Identifying the agricultural imprint on the global N_2O budget using stable isotopes

T. Pérez,[1] S. E. Trumbore,[1] S. C. Tyler,[1] P. A. Matson,[2] I. Ortiz-Monasterio,[3] T. Rahn,[4] and D. W. T. Griffith[5]

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^{-2} h^{-1}) and in $\delta^{15}N$ of N_2O emitted (from -46‰ to +5‰ relative to atmospheric N_2) 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_2O production shifts from nitrification (week 1) to denitrification (week 2). Isotopic signatures of N_2O emissions were not always in accord with other proxies (such as NO/N_2O emission ratio or water-filled pore space) used to estimate the relative importance of nitrification and denitrification as N_2O sources. 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_2O production in this system. Further clues as to the source of N_2O come from the positional dependence of ^{15}N in the emitted N_2O, 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 we measured for N_2O emitted from N-fertilized agricultural systems is more depleted than observed $\delta^{15}N$ values for N_2O emitted from more N-limited forest soils. Assuming that one half of the total agricultural N_2O 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_2O during this century of as much as 3‰ for ^{15}N. Although many uncertainties remain, we suggest that measurements of $\delta^{15}N$-N_2O in firn air will provide constraints on how the N_2O budget has changed during the past century.

1. Introduction

Nitrous oxide (N_2O) is a greenhouse gas primarily produced by bacteria in soils and oceans during the processes of nitrification and denitrification. The principal global N_2O sources are tropical rain forest soils, agricultural fields, and oceans, whereas the major sink is stratospheric destruction [*Khalil and Rasmussen*, 1992]. Most of the observed increase of N_2O in the troposphere (~0.25% per year) has been attributed to increased N_2O 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_2O budget have been hampered by the limited number of emission studies coupled with the high spatial and temporal variability associated with N_2O fluxes [*Cicerone*, 1989; *Prather et al.*, 1995].

[1]Department of Earth System Science, University of California, Irvine, California.

[2]Department of Geological and Environmental Sciences, Stanford University, Stanford, California.

[3]Centro Internacional de Mejoramiento de Maíz y Trigo, Mexico City, Mexico.

[4]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California.

[5]Department of Chemistry, University of Wollongong, Wollongong, New South Wales, Australia.

Recent publications suggest that the use of stable isotopes of N and O in atmospheric N_2O and its sources may better constrain the global N_2O budget [*Cliff 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_2O assumes that "light" (or ^{15}N depleted) N_2O from sources such as soils and the ocean surface are balanced by "heavy" (or ^{15}N enriched) N_2O 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_2O production and the changing importance of agriculture in the global N_2O 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

Depth cm	pH H_2O	CEC (NH_4Oac) cmol$_c$ kg^{-1}	Clay %	Silt %	Sand %	Soil Texture
0-15	8.5	41.10	48.5	20.3	31.2	clay
15-30	8.6	41.82	51.2	15.0	33.8	clay
30-60	8.3	44.76	48.7	17.5	33.8	clay
60-90	8.0	51.01	44.9	28.9	26.2	clay

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 agriculturaluse. 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 typic caliciorthid (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 four to five times more before the fields are harvested in April-May. Previous studies of gaseous N loss from these fields [*Matson 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 loss 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

3.2.1. 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 ^{18}O composition of soil H₂O, total soil carbon and nitrogen content, the NH₄⁺ and NO₃⁻ concen-
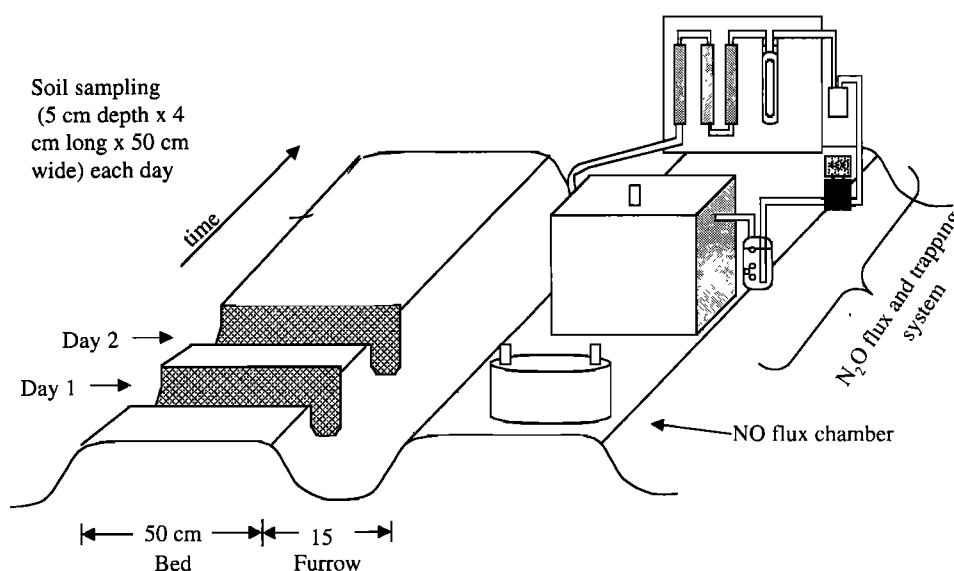

Figure 1. Diagram illustrating the dimensions of soil beds and furrows, soil sampling techniques, NO and N₂O flux chambers, and the N₂O isotope trapping system.
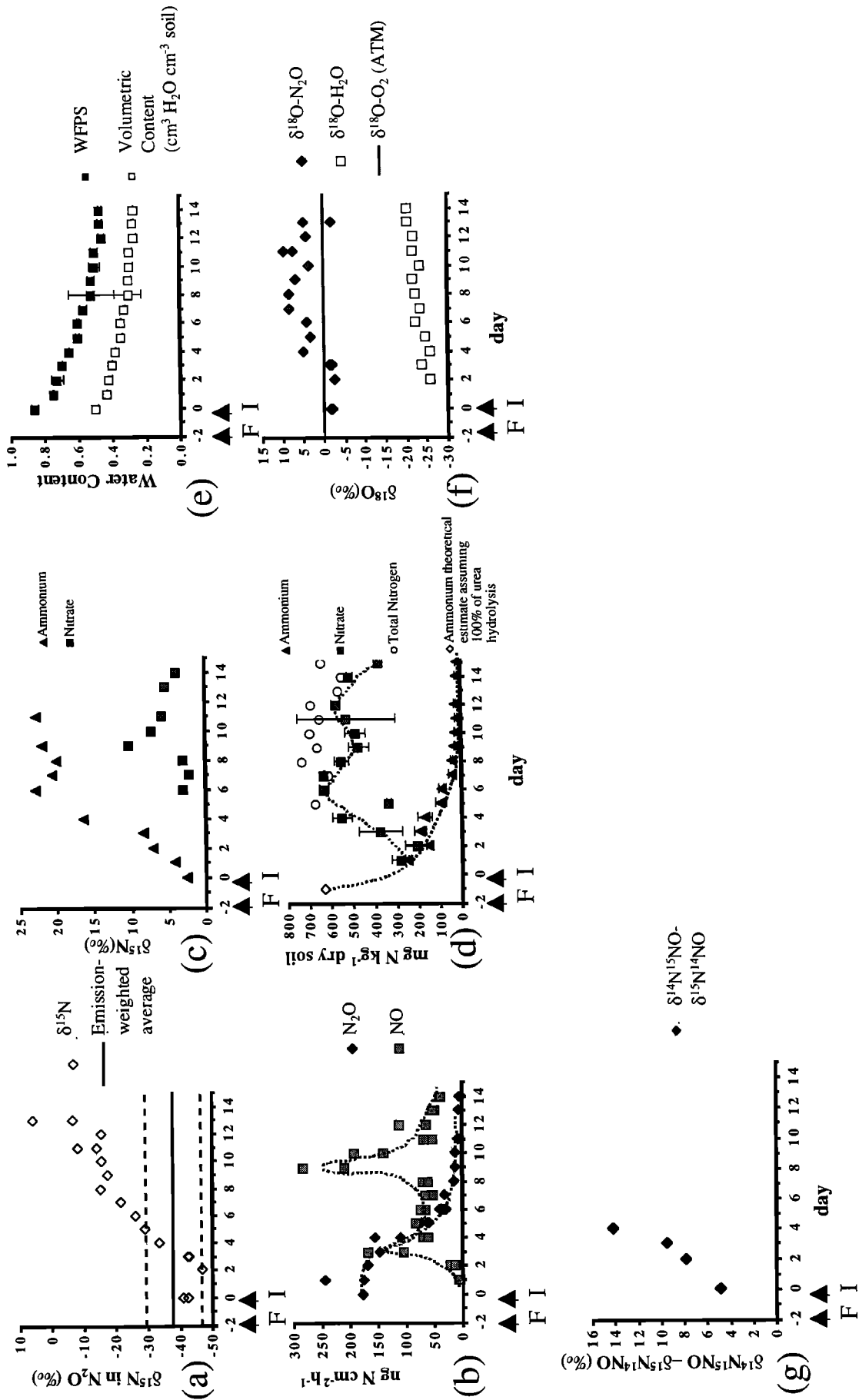
Figure 2. Parameters measured during a 15-day period following urea fertilization and irrigation: (a) $\delta^{15}N$ in N₂O; (b) N₂O and NO emissions; (c) $\delta^{15}N$ in NH₄⁺ and NO₃⁻; (d) NH₄⁺, NO₃⁻; total nitrogen abundance, and an estimate of initial urea-derived NH₄⁺ concentration (assuming that all urea applied was hydrolyzed and converted to NH₄⁺); (e) water content (0-5 cm); (f) $\delta^{18}O$ in N₂O and H₂O; and (g) $\delta^{15}N^{14}NO - \delta^{14}N^{15}NO$.

tration 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 1.11 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 NH_4^+ and NO_3^- at the University of California Irvine (UCI). Concentrations of NH_4^+ and NO_3^- were determined using the salicylate–hypochlorite and modified Griess-Illosvay methods, respectively [Mulvaney, 1996]. Reported NO_3^- concentrations are the sum of NO_3^- + NO_2^-. Both ions were measured using a spectrophotometer (HACH DR/2010).

3.2.2. Total carbon and nitrogen content. Soil samples taken every day from 0-5 cm depth were dried at 60°C for 24 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 $\delta^{15}N$ measurements in NO_3^- and NH_4^+ and $\delta^{18}O$-H_2O from soil water. Natural abundance ^{15}N in the inorganic nitrogen pool (NH_4^+ and NO_3^-) was analyzed at R. Mulvaney's laboratory (University of Illinois, Urbana) using methods described elsewhere [Mulvaney, 1997]. Water soil extraction and ^{18}O and isotopic composition were determined by conventional methods [Allison et al., 1983; Socki et al., 1992].

3.3. Trace Gas Emissions and Isotopic Measurements

N₂O and NO fluxes were measured between 1100 am to 0200 pm local time to minimize diel effects on fluxes.

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 CO_2 free and dry ambient air (≈680 mL min^{-1}) and soil chamber air (≈100 mL min^{-1}) was passed through a CrO_3 converter that oxidized NO to NO_2. NO_2 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 N_2, 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 $\delta^{15}N$ and $\delta^{18}O$-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 ^{15}N and ^{18}O were collected after syringe sampling by circulation of air from the chamber through a trapping system. The N₂O was trapped using a molecular sieve 5A trap, then transported to UCI 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 ^{15}N positioning of N₂O isotopomers. We determined the changes in ^{15}N positioning of N₂O isotopomers by Fourier transform infrared (FTIR) spectrometry [Esler 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 isobaric isotopomers ($\delta^{14}N^{15}NO$ - $\delta^{15}N^{14}NO$) after Yoshida and Toyoda [2000].

3.3.4. Isotope units. Isotopic data are reported as δ values, where $\delta=[(R_{sample}/R_{standard})-1] \, 1000$, and R_{sample} and $R_{standard}$ are $R = {}^{15}N/{}^{14}N$ or ${}^{18}O/{}^{16}O$ for sample and standard, respectively. Delta values are reported as deviations from $\delta^{15}N$ of atmospheric N₂ and $\delta^{18}O$ of atmospheric O₂. The conversion for the $\delta^{18}O_{atm}$ standard to SMOW standard is $\delta^{18}O_{atm}=\{-23 +[\delta^{18}O_{SMOW}/1.0235]\}$ [Kim and Craig, 1990].

4. Results

4.1. The $\delta^{15}N$ in Emitted N₂O

During week 1 (starting from the day of irrigation) when N₂O emissions were the highest (Figure 2b), both $\delta^{15}N$ and $\delta^{18}O$ values were very light (depleted in the heavy isotope) (Figures 2a and 2f). The first two measurements immediately following irrigation showed heavier $\delta^{15}N$ values (the average $\delta^{15}N$-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 $\delta^{15}N$-N₂O increased, as did the $\delta^{15}N$ signature of NH_4^+.

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 $\delta^{15}N$ in Inorganic Nitrogen (NH_4^+, NO_3^-) and Total Carbon and Nitrogen Content

The $\delta^{15}N$ values of NH_4^+ extracted from 0- to 5-cm soil become enriched during the 1st week following irrigation (Fig-

ure 2c). This increase is due to the preference by the soil bacterial population for the lighter (^{14}N) isotope as the nitrogen pool is consumed [*Nadelhoffer and Fry*, 1994]. The δ^{15}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 ≈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 δ^{15}N signature of NO$_3^-$ is depleted compared to δ^{15}N-NH$_4^+$, suggesting that most of the NO$_3^-$ is produced from NH$_4^+$ via nitrification. The isotopic signature of ^{15}N in NO$_3^-$ during 1st week of the experiment is not available. During the 2nd week the δ^{15}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 very low organic matter content (organic C was 9.03 ± 0.3 gC 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 δ^{15}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 δ^{18}O of Emitted N2O and Soil H2O

Water-filled pore space (WFPS) decreased from 0.86 to 0.46 in 15 days (Figure 2e). The δ^{18}O-H2Ovalues increased from −26 to −20‰ (Figure 2f). During the same time, δ^{18}O-N2O increased from −3‰ to +9‰ (Figure 2f). The overall magnitude of the δ^{18}O-N2O increase was not as great as that observed for δ^{15}N-N2O (Figure 2a).

4.5. The ^{15}N Positioning of N2O Isotopomer

Changes in the ^{15}N/^{14}N ratio of N2O emitted from the day of irrigation until 4 days later were accompanied by a significant change in the relative positional ^{15}N values in the N2O molecules as shown in Figure 2g. Isotopomeric site preference shifted 9.3‰ over the first 4 days after irrigation, in the sense that the N2O molecules were heavier by 9.3‰ in the central ^{15}N relative to the terminal ^{15}N on the 4th day compared to the day of irrigation. The actual site preference ranged from +4.9‰ to + 14.2‰ relative to the N2O 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 *Yoshida and Toyoda* [2000] for soil and oceanic N2O sources, −0.5‰ to +15‰.

5. Discussion

5.1. Differentiation Between Nitrification and Denitrification as Sources of N2O Using Stable Isotopes
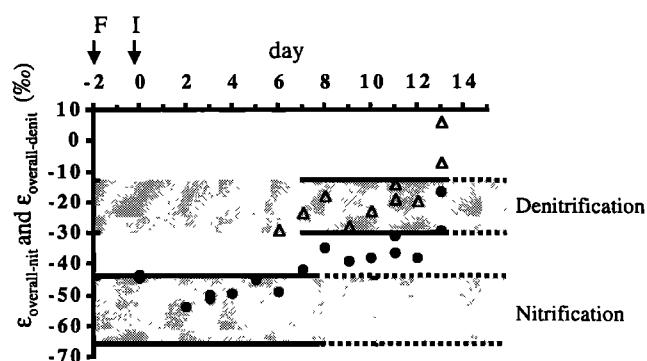

Figure 3. Calculated overall bacterial community instantaneous enrichment factors ε, for each day, assuming that the emitted N2O was produced by either nitrification ($\varepsilon_{overall-nit}$= δ^{15}N-N2O$_{emitted}$- δ^{15}N-NH$_4^+$, solid circles) or denitrification ($\varepsilon_{overall-denit}$= δ^{15}N-N2O$_{emitted}$-δ^{15}N-NO$_3^-$, open triangles). Shaded areas indicate the range of enrichment factors for nitrification and denitrification available in the literature [*Barford et al.*, 1999; *Ueda et al.*, 1999; *Wada and Ueda*, 1996; *Yoshida*, 1988, and references therein]. Dashed lines indicate the time period when there is disagreement between our calculated $\varepsilon_{overall}$ values and literature ε values for nitrification and denitrification.

Spatial and temporal variability in the δ^{15}N of N2O emitted from soils is caused by variations in substrate availability, the isotopic content of substrate, and shifts in microbial processes controlling N2O production and consumption [*Pérez et al.*, 2000]. The δ^{15}N signature of N2O emitted from the Yaqui Valley agricultural field (Figure 2a) showed dramatic shifts over time, ranging from highly depleted values (-46‰) during the 1st week when N2O emissions were the highest, to enriched values (+5‰) at the end of the 2nd week when emissions were low.

N2O is produced as a reaction byproduct or intermediate during nitrification (NH$_4^+$→NO$_3^-$) and denitrification (NO$_3^-$→N2). Both processes produce N2O molecules with distinct isotope signatures. The difference between δ^{15}N of emitted N2O and the substrate NH$_4^+$ or NO$_3^-$ is expressed as an enrichment factor ε, where ε = 1000 (α-1) and α is the isotopic fractionation factor of the reaction {α = $R_{product}/R_{substrate}$; R = (^{15}N/^{14}N$_{sample}$)/(^{15}N/^{14}N$_{standard}$)}. Published enrichment factors for nitrification (NH$_4^+$→N2O), ε_{nit}, range from -45 to -66‰ [*Ueda et al.*, 1999; *Yoshida*, 1988]. N2O produced via denitrification by soil denitrifiers has two characteristic enrichment factors reflecting the role of N2O as an intermediate in this process: $\varepsilon_{denit-1}$ of -13‰ to -28‰ for the NO$_3^-$ to N2O step [*Barford et al.*, 1999; *Wada and Ueda*, 1996 and references therein] and $\varepsilon_{denit-2}$ of -13‰ to -27‰ for the N2O to N2 step [*Barford et al.*, 1999; *Wada and Ueda*, 1996]. Therefore, if the substrates (NH$_4^+$ and NO$_3^-$) have a ^{15}N isotopic signature equal to 0‰, we expect to see differences in the isotopic signature of emitted N2O, with nitrification producing N2O 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 N2O reduced to N2 (which would further enrich the ^{15}N values of unconsumed N2O; 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 N_2O. We used measured $\delta^{15}N$ values for NH_4^+ and NO_3^- substrates (Figure 2c) and emitted N_2O (Figure 2a) to calculate instantaneous enrichment factors ($\varepsilon = \delta^{15}N_{product} - \delta^{15}N_{substrate}$) [*Goericke et al.*, 1994] for N_2O each day assuming that the entire N_2O production was either via nitrification ($\varepsilon_{overall-nit} = \delta^{15}N-N_2O_{emitted} - \delta^{15}N-NH_4^+$) or denitrification ($\varepsilon_{overall-denit} = \delta^{15}N-N_2O_{emitted} - \delta^{15}N-NO_3^-$) (Figure 3). We calculated $\varepsilon_{overall-denit}$ values for the 2nd week of the experiment only, because $\delta^{15}N-NO_3^-$ values were not available during the 1st week.

During the 1st week following irrigation, the calculated $\varepsilon_{overall-nit}$ 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 N_2O emission rates observed were associated with $\delta^{15}N_2O$ values that were several per mil more enriched (-42‰) than those measured on the three subsequent days (-46 to -42‰) (Figure 2a). This enrichment may have been caused by addition of some N_2O produced via denitrification. During the 2nd week, when N_2O fluxes decreased and much of the NH_4^+ had been converted to NO_3^- (Figure 2d), instantaneous $\varepsilon_{overall-nit}$ values fell above the range of published ε_{nit} values. In contrast, instantaneous $\varepsilon_{overall-denit}$ values were within the range of ε_{denit} in the literature during most of the 2nd week. Despite the fact that we cannot calculate values for $\varepsilon_{overall-denit}$ during the 1st week, these results suggest that most of the N_2O emission in the 1st week was derived from nitrification of abundant ammonium derived from the hydrolysis of urea. Once most of the NH_4^+ was converted to NO_3^- (Figure 2d), denitrification increased in importance and overall N_2O emissions decreased. Enrichment factors on the final day of the experiment did not correspond to published ranges of ε for either nitrification or denitrification; either N_2O was being produced outside of the range of published fractionation factors or another mechanism was controlling the isotopic signature of N_2O at that time.

5.2. Differentiation Between Nitrification and Denitrification as Sources of N_2O Using N_2O/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 N_2O emitted from soils are generally assumed to be derived primarily from nitrification and denitrification, respectively. Hence N_2O/NO emission ratios <1 are usually observed when soils are mesic or dry (WFPS <0.65) and nitrification is the dominant process, while high N_2O/NO ratios indicate more anaerobic conditions at higher soil water content (WFPS >0.65) with denitrification the dominant process [*Davidson*, 1993]. Our N_2O and NO emission results (Figure 2b) show a dramatic decrease in the N_2O/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 N_2O only for the first 2 to 3 days following irrigation, while nitrification was the dominant source of N_2O 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 N_2O source shifts 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 N_2O 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 ^{15}N labeling technique and found that the emitted N_2O was produced equally by denitrification and nitrification during the 1st week and nitrification during week 2. Our interpretation based on N_2O/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 N_2O 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 N_2O/NO ratio and WFPS (see section 5.2). The isotope data suggest that nitrification is the most important source of N_2O during the 1st week following irrigation (with the exception of some denitrification the 1st day), followed by denitrification as the dominant N_2O source during week 2. In contrast, N_2O/NO ratios suggest that denitrification is as important a source of N_2O 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 N_2O during the whole 2-week period, but the enrichment factors for nitrification increase with progressively limiting substrate availability (NH_4^+).

The first explanation relies on vertical separation of nitrification and denitrification in the soil column. We suggest that nitrification is the major source of N_2O 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 O_2 at the air-soil interface, will allow aerobic conditions for nitrification to occur. Initial N_2O/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 N_2O/NO emission ratios support nitrification as the major N_2O source, although WFPS (integrated over the top 10 cm of soil) remains high. Denitrification occurring at depth may contribute to, but does not dominate N_2O emissions the 1st week after irrigation, either because the NO_3^- substrate is increasing during this time or because denitrification may reduce N_2O to N_2 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 N_2O, 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 WFPS remains high and where the necessary NO_3^- substrate has leached from surface layers [*Riley et al.*, 2001], is responsible for N₂O 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 N₂O 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 N₂O emitted during the 1st week after irrigation is mostly derived from nitrification and is produced near the soil surface, while N₂O emitted during the 2nd week derives from denitrification in deeper soil layers where anaerobic conditions prevail. During the 2nd week, sources of NO and N₂O are decoupled into different vertical layers of the soil.

The second plausible explanation for the N₂O isotope shift is that nitrification continues to be the most important process producing N₂O during the whole 2-week period following irrigation, but the N₂O 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 N₂O 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 N₂O 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 (N₂O) 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 N₂O 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 positioning of N₂O isotopomers can potentially reflect shifts in microbial metabolism that influence the N₂O emissions from the soil and might provide information for differentiating between the two hypotheses. The average enrichment of $\delta^{15}N$ in N₂O 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 N₂O emissions on the 1st day, when soil WFPS was very high (0.86). The site preference (central minus terminal ^{15}N abundance) of the N₂O isotopomers on irrigation day (when denitrification is suggested by isotopic signature to contribute to N₂O production) was lower by ~9‰ than the value found 4 days later, when nitrification appears to be a more important contributor to N₂O production. This would imply that nitrifiers produce N₂O 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 N₂O in soils. We were unable to collect sufficient N₂O 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.
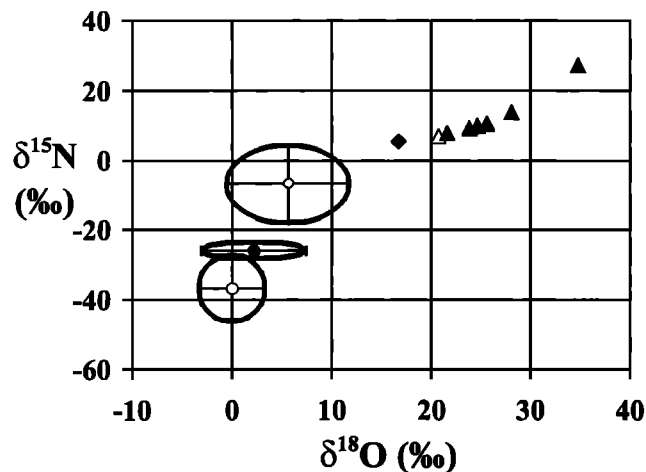

Figure 4. Average emission-weighted N₂O isotopic signatures for all agricultural fields (open circle) and unfertilized tropical rain forest soils from Costa Rica (solid circle) [*Pérez et al.*, 2000] and Brazil (open diamond) [*Pérez et al.*, 2000]. The isotopic signatures for N₂O emitted from the surface ocean (solid diamond) [*Dore et al.*, 1998], tropospheric N₂O (open triangle) and stratospheric N₂O (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 N₂O/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 N₂O and NO over the entire depth interval to assess these effects.

5.4. Identifying the Oxygen Source of Emitted N₂O Using Stable Isotopes

The $\delta^{18}O$-N₂O 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 N₂O formation from nitrification is greater than that of oxygen from water. There are no published ^{18}O enrichment factors for the NH_4^+ to N₂O nitrification step. Because it is an oxidation process, the $\delta^{18}O$ of N₂O produced should be more depleted in ^{18}O than the substrates (H_2O and O_2). However, we found that the ^{18}O in the emitted N₂O 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 N₂O were derived from atmospheric O_2 and the rest came from environmental water [*Yoshinari and Wahlen*, 1985]. Our results suggest that whether the pathway of N₂O production is (1) abiological oxidation of NH_2OH ($NH_2OH \rightarrow NOH \rightarrow N_2O$), (2) "nitrifier denitrification" ($NO_2^- \rightarrow NO \rightarrow N_2O$), and/or (3) denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O$) [*Wada and Ueda*, 1996, and refer-

ences therein], the bacteria are more likely to use the O_2-derived oxygen in each of the N_2O precursors. In the future it will be necessary to characterize the ^{18}O isotopic composition of N_2O precursors to have a better understanding of the $\delta^{18}O$-N_2O 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 N_2O and NO in this system occurred during the period we studied, following initial fertilization and irrigation and before planting. We therefore used the emission-weighted $\delta^{15}N$-N_2O average ($\delta^{15}N_{weighted}$) as the best estimate of the isotopic fingerprint representative of the N_2O emitted throughout the year at this site

$$\delta^{15}N_{weighted} = \frac{\sum_{i=1}^{n} \delta^{15}N_i \times F_i}{\sum_{i=1}^{n} F_i}$$

where $\delta^{15}N_i$ and F_i are the $\delta^{15}N$-N_2O and the N_2O emission for a given day, respectively. The calculated $\delta^{15}N_{weighted}$ was $-37.9 \pm 8.6‰$ ($\pm$ standard deviation, $n = 17$) (Figure 2a).

The only other fertilizer study we are aware of that tracked natural abundance stable isotopes in N_2O emissions was conducted in an NH_4NO_3–fertilized papaya plantation in Costa Rica. There, the $\delta^{15}N$ of N_2O emissions following fertilization were similar to those observed in this study ($\delta^{15}N$-N_2O = -30.0 $\pm 5.6‰$, $\pm$ standard deviation, $n = 2$; N_2O flux: 28.91-117.17 ng N cm^{-2} h^{-1})[*Pérez et al.*, 2000]. As in the Yaqui Valley study, N_2O emissions following fertilizer application in Costa Rica dramatically exceeded those for unfertilized soils. The $\delta^{15}N$-N_2O values have also been reported for a fertilized temperate lawn near San Diego, California ($\delta^{15}N$-N_2O = -24.5 $\pm 1.4‰$, $\pm$ standard deviation, $n = 2$; N_2O flux: 15 ng N cm^{-2} h^{-1}) [*Casciotti et al.*, 1997]. If we include these studies and calculate an overall ^{15}N isotope emission-weighted average for all three fertilized sites, the $\delta^{15}N$ of N_2O emitted is $-36.6 \pm 9.2‰$ ($\pm$ standard deviation, $n = 21$). This value is 10‰ to 30‰ depleted in ^{15}N compared to unfertilized tropical forest soils, which have reported emission-weighted average $\delta^{15}N$ values of $-26 \pm 2.5‰$ ($\pm$ standard deviation, $n = 3$) (Costa Rican forest, ultisol and inceptisol soils) and $-6.6 \pm 11.3‰$ ($\pm$ 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 N_2O emissions and decrease the $\delta^{15}N$ of emitted N_2O (Figure 4).

Application of N fertilizer to agricultural soils dramatically increases N_2O 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/c/s.dll/nph-db.pl). Developing countries are located mostly in tropical and subtropical

regions where N_2O emission rates from soils are generally the highest. Our results show that the high N_2O emissions following fertilization of subtropical (Mexico; this work) and tropical (Costa Rica [*Pérez et al.*, 2000]) agricultural soils are significantly depleted in the ^{15}N isotope compared to unamended soils. Therefore we expect a decrease in the ^{15}N of tropospheric N_2O if global agricultural N fertilizer application is a significant contributor to the observed increase in the mixing ratio of tropospheric N_2O. We further expect that changes in tropospheric $\delta^{15}N$-N_2O may be large enough to be useful in estimating the magnitude of the global N_2O agricultural source.

Rahn and Wahlen [2000] published a model predicting the changes in ^{15}N of tropospheric N_2O assuming $\delta^{15}N$ values for various sources. Their estimate, which assumed agricultural intensification increased the amount of N_2O emitted but did not change its isotopic signature from natural soil emissions, predicted a decrease in $\delta^{15}N$-N_2O of 1.6‰ to 1.9‰ 1900 AD. If we use the same model but change the isotopic signature of N_2O emitted from new agricultural sources to reflect our result of decreased $\delta^{15}N$ for N_2O derived from this study, we predict a decrease of 5‰ in $\delta^{15}N$-N_2O. 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 (N_2O derived from nitrate leaching and runoff). Stable isotope measurements do not yet exist for these sources, which together can make up approximately two-thirds of the total increased N_2O emissions due to agriculture [*Kroeze et al.*, 1999; *Mosier et al.*, 1998]. A more realistic estimate of $\delta^{15}N$ changes in the troposphere, derived assuming that only half of the increased N_2O 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 $\delta^{15}N$ values compared to natural soil emissions), predicts a net decrease in the isotopic signature of tropospheric N_2O from preindustrial times to the present of 2.2‰ to 3.0‰ for ^{15}N (here the range reflects values assuming the average plus and minus one standard deviation of $\delta^{15}N_{weighted}$ = -36.6 $\pm 9.2‰$). Assuming that present rates of fertilizer use continue into the future, we predict continued changes in tropospheric N_2O at rates of -0.04‰ to -0.06 ‰ yr^{-1} for $\delta^{15}N$. While our estimate of recent changes in the isotopic signature of tropospheric N_2O is admittedly uncertain, our results suggest that N_2O trapped in firn air in polar regions will show significant and measurable changes in the N_2O isotopic composition when developments in analytical methods permit its measurement. If the uncertainty in the N_2O isotopic measurement is reduced to 0.1‰ for ^{15}N (presently it is $\pm 0.2‰$) [*Dore et al.*, 1998; *Naqvi 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 N_2O isotopic shift may be observable over several years of monitoring.

6. Conclusions

The observed changes in ^{15}N of N_2O and changes in the position of nitrogen isotopomers in the N_2O molecules following fertilization and irrigation of a subtropical agricultural field demonstrate shifts in the microbial processes producing N_2O with time. Instantaneous enrichment factors for nitrifica-

tion and denitrification calculated for the sampling period suggest that the microbial N_2O production shifts from nitrification (week 1 after irrigation) to denitrification (week 2 after irrigation). These data are reconciled with evidence from N_2O/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 N_2O production in deeper layers of the soil. The $\delta^{18}O$-N_2O values suggest that incorporation of oxygen from molecular O_2 during N_2O 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 ^{18}O are available in the literature and $\delta^{18}O$-NO_3^- values are not available for this study. For the same reasons we also refrain from incorporating $N_2^{18}O$ in our modeling results.

The overall $\delta^{15}N$ 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 ^{15}N depletion for N_2O following the use of inorganic fertilizer suggests that changes in tropospheric $\delta^{15}N$-N_2O should have a measurable imprint from the increased use of inorganic N fertilizers in agriculture. Attempts to constrain the changes in tropospheric N_2O since preindustrial times using isotopes will require better characterization of the isotopic composition of all N_2O agricultural sources.

References

Allison, G. B. , C. J. Barnes, and M . W. Hughes, The distribution of deuterium and ^{18}O in dry soils, 2, Experimental, *J. Hydrol.*, *64*, 377-397, 1983.

Barford, C. C., J. P. Montoya, M. A. Altabet, and R. Mitchell, Steady-state nitrogen isotope effect of N_2 and N_2O production in *Paracoccus denitrificans*, *Appl. Environ. Microbiol.*, *65*(3), 989-994, 1999.

Casciotti, K., T. Rahn, and M. Wahlen, Stable isotopes of N and O in nitrous oxide emissions from fertilized soils, *Eos Trans., AGU, 78*(46), Fall Meet. Suppl., F58, 1997.

Cicerone, R. J., Analysis of sources and sinks of atmospheric nitrous oxide (N_2O), *J. Geophys. Res.*, *94*, 18 , 265-18, 271, 1989.

Cliff, S. S., and M. H. Thiemens, The $^{18}O/^{16}O$ ratios in atmospheric nitrous oxide, A mass independent anomaly, *Science*, *278*, 1774-1776, 1997.

Davidson, E. A., Soil water content and the ratio of nitrous oxide and nitric oxide emitted from soil, in *Biogeochemistry of Global Change Radiatively Active Trace Gases: Selected Papers From the Tenth International Symposium on Environmental Biogeochemistry, San Francisco, August 19-24, 1991*, edited by R. S. Oremland, pp. 369-386, Chapman Hall, New York, 1993.

Davidson, E. A., P. M. Vitousek, P. A. Matson, R. Riley, G. Garcia-Mendez, and J. M. Maass, Soil emissions of nitric oxide in a seasonally dry tropical forest of Mexico, *J. Geophys. Res.*, *96*, 15,439-15,445, 1991.

Davidson, E. A., P. A. Matson, P. M. Vitousek, R. Riley, K. Dunkin, G. García-Méndez, and J. M. Maass, Processes regulating soil emissions of NO and N_2O in a seasonally dry tropical forest, *Ecology*, *74*(1), 130-139, 1993.

Dore, J. E., B. N. Popp, D. M. Karl, and F. J. Sansone, A large source of atmospheric nitrous oxide from subtropical North Pacific surface waters, *Nature, 396*, 63-66, 1998.

Esler, M. B., D. W. T. Griffith, F. Turatti, S. R. Wilson, and T. Rahn, N_2O concentration and flux measurements and complete isotope characterization using FTIR spectroscopy, *Chemosphere*, in press, 2001.

Firestone, M. K., and E. A. Davidson, Microbiological basis of NO and N_2O production and consumption in soil, in *Exchange of Trace Gases Between Ecosystems and the Atmosphere*, edited by M. O. Andreae and D.S. Schimel, pp. 7-21, John Wiley, New York, 1989.

Galloway, J. N., W. H. Schlesinger, H. Levy, A. Michaels, and J. L. Schnoor, Nitrogen fixation: Anthropogenic enhancement-environmental response, *Global Biogeochem. Cycles*, *9*(2), 235-252, 1995.

Goericke, R., J. P. Montoya, and B. Fry, Physiology of isotopic fractionation in algae and cyanobacteria, in *Stable Isotopes in Ecology and Environmental Science*, pp. 187-221, Blackwell Sci., Cambridge, Mass., 1994.

Graham, R. D., D. Senadhira, and I. OrtizMonasterio, A strategy for breeding staple-food crops with high micronutrient density, *Soil Science Plant Nutrition*, *43*, 1153-1157, 1997.

Khalil, M. A., and R. A. Rasmussen, The global sources of nitrous oxide, *J. Geophys. Res.*, *97*, 14,651-14,660, 1992.

Kim, K. -R., and H. Craig, Two isotope characterization of N_2O in Pacific Ocean and constraints on its origin in deep water, *Nature*, *347*, 58-60, 1990.

Kim, K. -R., and H. Craig, Nitrogen-15 and oxygen-18 characteristic of nitrous oxide: A global perspective, *Nature*, *262*, 1855-1857, 1993.

Kroeze, C., A. Mosier, and L. Bouwman, Closing the global N_2O budget: A retrospective analysis 1500-1994, *Global Biogeochem. Cycles*, *13*(1), 1-8, 1999.

Machida, T., and T. Nakazawa, Increase in the atmospheric nitrous oxide concentration during the last 250 years, *Geophys. Res. Lett.*, *22*(21), 2921-2924, 1995.

Matson, P. A., C. Billow, S. Hall, and J. Zachariassen, Fertilization practices and soil variations control nitrogen oxide emissions from tropical sugar cane, *J. Geophys. Res.*, *101*, 18,533-18,545, 1996.

Matson, P. A., N. Rosamond, and I. Ortiz-Monasterio, Integration of environmental. agronomic, and economic aspects of fertilizer management, *Science*, *280*, 112-115, 1998.

Minami, K., Emissions of nitrous oxide (N_2O) from agroecosystem, *Jpn. Agric. Res. Q.*, *21*, 23-27, 1987.

Mosier, A., C. Kroeze, C. Nevison, O. Oenema, S. Seitzinger, and O. vanCleemput, Closing the global N_2O budget: Nitrous oxide emissions through the agricultural nitrogen cycle - OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology, *Nutrient Cycling Agroecosyst.*, *52*(2-3), 225-248, 1998.

Mulvaney, R. L., Nitrogen-Inorganic Forms, in *Methods of Soil Analysis: Part 3 Chemical Methods*, edited by D. L. Sparks, pp. 1123-1184, Soil Sci. Soc. of Am., Madison, Wisc., 1996.

Mulvaney, R. L., Accelerated diffusion methods for inorganic-nitrogen analysis of soil extracts and water, *Soil Sci. Soc. Am. J.*, *61*, 936-942, 1997.

Nadelhoffer, K. J., and B. Fry, Nitrogen isotope studies in forest ecosystems, in *Stable Isotopes in Ecology and Environmental Science*, edited by K. Lajtha and R. H. Michener, pp. 22-44, Oxford Univ. Press, New York, 1994.

Naqvi, S. W. A., T. Yoshinari, A. Jayakumar, M. A. Altabet, P. V. Narvekar, A. H. Devol, J. A. Brandes, and L. A. Codispoti, Budgetary and biogeochemical implications of N_2O isotope signatures in the Arabian Sea, *Nature*, *394*, 462-464, 1998.

Ortiz-Monasterio, J. I., R. J. Pena, K. D. Sayre, and S. Rajaram, CIMMYT's genetic progress in wheat grain quality under four nitrogen rates, *Crop Sci.*, *37*(3), 892-898, 1997a.

Ortiz-Monasterio, J. I., K. D. Sayre, S. Rajaram, and M. McMahon, Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates, *Crop Sci.*, *37*(3), 898-904, 1997b.

Panek, J. A., P. A. Matson, I. Ortiz-Monasterio, and P. Brooks, Distinguishing nitrification and denitrification sources of N_2O in a Mexican wheat system using N-15, *Ecol. Appl.*, *10*(2), 506-514, 2000.

Pérez, T., S. E. Trumbore, S. C. Tyler, E. A. Davidson, M. Keller, and P. B. de Camargo, Isotopic variability of N_2O emissions from tropical forest soils, *Global Biogeochem. Cycles*, *14*(2), 525-535, 2000.

Prather, M., R. Derwent, D. Ehhalt, P. Fraser, E. Sanhueza, and X. Zhou, Other trace gases and atmospheric chemistry, in *Climate Change 1994: Radiative Forcing of Climate Change and an Evaluation of the IPCC IS92 Emission Scenarios.*, edited by J. T. Houghton, et al., pp. 89-92, Cambridge Univ. Press, New York, 1995.

Rahn, T., and M. Wahlen, Stable isotope enrichment in stratospheric nitrous oxide, *Science, 278*, 1776-1778, 1997.

Rahn, T., and M. Wahlen, A reassessment of the global isotope budget of atmospheric nitrous oxide, *Global Biogeochem. Cycles, 14*(2), 537-543, 2000.

Riley, W. J., I. Ortiz-Monasterio, and P. A. Matson, Nitrogen leaching and soil nitrate, and ammonium levels in an irrigated wheat system in northern Mexico, *Nutrient Cycling in Agroecosyst.*, in press, 2001.

Socki, R. A., H. R. Karlsson, and E. K. J. Gibson, Extraction technique for the determination of oxygen-18 in water pre-evacuated glass vials, *Anal. Chem., 64*, 829-831, 1992.

Ueda, S., C. -S. Go, Y. Suwa, Y. Matsui, F. Yamaguchi, T. Shoji, K. Noto, T. Sumino, A. Tanaka, and Y. Matsufuji, Stable isotope fingerprint of N2O produced by ammonium oxidation under laboratory and field conditions, in *International Workshop on the Atmospheric N2O Budget: An Analysis of the State of Our Understanding of Sources and Sinks of Atmospheric N2O*, Japan Nat. Inst. of Agro-Environ. Sci., Tsukuba, Japan, 1999.

Wada, E., and S. Ueda, Carbon, nitrogen, and oxygen isotope ratios of CH4 and N2O on soil ecosystems, in *Mass Spectrometry of Soils*, edited by T. W. Boutton and S. -I. Yamasaki, pp. 177-204, Marcel Dekker, New York, 1996.

Yoshida, N., ^{15}N-depleted N2O as a product of nitrification, *Nature, 335*, 528-529, 1988.

Yoshida, N., and S. Toyoda, Constraining the atmospheric N2O budget from intramolecular site preference in N2O isotopomers, *Nature, 405*(6784), 330-334, 2000.

Yoshinari, T., and M. Wahlen, Oxygen isotope ratios in N2O from nitrification at waste water facility, *Nature, 317*, 349-350, 1985.

Yoshinari, T., M. A. Altabet, S. W. A. Naqvi, L. Codispoti, A. Jayakumar, M. Kuhland, and A. Devol, Nitrogen and oxygen isotopic composition of N2O from suboxic waters of the eastern tropical North Pacific and the Arabian Sea - Measurement by continuous-flow isotope-ratio monitoring, *Mar. Chem., 56*(3-4) 253-264, 1997.

Yung, Y. L., and C. E. Miller, Isotopic fractionation of stratospheric nitrous oxide, *Science, 278*, 1778-1780, 1997.

D. W. T. Griffith, Department of Chemistry, University of Wollongong, Wollongong NSW 2522 Australia. (david_griffith@uow.edu.au)

P. A. Matson, Department of Geological and Environmental Sciences, Stanford University, Stanford, CA 94305. (matson@pangea.Stanford.edu)

I. Ortiz-Monasterio, Centro Internacional de Mejoramiento de Maíz y Trigo, Apdo. Postal 6-641, Mexico D.F. 06600, Mexico. (I.ORTIZ-MONASTERIO@CGIAR.ORG)

T. Pérez, S. E. Trumbore, and S. C. Tyler, Department of Earth System Science, University of California, Irvine, CA 92697. (tjperez@uci.edu; setrumbo@uci.edu; styler@uci.edu)

T. Rahn, Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125. (trahn@gps.caltech.edu)