrification as Sources of N₂O Using N₂O/NO Ratios and Soil Water Content

Soil water content regulates the redox condition in soils and hence controls the degree to which nitrification (an aerobic process) and denitrification (an anaerobic process) can occur. In addition, the NO and N₂O emitted from soils are generally assumed to be derived primarily from nitrification and denitrification, respectively. Hence N₂O/NO emission ratios <1 are usually observed when soils are mesic or dry (WFPS <0.65) and nitrification is the dominant process, while high N₂O/NO ratios indicate more anaerobic conditions at higher soil water content (WFPS >0.65) with denitrification the dominant process [Davidson, 1993]. Our N₂O and NO emission results (Figure 2b) show a dramatic decrease in the N₂O/NO ratios over the 2-week period following irrigation (values dropped from 42 on day 1 to 1.1 on day 3 and decreased to <0.57 during the 2nd week following irrigation). These results suggest that denitrification was an important source of N₂O only for the first 2 to 3 days following irrigation, while nitrification was the dominant source of N₂O after the 5th day. Soil conditions measured during the same time suggest anaerobic soil conditions continued through about day 5 (when 0-10 cm WFPS decreased to 0.6; at the end of the 2-week period it was 0.46; see Figure 2e). Both proxies suggest that the primary N₂O source shifted from denitrification in the first few days to nitrification following the 5th day after irrigation; the 2-day period between days 3 and 5 when WFPS is high but nitrification appears to be the dominant N₂O source could be explained if the first few centimeters of the soil dried more than the deeper layers. Panek et al. [2000] analyzed the same soils and fertilization procedure using an ¹⁵N labeling technique and found that the emitted N₂O was produced equally by denitrification and nitrification during the 1st week and nitrification during week 2. Our interpretation based on N₂O/NO ratios agrees with that of Panek et al. [2000].

5.3. Reconciling Stable Isotope Data With Other Proxies for Nitrification and Denitrification

Our interpretation of the processes responsible for N₂O emissions from soils during the 2 weeks following irrigation based on stable isotope data (section 5.1) clearly does not always agree with interpretations based on other proxies such as the N₂O/NO ratio and WFPS (see section 5.2). The isotope data suggest that nitrification is the most important source of N₂O during the 1st week following irrigation (with the exception of some denitrification the 1st day), followed by denitrification as the dominant N₂O source during week 2. In contrast, N₂O/NO ratios suggest that denitrification is as important a source of N₂O as nitrification during the first few days following irrigation, while nitrification dominates after about day 5. The apparent inconsistency of the interpretations based on stable isotopes and other indicators of nitrification and denitrification can be reconciled if (1) nitrification is taking place throughout the experiment at the soil-air interface where drying or equilibration allows aerobic microbial activity, while denitrification becomes increasingly important at depth in the soil where WFPS remains high; or (2) nitrification continues to be the most important process producing N₂O during the whole 2-week period, but the enrichment factors for nitrification increase with progressively limiting substrate availability (NH₄⁺).

The first explanation relies on vertical separation of nitrification and denitrification in the soil column. We suggest that nitrification is the major source of N₂O emitted during the first week following irrigation, in accord with the isotope measurements. Drying of the very top of the soil, or rapid equilibration with atmospheric O₂ at the air-soil interface, will allow aerobic conditions for nitrification to occur. Initial N₂O/NO emission ratios may be high even with nitrification occurring if the NO reacts with water before it can be emitted to the air above the soil [Firestone and Davidson, 1989]. The increase in NO emissions toward the middle of the 1st week occurs as the surface dries further; from day 3 to the end of the 1st week both isotopes and N₂O/NO emission ratios support nitrification as the major N₂O source, although WFPS (integrated over the top 10 cm of soil) remains high. Denitrification occurring at depth may contribute to, but does not dominate N₂O emissions the 1st week after irrigation, either because the NO₃⁻ substrate is increasing during this time or because denitrification may reduce N₂O to N₂ before it can be emitted [Panek et al., 2000].

During the 2nd week after irrigation, the isotope data suggest denitrification as the major source of N₂O, but NO emissions are the highest observed for the 2-week period. We sug-
gest that denitrification occurring deeper in the soil (below 10 cm), where WPFS remains high and where the necessary NO$_3^-$ substrate has leached from surface layers [Riley et al., 2001], is responsible for N$_2$O emissions, while NO emissions primarily derive from continued nitrification in surface layers. While Panek et al. [2000] suggested that nitrification was the dominant process producing N$_2$O during week 2, their labeling study was done at the soil surface and does not account for processes occurring deeper than 10 cm. We conclude that N$_2$O emitted during the 1st week after irrigation is mostly derived from nitrification and is produced near the soil surface, while N$_2$O emitted during the 2nd week derives from denitrification in deeper soil layers where anaerobic conditions prevail. During the 2nd week, sources of NO and N$_2$O are decoupled into different vertical layers of the soil.

The second plausible explanation for the N$_2$O isotope shift is that nitrification continues to be the most important process producing N$_2$O during the whole 2-week period following irrigation, but the N$_2$O produced from nitrification becomes more enriched with progressively limiting substrate availability (NH$_4^+$). We found excellent agreement comparing the variation in $\delta^{15}$N and $\delta^{18}$O of emitted N$_2$O with the fraction of NH$_4^+$ remaining in the soil during the 1st week, with the same observations obtained in a study done using a chemostat culture of ammonium oxidizing bacteria [Ueda et al., 1999]. We therefore postulate that nitrogen isotope enrichment fractionation of N$_2$O produced in soil systems with fertilizer-enhanced nitrogen pools is greater than for systems in which the substrate is at or near limiting levels. In other words, the product (N$_2$O) will always have the lightest $^{15}$N isotopic values when the amount of substrate (NH$_4^+$) is unlimited. On the other hand, when nitrogen availability is limited, our results suggest that overall isotope enrichment for nitrification is less negative. In this limiting case the N$_2$O isotopic composition is closer to the isotopic composition of its substrates.

Both hypotheses, the separation of nitrification and denitrification by depth and changes in the enrichment factor for nitrification depending on substrate availability, are plausible, and we cannot rule either out at this time. Changes in $^{15}$N enrichment of N$_2$O isotopomers can potentially reflect shifts in microbial metabolism that influence the N$_2$O emissions from the soil and might provide information for differentiating between the two hypotheses. The average enrichment of $\delta^{15}$N in N$_2$O produced on the irrigation day (-42‰) compared to the subsequent 3 days (-46 to -42‰) (Figures 2a and 3) is consistent with denitrification being a small contributor to N$_2$O emissions on the 1st day, when soil WPFS was very high (0.86). The site preference (central minus terminal $^{15}$N abundance) of the N$_2$O isotopomers on irrigation day (when denitrification is suggested by isotopic signature to contribute to N$_2$O production) was lower by ~9‰ than the value found 4 days later, when nitrification appears to be a more important contributor to N$_2$O production. This would imply that nitrifiers produce N$_2$O more enriched in $^{15}$N in the central position than do denitrifiers. Although very preliminary, this observation suggests that microbial processes have a distinct positional dependence in their $^{15}$N fractionation, which may provide a valuable new and independent isotopic marker for distinguishing the processes producing N$_2$O in soils. We were unable to collect sufficient N$_2$O to measure the isotopomers during the 2nd week after irrigation; such measurements would certainly help to distinguish between the two hypotheses and should be emphasized in the future.

Figure 4. Average emission-weighted N$_2$O isotopic signatures for all agricultural fields (open circle) and unfertilized tropical rain forest soils from Costa Rica (solid circle) [Pérez et al., 2000] and Brazil (open diamond) [Pérez et al., 2000]. The isotopic signatures for N$_2$O emitted from the surface ocean (solid diamond) [Dore et al., 1988], tropospheric N$_2$O (open triangle) and stratospheric N$_2$O (solid triangles) [Rahn and Wahlen, 1997] are shown for comparison. The size of the ovals represents the standard deviation (1σ) of the $\delta^{15}$N and $\delta^{18}$O emission-weighted averages from soils.

We conclude that in agroecosystems where nitrogen pools and water content change dramatically through the soil column, the use of N$_2$O/NO and bulk soil characteristics as proxy for differentiation of nitrification versus denitrification may not be adequate, because it assumes that both gases are being produced uniformly throughout the soil column. Future studies should recognize the possibility of vertical heterogeneity in trace gas production suggested by the comparison of isotopic and flux data and should include measurements of the soil air mixing ratio of N$_2$O and NO over the entire depth interval to assess these effects.

5.4. Identifying the Oxygen Source of Emitted N$_2$O Using Stable Isotopes

The $\delta^{18}$O-N$_2$O values are close to those of molecular O$_2$ ($\delta^{18}$O-O$_2$ = 0‰) and enriched by 22‰ to 30‰ compared to soil water. This suggests that incorporation of oxygen from molecular O$_2$ during N$_2$O formation from nitrification is greater than that of oxygen from water. There are no published $^{18}$O enrichment factors for the NH$_4^+$ to N$_2$O nitrification step. Because it is an oxidation process, the $\delta^{18}$O of N$_2$O produced should be more depleted in $^{18}$O than the substrates (H$_2$O and O$_2$). However, we found that the $^{18}$O in the emitted N$_2$O is enriched compared to atmospheric O$_2$, which may indicate that the molecular oxygen in the soil air pore space itself became enriched by microbial consumption. Our results are in disagreement with previous work done in a waste water facility where less than half of the oxygen atoms in N$_2$O were derived from atmospheric O$_2$ and the rest came from environmental water [Yoshinari and Wahlen, 1984]. Our results suggest that whether the pathway of N$_2$O production is (1) abiological oxidation of NH$_2$OH (NH$_2$OH $\rightarrow$ NO$\rightarrow$N$_2$O), (2) "nitrifier denitrification" (NO$_2^-$ $\rightarrow$ NO$\rightarrow$N$_2$O), and/or (3) denitrification (NO$_3^-$ $\rightarrow$ NO$\rightarrow$N$_2$O) [Wada and Ueda, 1996, and ref-
ences therein], the bacteria are more likely to use the O2-derived oxygen in each of the N2O precursors. In the future it will be necessary to characterize the 18O isotopic composition of N2O precursors to have a better understanding of the δ18O-N2O signature from these soils.

5.5. Implications for the Global N2O Budget

A similar agricultural field in the Yaqui Valley has been studied by Matson et al. [1998] over ~85% of the entire crop cycle and under the same management regime. Matson et al. [1998] found that the majority of N loss as N2O and N2O in this system occurred during the period we studied, following initial fertilization and irrigation and before planting. We therefore used the emission-weighted δ15N-N2O average (δ15N_{weighted}) as the best estimate of the isotopic fingerprint representative of the N2O emitted throughout the year at this site

$$\delta^{15}\text{N}_{\text{weighted}} = \frac{\sum \delta^{15}\text{N}_i \times F_i}{\sum F_i}$$

where δ15N_i and F_i are the δ15N-N2O and the N2O emission for a given day, respectively. The calculated δ15N_{weighted} was -37.9 ± 8.6‰ (± standard deviation, n = 17) (Figure 2a).

The only other fertilizer study we are aware of that tracked natural abundance stable isotopes in N2O emissions was conducted in an NH4NO3-fertilized papaya plantation in Costa Rica. There, the δ15N of N2O emissions following fertilization were similar to those observed in this study (δ15N-N2O = -30.0 ±5.6‰, ± standard deviation, n = 2; N2O flux: 28.91-117.17 ng N cm^{-2} h^{-1}) [Pérez et al., 2000]. As in the Yaqui Valley study, N2O emissions following fertilizer application in Costa Rica dramatically exceeded those for unfertilized soils. The δ15N-N2O values have also been reported for a fertilized temperate lawn near San Diego, California (δ15N-N2O = -24.5 ±1.4‰, ± standard deviation, n = 2; N2O flux: 15 ng N cm^{-2} h^{-1}) [Casciotti et al., 1997]. If we include these studies and calculate an overall 15N isotope emission-weighted average for all four fertilized sites, the δ15N of N2O emitted is -36.6±9.2‰ (± standard deviation, n = 21). This value is 10‰ to 30‰ depleted in δ15N compared to unfertilized tropical forest soils, which have reported emission-weighted average δ15N values of -26±2.5‰ (± standard deviation, n = 3) (Costa Rican forest, ultisol and inceptisol soils) and -6.6±11.3‰ (± standard deviation, n = 14) (Brazilian forest, oxisol soils) [Pérez et al., 2000]. Although we observe large spatial variability in natural systems [Pérez et al., 2000] and dramatic variations with time after fertilization in agricultural systems, the overall effect of synthetic fertilizer N application is to increase N2O emissions and decrease the δ15N of emitted N2O (Figure 4).

Application of N fertilizer to agricultural soils dramatically increases N2O emissions [Kroeze et al., 1999; Prather et al., 1995]. The global N fertilizer production is expected to increase ~60% by the year 2020; two thirds of that increase will occur in Asia [Galloway et al., 1995]. Urea represents 48% of the world synthetic fertilizer use and from that amount, 41% is used in the developing countries (Food and Agricultural Organization, FAO), FAOSTAT Statistical database, 1999, available at http://apps.fao.org/CS/DLL/NPH-DH.pl). Developing countries are located mostly in tropical and subtropical regions where N2O emission rates from soils are generally the highest. Our results show that the high N2O emissions following fertilization of subtropical (Mexico; this work) and tropical (Costa Rica [Pérez et al., 2000]) agricultural soils are significantly depleted in the 15N isotope compared to unfertilized soils. Therefore we expect a decrease in the 15N of tropospheric N2O if global agricultural N fertilizer application is a significant contributor to the observed increase in the mixing ratio of tropospheric N2O. We further expect that changes in tropospheric δ15N-N2O may be large enough to be useful in estimating the magnitude of the global N2O agricultural source.

Rahn and Wahlen [2000] published a model predicting the changes in 15N of tropospheric N2O assuming δ15N values for various sources. Their estimate, which assumed agricultural intensification increased the amount of N2O emitted but did not change its isotopic signature from natural soil emissions, predicted a decrease in δ15N-N2O of 1.6‰ to 1.9‰ 1990 AD. If we use the same model but change the isotopic signature of N2O emitted from new agricultural sources to reflect our result of decreased δ15N for N2O derived from this study, we predict a decrease of 5‰ in δ15N-N2O. This is likely an overestimate, since agricultural intensification has involved not only the synthetic N fertilizer application studied here but also the application of organic and animal waste fertilizers and associated indirect emissions (N2O derived from nitrification and runoff). Stable isotope measurements do not yet exist for these sources, which together may make up approximately two-thirds of the total increased N2O emissions due to agriculture [Kroeze et al., 1999; Mosier et al., 1998]. A more realistic estimate of δ15N changes in the troposphere, derived assuming that only half of the increased N2O emission from agriculture during the past century has been from application of ammonium-based inorganic fertilizers, nitrogen fixation, and other direct emissions that are largely derived from nitrification (and therefore associated with a large depletion in δ15N values compared to natural soil emissions), predicts a net decrease in the isotopic signature of tropospheric N2O from preindustrial times to the present of 2.2‰ to 3.0‰ for 15N (here the range reflects values assuming the average plus and minus one standard deviation of δ15N_{weighted} = -36.6 ± 9.2‰). Assuming that present rates of fertilizer use continue into the future, we predict continued changes in tropospheric N2O at rates of 0.04‰ to 0.06‰ yr^{-1} for δ15N. While our estimate of recent changes in the isotopic signature of tropospheric N2O is admittedly uncertain, our results suggest that N2O trapped in firm air in polar regions will show significant and measurable changes in the N2O isotopic composition when developments in analytical methods permit its measurement. If the uncertainty in the N2O isotopic measurement is reduced to 0.1‰ for 15N (presently it is ± 0.2‰) [Dore et al., 1998; Nagy et al., 1998; Pérez et al., 2000; Rahn and Wahlen, 1997; Yoshinari et al., 1997] and if the rate of isotope decrease calculated from this work is an appropriate estimate, then a tropospheric N2O isotopic shift may be observable over several years of monitoring.

6. Conclusions

The observed changes in 15N of N2O and changes in the position of nitrogen isotopomers in the N2O molecules following fertilization and irrigation of a subtropical agricultural field demonstrate shifts in the microbial processes producing N2O with time. Instantaneous enrichment factors for nitrifica-
tion and denitrification calculated for the sampling period suggest that the microbial N₂O production shifts from nitrification (week 1 after irrigation) to denitrification (week 2 after irrigation). These data are reconciled with evidence from N₂O/NO ratios if nitrification and denitrification are decoupled spatially in the 2nd week, with nitrification dominating NO production near the soil surface and denitrification dominating N₂O production in deeper layers of the soil. The δ¹⁵N-N₂O values suggest that incorporation of oxygen from molecular O₂ during N₂O formation by both nitrification and denitrification pathways is greater than that of oxygen from water. However, we cannot at this time determine the relative contribution of the different oxygen sources, because no enrichment factors for δ¹⁸O are available in the literature and δ¹⁸O-N₂O values are not available for this study. For the same reasons we also refrain from incorporating N₂O¹⁸O in our modeling results.

The overall δ¹⁵N signature we measure from N-fertilized agricultural systems is more depleted than those observed for more N-limited forest soils. The relationship we observe between very high emission rates and δ¹⁵N depletion for N₂O following the use of inorganic fertilizer suggests that changes in tropospheric δ¹⁵N-N₂O should have a measurable imprint from the increased use of inorganic N fertilizers in agriculture. Attempts to constrain the changes in tropospheric N₂O since preindustrial times using isotope ratios will require better characterization of the isotopic composition of all N₂O agricultural sources.

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