Can N_2O stable isotopes and isotopomers be useful tools to characterize sources and microbial pathways of N_2O production and consumption in tropical soils?

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[1] Nitrous oxide (N_2O) is an important greenhouse gas in which the main sources are tropical rainforest and agricultural soils. N_2O is produced in soils by microbial processes, which are enhanced by the application of nitrogenous fertilizers. The soil N_2O bulk isotopic composition ($\delta^{15}N^{bulk}$ and $\delta^{18}O$) and the "site-specific," or intramolecular, ^{15}N isotopic composition, i.e., the $^{15}N/^{14}N$ ratio at the cenral (α) or terminal (β) nitrogen position, expressed in this study as $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ could help identify both the sources (natural and anthropogenic) and microbial pathways of N_2O production and consumption prior to emission.We report new isotope measurements of soil N_2O emissions and from soil air collected during the rainy season in a mature tropical forest (Tapajos National Forest, Para, Brazil) and in a tropical agricultural corn field ("Fundo Tierra Nueva," Guárico State, Venezuela). The statistically different $\delta^{15}N^{bulk}$ emission weighted average between the mature forest ($-18.0‰ \pm 4.0‰$, $n = 6$) and agricultural corn field ($-34.3‰ \pm 12.4‰$, $n = 17$) suggests that the $\delta^{15}N^{bulk}$ data are useful for distinguishing N_2O fluxes from fertilized agricultural and natural "background" soils. They also demonstrate that the site-specific $\delta^{15}N$ measurements have the potential to provide a new tool to differentiate between the production and consumption N_2O microbiological processes in soils. This study further demonstrates that the observed correlations (or lack thereof) between $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, and $\delta^{18}O$ can be used to estimate the relative proportion of N_2O that would have been emitted to the air but was consumed via reduction of N_2O to N_2 within the soil.

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1. Introduction

[2] Nitrous oxide (N_2O) is an important greenhouse gas with a mean positive radiative forcing representing 6% of the total greenhouse gas contribution. Currently, its atmospheric concentration is ~323 ppbv (NOAA, CATS data), which is 15% larger than preindustrial concentrations [e.g., *Forster et al.*, 2007]. N_2O can also affect the balance of stratospheric ozone since its photolysis in the stratosphere is the major source of nitric oxide (NO), which participates in catalytic cycles of ozone destruction. The largest sources of N_2O are agricultural and natural soils, which represent more than 50% of the total, with tropical soils responsible for roughly 2/3 of the natural total soil source. The increase of N_2O in the troposphere of ~0.25% yr^{-1} [e.g., *Denman et al.*, 2007] has been attributed to large increases in the application of inorganic fertilizer application during the last century. This increase should be accompanied by a temporal change in N_2O isotopic compositions since the trend in concentration results from a continuing imbalance between the sources and sinks which have distinctly different isotopic signatures. If the isotopic fingerprints of the N_2O soil sources to the atmosphere are known, then we can explain any observed trends in the isotopic composition of tropospheric N_2O and estimate the magnitude of temporal changes in the sources. This information is also valuable for establishing mitigation strategies of N_2O reduction from agricultural soils.

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[3] *Pérez et al.* [2000, 2001] found that N$_2$O emitted from subtropical agricultural soils was significantly depleted in ^{15}N^{bulk} compared to tropical natural rainforest soils and suggested that such a difference in the isotopic signatures could be used to establish the relative contribution of these sources to tropospheric N$_2$O. Observations showing decreasing trends in N$_2$O isotopic compositions from the 1700s to the early 2000s from measurements on archived air samples [*Röckmann and Levin*, 2005; *Park et al.*, 2005, 2008], firn air [e.g., *Bernard et al.*, 2006; *Park et al.*, 2008; *Röckmann et al.*, 2003], and air extracted from ice cores [e.g., *Röckmann et al.*, 2003; *Sowers*, 2001], supported the prediction of *Pérez et al.* [2001] that the increase in the atmospheric N$_2$O burden is a result of agricultural activities that produce isotopically light N$_2$O. Tropical soils, the largest N$_2$O source to the atmosphere, are widely subjected to rapid land use changes, particularly conversion to agricultural land and pasture and addition of inorganic fertilizer [*Lambin et al.*, 2003, and references therein], with deforestation rates for tropical forests of about 2%–5% yr^{-1}. This implies that even larger shifts in the isotopic composition of tropospheric N$_2$O are to be expected.

[4] One issue that was raised in previous work was the usefulness of stable isotope determinations in differentiating mechanisms for production versus consumption of N$_2$O in soils. N$_2$O emitted from soils is biologically produced by nitrifying and denitrifying bacteria. During nitrification, NH$_4^+$ or NH$_3$ are oxidized to NO$_3^-$ via NO$_2^-$; N$_2$O is produced as a side reaction of the biological oxidation of NH$_2$OH, which is the first intermediate substance in NH$_3$ oxidation [*Otte et al.*, 1999]. N$_2$O production can also result from reduction of NO$_2^-$ to N$_2$O most probably by NH$_3$ oxidizers in a process known as "*nitrifier denitrification.*" During denitrification under more anaerobic conditions, NO$_3^-$ is reduced to N$_2$ via the enzyme nitrous oxide reductase; N$_2$O is an intermediate in the full sequence of reactions in the reduction of NO$_3^-$ to N$_2$ and can leak out of the soil, thereby avoiding further reduction [*Davidson*, 1991, and references therein]. The ratio of N$_2$O to N$_2$ emitted depends on environmental conditions. For instance, larger N$_2$O/N$_2$ ratios are dependent of low pH, and/or the presence of O$_2$ (which are known to suppress the function of nitrous oxide reductase) and abundant NO$_3^-$ (which enhance the rate of production of N$_2$O). It has been suggested that stable isotopes could be used to differentiate between these pathways. Indeed, the measured enrichment factors, ε, for ^{15}N^{bulk} (where ε is effectively the difference between the δ^{15}N of emitted N$_2$O and the precursor N source) from studies of both pure cultured bacteria and soils are larger for nitrification (NH$_4^+$ to N$_2$O) than for denitrification (NO$_3^-$ to N$_2$O), as shown from ε values found in the literature (Figure 1).

[5] The utility of bulk δ^{15}N values for distinguishing nitrification versus denitrification as the source of emitted N$_2$O, however, have been hampered by several complicating factors [*Baggs*, 2008]. First, the enrichment factors for nitrification and denitrification, and thus the bulk δ^{15}N values of the emitted N$_2$O, depend on the substrate (NH$_4^+$, NO$_2^-$ or NO$_3^-$) and on the substrate's ^{15}N content; thus each of these must be measured in order to determine or estimate a value for ε for each site. Second, the isotopic compositions

of the soil N$_2$O pool are also influenced by the degree to which N$_2$O may have been reduced to N$_2$ (during denitrification). Pure bacterial cultures as well as soil incubation studies have shown that the residual N$_2$O becomes enriched in ^{15}N as an increasing fraction is consumed via denitrification (Figure 1). In soils, the main factor that influences this reduction is oxygen availability, which is regulated by soil water content, soil texture and the supply and form of N in soil. Therefore, it is difficult to predict the degree of reduction of N$_2$O in a particular soil. Finally, the enrichment factors are either not constant (e.g., for denitrification) or not different enough to distinguish among processes (e.g., for nitrification). For example, it has been shown that the enrichment factors for ^{15}N and ^{18}O during N$_2$O reduction to N$_2$ decrease with increasing the reaction rate, thus producing smaller depletions in ^{15}N and ^{18}O in the products [*Vieten et al.*, 2007]. This variability in the magnitude of the enrichment factors prevents the partitioning of the relative contribution of each process to be determined by a simple isotope mass balance approach. Furthermore, differentiating nitrifier denitrification from denitrification is not possible since the enrichment factors for ^{15}N^{bulk} are very similar (Figure 1).

[6] Similar complications are encountered when trying to use ^{18}O signatures for soil N$_2$O in order to differentiate the microbial processes of N$_2$O production and consumption. The main problem in this case is that measurements of ^{18}O enrichment factors are scarce and highly variable. For denitrification (NO$_3^-$ to N$_2$O), for example, the values range from −1‰ to 43‰ [*Casciotti et al.*, 2002; *Snider et al.*, 2008; *Toyoda et al.*, 2005] relative to the O source (in this case, NO$_3^-$), whereas that for N$_2$O to N$_2$ reduction is −5‰ to −42‰. With such a large range of values, it is difficult to differentiate microbial processes using the oxygen isotope ratios alone. Furthermore, a recent study has shown that the δ^{18}O values of N$_2$O are influenced by oxygen exchange between the N$_2$O precursors and water [*Snider et al.*, 2008]. This work shows that 64 to 94% of the oxygen atoms in the N$_2$O precursors exchanged with oxygen atoms in water, effectively obscuring the original information about the source of ^{18}O in the N$_2$O molecule.

[7] Alternatively, the site-specific ^{15}N isotopic composition of N$_2$O has been proposed as a new tool for distinguishing the different microbial pathways of N$_2$O production. In our previous soil incubation experiments conducted on Brazilian natural forest soils [*Pérez et al.*, 2006], we found significant differences in site preference values (defined as δ^{15}N$^{\alpha}$ − δ^{15}N$^{\beta}$) for N$_2$O produced for nitrification and denitrification: −16.8‰ ± 8.4‰ versus 9.4‰ ± 8.1‰ relative to atmospheric N$_2$ (using *Toyoda and Yoshida* [1999] scale (see section 2), respectively. This difference suggests that reactions involved in denitrification result in ^{15}N enrichment at the central N relative to the terminal N, while reactions during nitrification result in smaller ^{15}N discrimination between the central N and the terminal N under our experimental conditions. An advantage of this new tracer is that it can potentially be used independently to differentiate between each microbial process without requiring knowledge of the ^{15}N composition of the substrates. Recent studies conducted in the laboratory cultures consisting of one or two
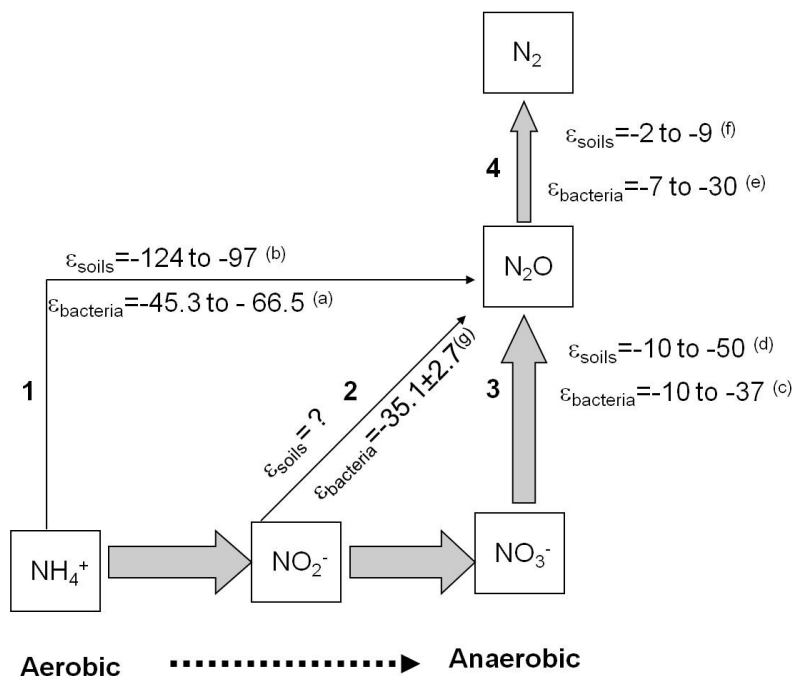
Figure 1. Microbial pathways of N_2O production: 1, nitrification; 2, nitrifier denitrification; 3, denitrification (NO_3^- to N_2O); 4, denitrification (N_2O to N_2). Numbers given are the range in $^{15}N^{bulk}$ enrichment factors (ε), calculated by different means for each microbial process as determined in either pure culture bacteria or soil studies: $\varepsilon = (\alpha-1) \times 100$, where $\alpha = R_{product}/R_{substrate}$; $\varepsilon = (-1000 \times \ln\alpha)/\ln f) \times 100$, where f = fraction of remaining substrate; $\varepsilon = \delta^{15}N_{product} - \delta^{15}N_{substrate}$). References: a, [*Sutka et al.*, 2006; *Ueda et al.*, 1999; *Yoshida et al.*, 1984]; b, [*Pérez et al.*, 2006]; c, [*Barford et al.*, 1999; *Snider et al.*, 2008; *Sutka et al.*, 2006; *Thompson et al.*, 2004; *Toyoda et al.*, 2005; *Yoshida et al.*, 1984]; d, [*Mariotti et al.*, 1981, 1982; *Menyailo and Hungate*, 2006; *Ostrom et al.*, 2007; *Pérez et al.*, 2006; *Snider et al.*, 2008; *Wada et al.*, 1991; *Well et al.*, 2006]; e, [*Barford et al.*, 1999; *Ostrom et al.*, 2007; *Thompson et al.*, 2004; *Wahlen and Yoshinari*, 1985; *Webster and Hopkins*, 1996; *Yoshida et al.*, 1984]; f, [*Mandernack et al.*, 2000; *Menyailo and Hungate*, 2006; *Ostrom et al.*, 2007; *Vieten et al.*, 2007]; g, [*Sutka et al.*, 2003, 2004].

species also showed significant differences in the site preference values for nitrification versus denitrification [*Sutka et al.*, 2006, 2003, 2004; *Thompson et al.*, 2004; *Toyoda et al.*, 2005]. The site preference values derived for denitrification are roughly in good agreement among the existing soils and bacteria studies, while those derived for nitrification appear to differ between those measured in the pure laboratory cultures and those in the incubation experiments of the natural environmental soils.

[8] In this study, we report new measurements of the bulk and site-specific ^{15}N and ^{18}O isotopic compositions of N_2O from two sites: a mature tropical forest and a fertilized tropical agricultural field. The isotopic data (including those for precursor N species) are used to identify the main microbial pathways of N_2O production and consumption in these soils, and provide a first opportunity to compare interpretations of bulk isotope and isotopomer ratios in a field setting. In addition to isotopes, we measured a number of physical and chemical parameters, such as soil type, water content, organic carbon and nitrogen contents, and substrate concentrations and isotopic compositions, all of which

provide constraints on our interpretation of N_2O production and consumption pathways. These new observations will be discussed for the purpose of better characterization of N_2O isotopic signatures from the emission sources and potential differentiation of the microbial mechanisms of N_2O production and consumption.

2. Data and Methodology

2.1. Study Sites

[9] Air samples for determination of the isotopic signature of N_2O emitted from the soil surface and in soil air at different depths were collected in March 2002 during the rainy season in the Amazon tropical rainforest (Tapajos National Forest (TNF) near Santarém, Para state, Brazil, 2°64'S, 54°59'W) and in June 2005 in a tropical agricultural cornfield ("Fundo Tierra Nueva," Guárico State, Venezuela 9°23'N, 66°38'W).

[10] The Brazilian Amazon samples were collected across a soil texture gradient from areas of active measurement of soil nitrogen trace gas fluxes located in the TNF, near
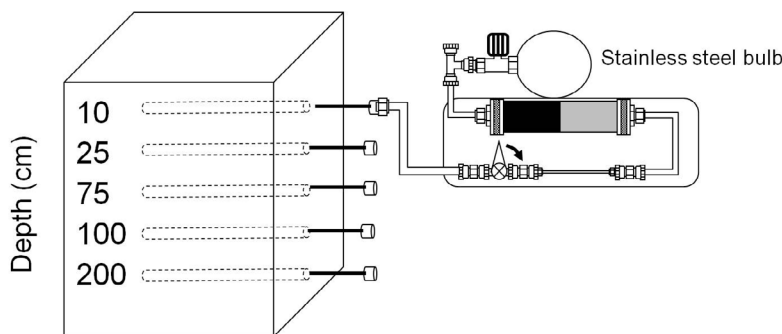
Figure 2. Soil air sampling system for N$_2$O stable isotope analysis.

Santarém, Para state, Brazil (2°64′S, 54°59′W) during one day of the rainy season (23 March 2002). Different soil types located at km-83 site from three primary forest sites were collected: a clay-rich soil (Oxisol), a sandy clay loam (Ultisol) and sandy loam (Ultisol) described elsewhere [*Silver et al.*, 2000; *Telles et al.*, 2003] and one set of Oxisol soil samples from a forest dry down experiment (seca floresta) located in the km-63 site of the TNF (for this site details see *Nepstad et al.*, 2007). Soil bulk density and texture were determined at 0–5, 10, 25, 75, 100 and 200 cm depth at the km-83 site. The texture classification at the deeper layers shows that the sandy clay loam soil becomes a clay textured soil. N$_2$O and NO soil emissions are available for a 1 year period in the clay and sandy loam soils, and *Keller et al.* [2005] showed that the NO emission values were higher in the dry season while N$_2$O emission values were higher in the wet season. For the sandy clay loam, only N$_2$O fluxes were measured during the sampling period.

[11] The Venezuelan cornfield site was under a nontillage agricultural practice at the time of the sampling in 2005. The soil is classified as a Vertisol (Typic Haplusterts), and is characterized by high clay content. The area has two distinctive precipitation seasons, wet (May–October) and dry (November–April). The mean annual precipitation for the previous 5 years was 622 ± 97.3 mm. Because irrigation of the crops depends on precipitation, planting is scheduled during the months of highest precipitation in June–July. We measured nitrogenous surface gas emissions (N$_2$O and NO), total inorganic nitrogen, and KCl-extractable NH$_4^+$ and NO$_3^-$ as well as soil climate conditions (temperature and water content). Measurements were made after fertilization to estimate the gaseous and leaching nitrogen losses. We also measured surface CO$_2$ emissions to evaluate the relationship of microbial respiration to the emissions of nitrogenous trace gases (S. Marquina, manuscript in preparation, 2010). Soils were fertilized with 54 kgN ha^{-1} (N:P:K = 12:24:12, nitrogen as NH$_4^+$) and planted simultaneously by a planting machine with a furrow opener. In the furrow, the fertilizer and seeds are incorporated at depths from 0 to 10 cm. Thirty days after the first fertilization, soils were refertilized by broadcast addition of 18 kgN ha^{-1} (as ammonium nitrate). For stable isotope analysis we collected continuously for 35 days: from the first fertilization until five days after the second fertilization.

2.2. Soil Gas Sampling

[12] For the Brazilian clay, sandy clay loam, and sandy loam we dug a pit of 2 m depth in which we inserted 2 m of 1/8 in. O.D. stainless steel tube probes at 10, 25, 75, 100 and 200 cm on one wall of the pit. One end of the probe had holes to allow soil air flow in and the other end had a 1/8 in. Swagelok fitting with a septum to collect the air samples (see Figure 2). We used one of the pit walls to determine physical parameters such as the bulk density and water content and to collect soil samples for nutrient soil analysis. We sampled 20 ml soil air with a syringe at each depth, and the first 20 ml of air was flushed to avoid possible N$_2$O dilution within the probe (due to the probe length). The soil gas samples collected at 0, 10, 25, 75, 100 and 200 cm were used to determine the mixing ratios and stable isotopic compositions of N$_2$O, concentrations and isotopic compositions of total carbon (TC) and of total nitrogen (TN), concentrations of soil nutrients (NH$_4^+$ and NO$_3^-$) concentrations, and water content. δ^{15}N for NH$_4^+$ and NO$_3^-$ were determined from the KCl extracts of selected soil samples at depths of 0 to 5 and 5 to 10 cm using the methods described by *Sigman et al.* [1997] and *Holmes et al.* [1998]. For the seca floresta site, the same parameters were measured at depths of 10, 25, 50, 100, 200, 300, 700 and 1100 cm, where the trace gases concentrations have been monitored monthly (E. A. Davidson et al., unpublished results, 2002). Surface trace gas emissions for this site are also available [*Davidson et al.*, 2008]. In the Venezuelan agricultural field, the same procedure was applied and the same parameters were measured but at depths of 10, 25, 50, 75 and 100 cm.

2.3. Measurements of N$_2$O Isotopic Compositions

[13] The N$_2$O collection system for stable isotope analysis consisted of an evacuated stainless steel canister attached to a "Tee" with a septum in one end and a drierite/ascarite trap at the other end for removal of CO$_2$ and H$_2$O. The other end of the drierite/ascarite trap could be connected either to the chamber placed on the soil surface or to the soil probe at each particular depth (Figure 1). Evacuated 500 mL canisters were filled with the gas sample following the procedure described elsewhere [*Pérez et al.*, 2006]. After a 2 minute equilibration period, the canister valve was closed and the sample stored until it was analyzed at UCB (University of

California, Berkeley). The N_2O isotope measurements were performed using a Finnigan MAT 252 isotope ratio mass spectrometer operated in continuous flow mode coupled with an online Finnigan preconcentrator and gas chromatograph [*Park et al.*, 2004]. Two separate aliquots of ~100 ml STP (equivalent to ~2 to 7 nmol of N_2O depending on N_2O mixing ratio) were taken by expanding the sample in the stainless steel canister into an evacuated, two-valved analysis flask, one for the $\delta^{15}N^{bulk}$ and $\delta^{18}O$ measurements and one for the site-specific $\delta^{15}N^{\alpha}$ measurement. For $\delta^{15}N^{bulk}$ and $\delta^{18}O$, the N_2O^+ molecular ions at mass-to-charge (m/z) ratios of 44, 45 and 46 were measured. For $\delta^{15}N^{\alpha}$, the NO^+ fragment ion at m/z ratios of 30 and 31 were measured. The isotope ratios ($^{15}N/^{14}N$ and $^{18}O/^{16}O$ for the molecular ion and the $^{15}N/^{14}N$ ratio for the NO^+ fragment ion) were measured relative to those of a pure N_2O reference gas. For the raw $\delta^{15}N^{bulk}$ and $\delta^{18}O$ values obtained for N_2O emitted at the soil surface, we applied a correction for the contribution of the isotopic composition of tropospheric N_2O present at the time of the chamber closure. The isotopic values for N_2O collected at depth were not corrected for a contribution from tropospheric N_2O contribution since the N_2O mixing ratios were most of the time an order of magnitude larger than the tropospheric mixing ratios.

2.4. Isotope Units

[14] In general, isotope values are reported using δ notation

$$\delta X = \left(R_{sample}/R_{standard} - 1 \right) \times 1000\text{‰},$$

where R is the ratio of the heavy to the light isotope $[^HX]/[^LX]$ and $X = {}^{15}N$, ^{18}O, $^{15}N^{\alpha}$, and $^{15}N^{\beta}$. $R_{standard}$ is the isotope ratio in atmospheric N_2 for $\delta^{15}N$ and Vienna standard mean ocean water (VSMOW) for $\delta^{18}O$. Site-specific $\delta^{15}N$ values ($\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$) are also given relative to air N_2. Originally, calibration of the UCB working standard to the international air-N_2 isotope scale for the site-specific $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ was determined by a mass-spectrometric method based on addition of different amounts of doubly labeled $^{15}N_2O$ to a pure N_2O sample (see *Kaiser et al.* [2004] for a detailed discussion), but this method yielded different values for the site-specific ^{15}N isotope composition for tropospheric N_2O than a calibration method based on site-specific chemical conversion of HNO_3 and NH_4OH to N_2O [*Toyoda and Yoshida*, 1999; *Yoshida and Toyoda*, 2000] ($\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ of tropospheric N_2O were 16.4‰ ± 1.6‰ and −2.4‰ ± 1.6‰ relative to air-N_2, respectively, from the Tokyo group versus 27.2‰ ± 0.9‰ and −14.7‰ ± 1.0‰ relative to air-N_2, respectively, for the Berkeley group, despite the fact that $\delta^{15}N^{bulk}$ and $\delta^{18}O$ isotopic compositions of tropospheric N_2O are in good agreement between the two groups). *Westley et al.* [2007] suggested that the discrepancy is mainly due to isotope effects associated with the formation of NO^+ fragment ions from the different isotopic species of N_2O in the ion source of a mass spectrometer and that these effects reflect different conditions in various ion sources, raising questions about the accuracy of the purely mass spectrometric dilution technique. Recently, *Griffith et al.* [2009] measured the site preference (defined frequently as $\delta^{15}N^{\alpha}−\delta^{15}N^{\beta}$) of tropospheric N_2O

using a Fourier transform infrared spectroscopic technique yielding values that are in agreement with the *Toyoda and Yoshida* [1999] air-N_2 scale. Thus, we report $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ and site preference values, which are roughly a measure of the degree of discrimination between the two nitrogen sites, on the *Toyoda and Yoshida* [1999] scale.

3. Results

3.1. Inorganic Nitrogen (NH_4^+ and NO_3^-), Total Carbon, Total Nitrogen, and Water Content

[15] Average inorganic nitrogen contents from the Brazilian tropical forest soils at depths of 0–5 cm were smaller than those measured at the Venezuelan agricultural site (Table 1). In the Brazilian forest soils, nitrate was the dominant form of inorganic nitrogen, whereas the Venezuelan agricultural sites had approximately equal amounts of ammonium and nitrate (due to the effect of fertilizer addition in these soils, Table 1). For the Venezuelan agricultural site, the 0–5 cm depth averages of inorganic nitrogen content varied significantly during the 35 day sampling period. Immediately after fertilization events, the NH_4^+ and NO_3^- contents increased and then decreased by an order of magnitude as time progressed (S. Marquina et al., manuscript in preparation, XXXX). The range of values measured was 0.54 to 230 and 6.7 to 233.6 μg N grams of dry soil⁻¹ for NH_4^+ and NO_3^-, respectively.

[16] The total carbon content of the surface (0–5 cm) soils was similar at both sites (about 3%, Table 1). The Venezuelan agricultural soils are not tilled, which likely explains why this carbon content is higher than for typical agricultural soils. At both sites, organic carbon contents decreased with soil depth. The Venezuelan Vertisols show an increase in total C from 75 to 100 cm depth due to a calcium carbonate layer. Carbon isotope data are consistent with C3 forest vegetation in Brazil (with $\delta^{13}C = −28.7$‰) [*Martinelli et al.*, 1999] and with C4 crops and grasses ($\delta^{13}C = −16$‰) at the Venezuelan sites (Table 1), which have been the dominant vegetation coverage at this site for the past 50 years. A shift to higher $\delta^{13}C$ values is associated with the carbonate layer in the Vertisols; pedogenic carbonates in grassland soils have shown $\delta^{13}C$ values of −1‰ to −4‰ [*Cerling and Wang*, 1996].

[17] Soil total N content at the two sites had similar values in surface layers and dropped with depth (Table 1). Soil water content (expressed as water-filled pore space, WFPS) was larger at the Venezuelan site, both at the surface where values ranged from 57% to >100% and in deep soils where values ranged from 49% to >100%, than at the Brazilian site, which ranged from 12 to 41%, with the lowest value in the sandy loam soils and the highest in the clay soils. (Note that values larger than 100% are due to uncertainties in bulk density determinations typical of these Vertisol soils (S. Marquina, manuscript in preparation, 2010). The water contents increased with soil depth at all sites (Table S1 of auxiliary material).[1]

Table 1. Concentration and Stable Isotope Composition Average and Standard Deviation of Total and Soluble Inorganic Nitrogen and Total Carbon; the Range in $\delta^{15}N^{bulk}$ and $\delta^{18}O$ of N$_2$O From Surface Emission and Deep Soil Pore Space Air Samples

Site	Sample Type	n	NH$_4^+$/μg N gds^{-1}	NO$_3^-$/μg N gds^{-1}	δ^{15}N-NH$_4^+$ (‰)	δ^{15}N-NO$_3^-$[a] (‰)	TC[b] (%)	TN[b] (%)	C/N	δ^{13}C-TC[c] (‰)	δ^{15}N-TN[c] (‰)	δ^{15}N^{bulk}-N$_2$O Range[a] (‰)	δ^{18}O-N$_2$O Range[d] (‰)
Brazilian Amazon forest	Soil surface samples (0–5 cm)	3	3.9 ± 2.5	10.6 ± 5.4	22.3 ± 16.4	−4.1 ± 3.6	3.0 ± 2.0	0.2 ± 0.1	12.8 ± 1.1	−28.7 ± 0.5	8.9 ± 0.5	−22.4 to −4.7	3.3 to 20.1
	Soil depth samples	22	0.2 ± 0.3	4.5 ± 4.2	38.3 ± 23.8[e]	4.9 ± 4.4[f]	1.0 ± 1.0	0.1 ± 0.1	11.3 ± 2.5	−26.5 ± 0.9	11.2 ± 1.4	−8.5 to 2.6	35.1 to 43.4
Venezuelan agricultural field	Soil surface samples (0–5 cm)	16	80.7 ± 93.1	75.9 ± 76.2	15.4 ± 13.6[g]	−2.4 ± 8.4[h]	2.9 ± 0.4[i]	0.3 ± 0.0[i]	10.8 ± 1.0[i]	−16.3 ± 1.4[i]	5.8 ± 0.5[i]	−49 to 5.6	7.7 to 38
	Soil depth samples	16	1.6 ± 2.0	16.3 ± 20.7	22.1[j]	3 ± 13[k]	2.8 ± 1.3[l]	0.1 ± 0.1[l]	39.7 ± 32.1[l]	−8.9 ± 5.9[l]	4.3 ± 1.3[l]	−47 to 1.6	25 to 42.2

[a] Relative to atmospheric N$_2$.
[b] Here (%) = 100 × (grams of nitrogen (or carbon) × gds (grams of dry soil)).
[c] Relative to Pee Dee bellemnite.
[d] Relative to Vienna standard mean ocean water.
[e] Here n = 6 (10–300 cm depth).
[f] Here n = 21 (10–700 cm depth).
[g] Here n = 11.
[h] Here n = 12.
[i] Here n = 13.
[j] Here n = 1 (25 cm).
[k] Here n = 8 (10–100 cm depth).
[l] Here n = 15.

3.2. N$_2$O Soil Emissions and Soil Air Mixing Ratios

[18] The average of the N$_2$O soil surface emissions for the chambers for which we performed stable isotope analyses were 2.4 ± 2.0 ng N cm^{-2} h^{-1} (n = 6) for the Brazilian tropical forest and 40 ± 30 ng N cm^{-2} h^{-1} (n = 17) for the Venezuelan agricultural field (individual data points are given in Table 2). The observed N$_2$O forest soil emission value was in agreement with the average emission values taken over 2 years in the same location (7.9 ± 0.7 and 7.0 ± 0.6 ng N cm^{-2} h^{-1} for the Oxisol and 1.7 ± 0.1 and 1.6 ± 0.3 ng N cm^{-2} h^{-1} for the Ultisol for 2000 and 2001, respectively) [*Keller et al.*, 2005]. The average N$_2$O emission from the Venezuelan agricultural field during the study period of 35 days was 44 ± 56 ng N cm^{-2} h^{-1} (n = 195) (S. Marquina, manuscript in preparation, 2010).

[19] The N$_2$O mixing ratios in the soil air at different depths were an order of magnitude larger in the Venezuelan agricultural site than in the Brazilian forest soils (Figures 3a and 3b). For both sites, the largest mixing ratios were measured not in the surface layer but instead at deeper layers (between 25 and 100 cm), with lower values both below and above, indicating N$_2$O diffusion and/or consumption was occurring both into and out of the soils. Only on 6 July, at the Venezuelan site, was the mixing ratio higher in the surface layers (10 cm). This difference is attributed to an enhancement in N$_2$O production near the surface due to fertilizer application to the soil surface the day before (S. Marquina et al., manuscript in preparation, XXXX).

3.3. The δ^{15}N of the Substrates Total Nitrogen, Ammonium, and Nitrate

[20] The δ^{15}N values for total nitrogen were lower (i.e., total nitrogen was more depleted in ^{15}N) at the Venezuelan site than in the Brazilian Amazon Forest soils (p < 0.001), most likely implying effect of the addition of inorganic fertilizer of which ^{15}N isotopic value is about 2‰ (Table 1). The average δ^{15}N value of TN at depth was larger for the Brazilian soils than the Venezuelan site. The Venezuelan site average δ^{15}N-TN value at depth was not statistically different from the one observed at surface (Table 1). This implies that the applied inorganic fertilizer percolated through depth quite effectively at the Venezuelan site, whereas the Brazilian Amazon Forest soil nitrogen seems to have been recycled largely before it percolated to deeper layers.

[21] In both study sites, the δ^{15}N values for NH$_4^+$ are higher than those for NO$_3^-$ both at the surface and at depth (Table 1). This trend is expected due to the fact that NO$_3^-$ is derived from nitrification and its isotopic fingerprint will be depleted in the heavy isotopes relative to that for the substrate NH$_4^+$. At the Venezuelan site, after the first fertilization (in which nitrogen was added as NH$_4^+$), very isotopically light NO$_3^-$ was measured. This is mainly due to kinetic isotope effects in the oxidization of NH$_4^+$ to NO$_3^-$ during nitrification that result in ^{15}N-depleted NO$_3^-$ relative to the substrate. After the second fertilization, the δ^{15}N values of NH$_4^+$ and NO$_3^-$ were very similar. In this case, nitrogen addition was in the form of NH$_4$NO$_3$. Interestingly, 4 days later, as the NH$_4^+$ concentration diminished by an order of

Table 2. Brazilian TNF Amazon Forest and Venezuelan Agricultural Cornfield δ^{15}N and δ^{18}O Values of Individual N$_2$O Soil Surface Emissions and Water-Filled Pore Space Values

Site	Date	Soil Description	N$_2$O Surface Emission (ng cm^{-2} h^{-1})	δ^{15}N^{bulk} N$_2$O^a (‰)	δ^{18}O-N$_2$O^b (‰)	NH$_4^+$-μg N gds^{-1}	NO$_3^-$-μg N gds^{-1}	δ^{15}N-NH$_4^{+a}$ (‰)	δ^{15}N-NO$_3^{-a}$ (‰)	Water-Filled Pore Space
Amazon Forest soil emission samples	23 Mar 2002	Sandy soil	1.2	−4.7	3.3	1.2	4.6	3.8	−8.3	12
	23 Mar 2002	Clay soil	3.7	−18.3	10.3	6.1	12.1	34.8	−1.5	41
	23 Mar 2002	Transition soil	1.8	−14.1	6.9	4.6	15.0	28.2	−2.6	37
	23 Mar 2002	Transition soil	5.9	−22.4	20.1	4.6	15.0	28.2	−2.6	37
	18 Mar 2002	Seca Floresta Pit 1 (Poco 1)	1.0	−14.9	4.8					
	18 Mar 2002	Seca Floresta Pit 1 (Poco 1)	0.9	−21.0	8.7					
Venezuelan agricultural soil emission samples	1 Jun 2005	Chamber 4	18.4	5.6	30.5	3.4	12.0	10.7	6.1	65
	5 Jun 2005	Chamber 11	16.0	−22.5	30.6	148.1	81.4	13.8	−19.8	100
	9 Jun 2005	Chamber 6	22.3	1.7	37.6	130.8	130.5	29.4	−14.7	82
	12 Jun 2005	Chamber 8	33.8	−38.3	25.3	2.3	34.1	6.3	−8.6	57
	14 Jun 2005	Chamber 2	6.0	−20.4	26.3	0.5	15.2			59
	15 Jun 2005	Chamber 7	73.9	−34.4	27.0	21.4	28.7			107
	16 Jun 2005	Chamber 10	100.8	−48.9	26.0					100
	21 Jun 2005	Chamber 12	29.0	−11.6	32.7	4.7	18.6	21.6	−5.1	90
	22 Jun 2005	Chamber 2	13.4	−9.0	30.5	93.7	52.3	8.9	−4.0	73
	25 Jun 2005	Chamber 13	58.5	−21.0	34.7	1.4	6.7		7.4	110
	28 Jun 2005	Chamber 8	6.2	−28.6	20.7	2.4	12.9			93
	5 Jul 2005	Chamber 9	3.8	−20.2	7.7	196.1	73.5	0.5	3.4	73
	6 Jul 2005	Chamber 3	77.0	−46.6	21.0	216.9	233.6	5.1	0.3	117
	6 Jul 2005	Chamber 9	40.7	−18.3	24.3	230.0	126.4	5.1	0.3	117
	7 Jul 2005	Chamber 14	64.4	−15.8	32.5	216.9	233.6			133
	8 Jul 2005	Chamber 3	79.3	−42.8	21.2	20.1	137.9	47.1	1.5	133
	13 Aug 2005	Chamber 5	28.7	−16.0	38.0	2.6	16.4	21.0	4.4	110

^{a}Relative to atmospheric N$_2$.
^{b}Relative to Vienna standard mean ocean water.

magnitude, the δ^{15}N-NH$_4^+$ values were significantly isotopically enriched by about 50‰. This isotopic shift is most likely attributable to enhancement of nitrification activity. It is also interesting that the δ^{15}N-NO$_3^-$ values and the NO$_3^-$ soil content did not change significantly during this period, suggesting that NO$_3^-$ was an infinite pool for denitrifying soil bacteria; details will be given by S. Marquina (manuscript in preparation, 2010).

3.4. The δ^{15}N^{bulk} and δ^{18}O of Soil-Emitted N$_2$O and Soil Air Samples

[22] We report the isotopic composition of surface-emitted N$_2$O (e.g., δ^{15}N^{bulk}, δ^{18}O and ^{15}N site preference) as emission-weighted averages. N$_2$O fluxes are highly variable in space and time, and the isotopic signatures of emitted N$_2$O may covary with fluxes (see *Pérez et al.* [2000, 2001] and Table 3). Especially in fertilized systems, the isotopic composition of N$_2$O released will be primarily determined by the largest fluxes.

[23] For our Venezuelan agricultural study site, there was a statistically significant correlation of δ^{15}N^{bulk} ($R^2 = 0.43$, $n = 15$) and site preference values ($R^2 = 0.50$, $n = 17$) with N$_2$O surface emissions (Table 2). The emission-weighted δ^{15}N^{bulk} average for the Venezuelan agricultural site was

lower than that for the Brazilian natural forest site, consistent with previous work (Table 3) [*Pérez et al.*, 2000, 2001].

[24] The emission-weighted δ^{18}O average for N$_2$O fluxes from the TNF Amazon tropical forest soils of 12.2‰ ± 5.9‰ ($n = 6$) was significantly lower than that for the Venezuelan agricultural field of 26.3‰ ± 4.4‰ ($n = 17$) and those measured for previous in situ soil N$_2$O emissions from fertilized grassland soils (Table 3). Indeed, to our knowledge, these are the lowest emission-weighted δ^{18}O averages ever measured for soil-emitted N$_2$O.

[25] In the Brazilian TNF soils, the emission-weighted average values for δ^{15}N^{bulk} and δ^{18}O of N$_2$O emitted from the soil surface are lower than those of N$_2$O in soil pore space (Table 1). In the Venezuelan agricultural field, however, no significant difference between the surface-emitted and soil pore space N$_2$O samples for δ^{15}N^{bulk} were observed (Table 1). The δ^{15}N^{bulk} values for depth samples in the Venezuelan agricultural field were about 20‰ lower than those for the Brazilian tropical forest soils (Figures 3c and 3d). However, the δ^{18}O values of N$_2$O sampled from deeper soil layers were comparable to the measurements on the Brazilian TNF soils (Table 1).

[26] A linear trend between δ^{15}N^{bulk} and depth in the TNF Brazilian Amazon soils was not observed (see Figure 3c), while at the Venezuelan agricultural field for the 4 days
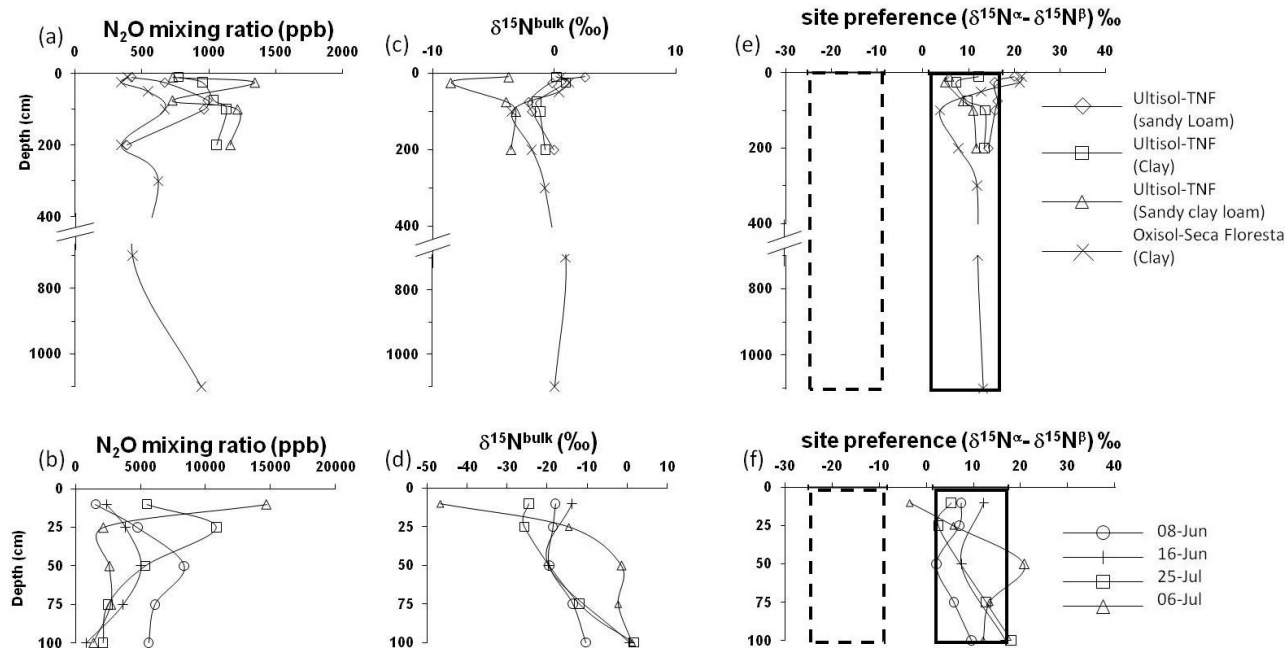
Figure 3. N$_2$O mixing ratio (ppbv), δ^{15}N^{bulk} versus air N$_2$ (‰), and site preference (‰ on the *Toyoda and Yoshida* [1999] air-N$_2$ scale) for (a, c, and e) soil air depth samples in the Brazilian TNF Amazon Forest soils and (b, d, and f) the Venezuelan agricultural cornfield soils, respectively. The rectangles represent the site preference values for denitrification (solid) and nitrification (dashed) obtained from the TNF Amazon Forest soil incubation experiments of *Pérez et al.* [2006].

when the soil air depth profiles were collected there is an increase in δ^{15}N^{bulk} values above and below the depth with the highest concentration (Figure 3d). These trends with depth were less pronounced for δ^{18}O. Enrichments in both ^{15}N and ^{18}O with depth were observed, after a heavy rain event (46 mm) on 25 July 2005, when the N$_2$O mixing ratio at the near-surface layers (10–25 cm) was between 5000 and 10000 ppb (Figure 3b).

3.5. Site-Specific δ^{15}N of Soil-Emitted N$_2$O and Soil Air Samples

[27] In contrast to the clear difference in δ^{15}N^{bulk} between natural and agricultural N$_2$O sources shown in section 3.4, the site-specific δ^{15}N-N$_2$O emission-weighted average values from the forest soil and the agricultural field in this study are not statistically different from each other (see Table 3). N$_2$O site preference values were similar for soil pore space sampled at different depths between the two sites (Figure 3). The few exceptions will be addressed below (section 4.2.2).

4. Discussion

4.1. Distinguishing N$_2$O Emitted From Tropical Forest Versus Agricultural Soils

[28] The TNF Amazon tropical forest soils have an emission-weighted average bulk δ^{15}N value of −18.0‰ ± 4.0‰ ($n = 6$). This average is within the range of the emission-weighted average values found by *Pérez et al.* [2000] in Amazon tropical forest soils from another mature Amazon Forest site (Paragominas, Para state), which averaged −6.6‰ ± 11.1‰ ($n = 14$). The bulk δ^{15}N value of N$_2$O emitted from both tropical forest sites is higher than the emission-weighted average δ^{15}N^{bulk} for N$_2$O emitted from the Venezuelan agricultural cornfield (−34.3‰ ± 12.4‰, $n = 17$). The lower δ^{15}N^{bulk}-N$_2$O values in the agricultural field are consistent with the fact that when nitrogen availability can be considered unlimited (e.g., just following nitrogen fertilizer addition), the production of N$_2$O either via nitrification or denitrification favors larger ^{15}N fractionation [*Mariotti et al.*, 1981]. N$_2$O emission values were higher and δ^{15}N^{bulk} was lower when substrate availability was larger (Table 2).

[29] The bulk δ^{15}N emission-weighted average found for the Venezuelan agricultural cornfield (−34.3 ± 12.4, $n = 17$) is almost identical to that found by *Pérez et al.* [2001] in a Mexican subtropical wheat field (−37.9‰ ± 8.6‰, $n = 17$), although these sites differed in climate (subtropical versus tropical), crop type (corn versus wheat), the amount and type of inorganic fertilizer applied, and overall agricultural practice (tillage, irrigation, timing of fertilization). These results suggest that the isotopically distinct signature of bulk ^{15}N for N$_2$O emitted from fertilized tropical or subtropical agricultural fields provides a tool to aid in distinguishing N$_2$O derived from background forest emissions versus anthropogenically altered, fertilized soil sources. Further-

Table 3. Flux-Weighted Averages of N$_2$O Isotopic Compositions From in Situ Measurements of Soil-Emitted Gas Samples[a]

Site	n	δ^{15}N-N$_2$O^{bulk} [b] (‰)	δ^{18}O-N$_2$O[c] (‰)	δ^{15}N$^{\alpha}$-N$_2$O[b] (‰)	δ^{15}N$^{\beta}$-N$_2$O[b] (‰)	Site Preference[b] (‰)	Reference
Brazilian Amazon tropical forest Soil (TNF, Para state)	6	−18.0 ± 4.0	12.1 ± 5.9	−12.4 ± 6.6	−22.2 ± 4.3	9.7 ± 7.9	This paper
Venezuelan tropical agricultural soil (NPK amendment)	17	−34.3 ± 12.4	26.3 ± 4.4	−32.8 ± 17.5	−34.3 ± 11.6	1.5 ± 21.0	This paper
Brazilian Amazon tropical forest soil (Paragominas, Para state)	14	−6.6 ± 11.1	29.3 ± 6.1	NA[d]	NA[d]		*Pérez et al.* [2000]
Costa Rican tropical forest (Heredia Province)	3	−26.0 ± 2.5	26.3 ± 5.2	NA[d]	NA[d]		*Pérez et al.* [2000]
Mexican subtropical agricultural soil (urea amendment)	4	−41.5 ± 4.0	23.3 ± 2.6	−37.3 ± 17.7	−46.3 ± 21.1	9.0 ± 2.9	*Pérez et al.* [2001]
Temperate grassland soil (urine amendment, North Wyke, United Kingdom)	5	−3.4 ± 3.2	42.0 ± 3.4	−0.1 ± 3.7	−6.8 ± 2.8	6.7 ± 1.9	*Yamulki et al.* [2001]
Temperate grassland soil (KNO$_3$ amendment, North Wyke, United Kingdom)[e]	14	−6.1 ± 6.1	26.0 ± 4.5	−5.0 ± 6.2	−7.1 ± 6.0	2.2 ± 0.8	*Bol et al.* [2003]
Brazilian Amazon tropical forest soil (TNF Ultisol nitrification)[e]	9	−91.6 ± 4.4	9.6 ± 1.9	−96.9 ± 7.3	−80.1 ± 4.1	−16.8 ± 8.4	*Pérez et al.* [2006]
Brazilian Amazon tropical forest soil (TNF Ultisol denitrification)	9	−39.9 ± 4.3	27.2 ± 1.6	−32.7 ± 7.1	−42.1 ± 4.0	9.4 ± 8.1	*Pérez et al.* [2006]

[a]For comparison, nitrifier denitrification and denitrification values from *Pérez et al.* [2006] and the results from other previous studies are given.
[b]Relative to atmospheric N$_2$.
[c]Relative to Vienna standard mean ocean water.
[d]NA, not analyzed.
[e]In the original publication [*Pérez et al.*, 2006], this value was attributed to nitrification; see section 4.3 for arguments supporting the assignment of this value to either nitrification or nitrifier denitrification.

more, given the much larger emissions of N$_2$O from N-fertilized soils and the expanding use of N fertilizers in the tropics [*Lambin et al.*, 2003], the overall δ^{15}N^{bulk} of tropospheric N$_2$O should thus continue to decline in the future.

[30] On the other hand, as mentioned in section 3.5, there is little difference between the emission-weighted average site-specific δ^{15}N values of N$_2$O for the Brazilian forest soils and those for the Venezuelan agricultural field. These values are also similar to those obtained in a temperate grassland [*Yamulki et al.*, 2001] and a subtropical agricultural field [*Pérez et al.*, 2001], which are the only other N$_2$O isotopomer data set from in situ isotopic determinations of soil emissions from agricultural fields available in the literature (see Table 3). These similarities appear to indicate that the site-specific δ^{15}N-N$_2$O values and the site preference values associated with N$_2$O soil sources (i.e., natural versus agricultural soils) are not distinct and, thus, are less useful than δ^{15}N^{bulk}-N$_2$O values are for partitioning the relative role that N-fertilized agricultural sources play in global N$_2$O emissions.

[31] For the oxygen sources of N$_2$O, δ^{18}O of N$_2$O was also proposed as a tool for identifying them [*Kool et al.*, 2007, and references therein], because the larger observed variability in δ^{18}O results compared to that for δ^{15}N hinted that it could potentially be a better tracer for differentiating N$_2$O sources [*Kim and Craig*, 1993]. However, it proved difficult to find effective mechanisms to explain the observed differences in δ^{18}O values measured on emitted N$_2$O. For this study, the observed low values of 12.2‰ ± 5.9‰ of emission-weighted ^{18}O-N$_2$O averages in the TNF Brazilian soils may be explained by the fact that oxygen in the NO$_3^-$ and N$_2$O precursors is exchanging with ambient water during denitrification. This has been previously proposed [*Kool et al.*, 2007] and experimentally demonstrated during a soil incubation study in Canadian soils in which the δ^{18}O-N$_2$O values were explained by 64% to 94% of the oxygen atoms in the N$_2$O precursors exchanged with those in water [*Snider et al.*, 2008]. Such a water exchange mechanism in our Brazilian TNF soils appears to be supported by the measured δ^{18}O-H$_2$O values for these soils of −4.7‰ during our sampling period. Overall, our results implies that δ^{18}O measurements alone are not very useful for determining the source of the oxygen in N$_2$O, mainly due to the large variability imposed by the ^{18}O atom exchange between water and the N$_2$O precursors.

4.2. Identifying Microbial Processes of N$_2$O Production in Soils

4.2.1. Site Preference Values for Nitrification- Versus Denitrification-Derived N$_2$O in the Literature

[32] In order to asses the usefulness of site preference values to differentiate microbial N$_2$O production and consumption in soils a detailed comparative analysis of the currently available information in the literature is required. As mentioned in the introduction, recently published site preference values from pure cultures and soil incubation studies have shown distinct site-specific nitrogen isotopic compositions for N$_2$O derived from nitrification and denitrification in soils (Figure 4). These studies suggest the potential utility of the site-preference measurements for distinguishing microbiological N$_2$O production mechanisms in soils without the need to also measure δ^{15}N of NH$_4^+$ or of NO$_3^-$ [*Pérez*, 2005; *Pérez et al.*, 2006].
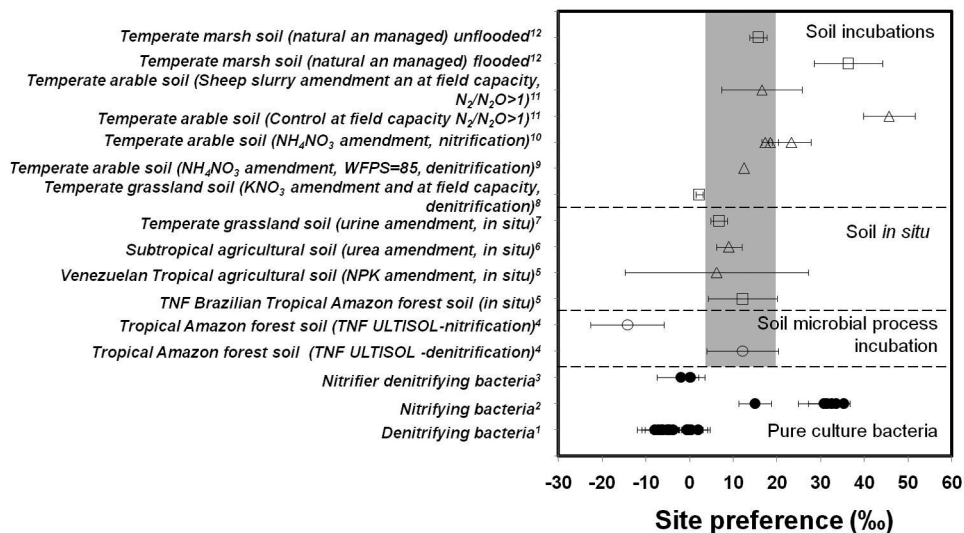
Figure 4. Site preference values (‰) for pure bacteria cultures, soil incubation studies, and in situ soil emission of the current study (emission-weighted averages), with 1σ uncertainties. Open and closed circles represent nitrification and denitrification derived by soils and bacteria culture studies, respectively. Natural and arable soils are represented by squares and triangles, respectively. References: 1, [*Sutka et al.*, 2006; *Thompson et al.*, 2004; *Ostrom et al.*, 2007]; 2 and 3, [*Sutka et al.*, 2006, 2003, 2004]; 4, [*Pérez et al.*, 2006]; 5, this paper; 6, [*Pérez et al.*, 2001]; 7, [*Yamulki et al.*, 2001]; 8, [*Bol et al.*, 2003]; 9, [*Well et al.*, 2006]; 10, [*Well et al.*, 2008]; 11, [*Cardenas et al.*, 2007]; 12, [*Bol et al.*, 2004]. Note the following: the *Toyoda et al.* [2005] value for the denitrifying bacteria *Pseudomonas fluorescence* was excluded; see section 4.2 for an explanation.

[33] However, a comparison of values for the site-specific isotopic compositions of emitted N₂O in this study with those from previous work cautions against oversimplification. The soil incubation study that produced an estimate for site preference for denitrification [*Pérez et al.*, 2006] was conducted on some of the same Brazilian soils used in this study (Ultisol) (Figure 4). The denitrification site preference reported for these soils by *Pérez et al.* [2006] coincides with the smaller values for nitrification in pure culture bacteria studies and with the upper range of values from denitrifying pure culture bacteria studies (Figure 4). On the other hand, the site preference signature for denitrification (i.e., reduction of NO_3^- to N₂O) in the TNF Amazon forest soils was 9.4‰ ± 8.1‰ [*Pérez et al.*, 2006], which is in the same range as results from other soil studies favoring denitrification conditions, such as incubation experiments for fertilized (NH₄NO₃) temperate arable soils favoring denitrification with variable water content [*Well et al.*, 2006], for temperate arable soils fertilized with sheep slurry under field capacity (with N₂/N₂O >1) [*Cardenas et al.*, 2007], and for temperate marsh soils under natural and managed unflooded conditions [*Bol et al.*, 2004] (see Figure 4). Only one soil incubation study that favored nitrification coincides with the upper limit of the *Pérez et al.* [2006] soil site preference value for denitrification [*Well et al.*, 2008]. Although, Well et al.'s soils were fertilized with NH_4^+, some denitrification indeed could not be excluded because NO_3^- concentration values at the end of the experiment were up to 3 times larger than initial values. In general, the site preference values

for denitrification derived or estimated from various soil studies lay within a narrower range than those for nitrification (see below). The largest denitrification site preference published to date is 23.4‰ ± 4.2‰ from an incubation study with *Pseudomonas fluorescence* bacteria [*Toyoda et al.*, 2005]. As the authors pointed out, under their experimental conditions abiotic N₂O formation could have taken place, and thus we excluded this value for comparison.

[34] In contrast, the soil nitrification-derived site preference value of −16.8‰ ± 8.4‰ ($n = 9$) from incubation studies [*Pérez et al.*, 2006], is very different from results from studies conducted on pure bacterial cultures, which range from 11‰ to 19‰ and 25‰ to 35‰, [*Sutka et al.*, 2006, 2003, 2004] (see Figure 4). They also differ from three temperate agricultural soil incubation studies performed under conditions that favored nitrification, with site preference values that ranged from 17.3‰ to 23.3‰ ($n = 9$) [*Well et al.*, 2008], also shown in Figure 4. However, the *Pérez et al.* [2006] values are closer in magnitude to values measured for nitrifier denitrifying bacteria under nitrite and nitrate reduction of −7‰ to 2‰ ($n = 26$) [*Sutka et al.*, 2006, 2003, 2004] (Figure 4).

[35] There are two possible explanations for these differences between the nitrification-derived site preference values found in the incubations by *Pérez et al.* [2006] and from other studies. First, the consortia of nitrifying bacteria from the TNF Amazon forest soils likely differ from the simple microorganism communities represented by the few nitrifying bacteria evaluated in the other studies. For

instance, *Yuan et al.* [2005] found that most of the nitrifying bacteria communities present in soils from different ecological regions of China were ammonium-oxidizing bacteria (*amoA*) and an order of magnitude more prevalent than nitrite-oxidizing bacteria. The sequence of *amoA* indicated that all of them were grouped into *Nitrosphira* clusters 1 and 3. Similar results were found in activated sludge and in coastal sand dunes [*Juretschko et al.*, 1998; *Kowalchuk et al.*, 1997]. Unfortunately, no previous ^{15}N site preference study of ammonium oxidation by *Nitrosphira* bacteria is available, (the only available data is for hydroxylamine oxidation process). A second explanation may be that, given the higher nitrate concentrations compared to ammonia in our tropical forest soils, the nitrifying organisms may have adapted to perform nitrifier denitrification by means of nitrite and nitrate reduction, resulting in site preference values of −7‰ to 2‰ [*Sutka et al.*, 2006, 2003, 2004], in close agreement with the *Pérez et al.* [2006] results. Therefore, we suggest that, based on the site preference data, the nitrification-derived site preference values found by *Pérez et al.* [2006] for our Brazilian soils could be representative of nitrifier denitrification. Unfortunately, no such measurement in soil has been reported in the literature to test such a conclusion.

[36] Finally, when soil conditions favor N₂O-to-N₂ reduction, N₂O site preference is largest [*Bol et al.*, 2004; *Cardenas et al.*, 2007] (Figure 4), implying that breaking the N-O bond enriches the nitrogen in the alpha position in the remaining N₂O molecules. The largest site preference values of 46‰ ± 6‰ were found by *Cardenas et al.* [2007] in unfertilized temperate arable soils under field capacity with N₂/N₂O > 1. It is interesting that even after the same soils were fertilized, the site preference value (17‰ ± 9‰) was still in a range similar to those for denitrification. Overall, N₂O reduction to N₂ in soils results in the largest site preference under nitrogen-limited conditions, and this effect is reduced when the substrate availability is no longer limited. This suggests that N₂O site preference in soils could be affected by substrate availability, as found in a *Pseudomonas aureofaciens* pure culture study under widely varying NO_3^- concentrations [*Thompson et al.*, 2004].

[37] Based on the arguments given above, the site preference value found by *Pérez et al.* [2006] for denitrification in the TNF Brazilian tropical rainforest soils could be considered representative for this process in soils due to its similar values found in previous soil incubation studies favoring denitrification. We suggest that differences in the nitrification site preference suggested by *Pérez et al.* [2006] compared to other studies might be explained by the predominance of nitrifier denitrification, though more work with natural soil bacterial consortia and studies of site preference for this pathway need to be conducted to test this hypothesis. In spite of the large uncertainties among the site preference studies for nitrification, the site preference could still be potentially useful for differentiating microbial processes in soils. However, is of particular importance to conduct soil incubation experiments aimed to characterize the N₂O isotopic site preference associated with microbial processes of N₂O production and consumption in order to reduce the current discrepancies.

4.2.2. Microbial Production Processes of N₂O Emitted From Tropical Forest and Agricultural Soils

[38] All the available field measurements of emission-weighted average values for site preference in N₂O (including this study) lie within the very narrow site preference range found for soil denitrification [*Pérez et al.*, 2006] (Figure 4, shaded area). For instance, the emission-weighted average site-specific $\delta^{15}N$ values measured in this work from the Brazilian TNF soils and the Venezuelan agricultural field show that N₂O is mostly produced by denitrification in both soils (using the site-specific signature for denitrification given above from *Pérez et al.* [2006]) (see Table 3 and Figure 4). Comparison of the site-specific values with those for N₂O measured within the soil pore space also suggest that denitrification is the most important microbial process for N₂O production in both soils at depth (Figures 3d and 3e). Deviations from the overlap in the denitrification (solid rectangle) and the site preference observed at specific depths can be explained using the concentration fluctuations in the soil profile. For example, the smallest site preference value in the soil depth profiles for the TNF Brazilian Amazon site was observed at the depth where the highest N₂O mixing ratio occurs (Figure 3e); site-specific $\delta^{15}N$ then increased toward the surface and toward deeper layers of the soil, showing a 4‰–20‰ enrichment. For the Venezuelan agricultural field on all but one day (6 July 2005), the same trend was observed (Figure 3d). However, the shifts in the site preference value with depth were less pronounced than in the Brazilian Amazon soils. The samples collected on 6 July 2005 at the Venezuelan agricultural field after the second fertilization (with NH_4NO_3) showed the smallest site preference (−3.5‰) at a depth of 10 cm where the N₂O mixing ratio was highest (~15000 ppb); the site preference then increased to its highest value (20.8‰), measured at the same site at 50 cm where the N₂O mixing ratio was about 2000 ppb (see Figures 3b and 3f). If the site preference values for nitrification and denitrification obtained from the TNF Brazilian Amazon soils (the only microbial process site preference data for soils available in the literature) [*Pérez et al.*, 2006] (Figure 4) are generalizable to the Venezuelan soils, then most of the N₂O produced in these soils would therefore be denitrification-derived, as shown in Figure 3f, which shows that most of the site preference values lie within the uncertainty of the site preference values obtained for denitrification (see Figures 3e and 3f). This is true for all but the Venezuelan soil depth profile on 6 July 2005, for which at 10 cm depth the site preference was −3.5‰. We calculated the relative contribution of nitrification or denitrification at this particular depth using the site preference value obtained from *Pérez et al.* [2006] and assuming no N₂O consumption has occurred at this depth given the high N₂O mixing ratio of 15000 ppb. We applied the following a mass balance approach:

$$SP_{10\ cm} = SP_{nit}(X) + SP_{denit}(1 - X),$$

where $SP_{10\ cm}$ is the measured site preference value for 10 cm depth, SP_{nit} and SP_{denit} are the site preference values for nitrification and denitrification, respectively [*Pérez et al.*, 2006], and X is the fraction of N₂O nitrification derived.

[39] The calculation yielded an estimate of 49% of N$_2$O nitrification-derived, which appears to be consistent with the idea that the observed high N$_2$O mixing ratio must be due to the enhancement both of nitrification (or nitrifier denitrification) and of denitrification in the soils, particularly since the second fertilization nitrogen addition was in the form of NH$_4$NO$_3$. This calculation should be taken cautiously due to the fact that is based on average of site preference values for nitrification and denitrification that have large uncertainties (Table 3). In spite of that, if in the future these uncertainties of SP estimates for nitrification and denitrification in soils can be narrowed, we can more effectively estimate the relative contribution of these processes in a particular soil.

[40] Overall, the interpretations put forth here suggest that site preference values could indeed be potentially useful for differentiating the microbial processes of N$_2$O production in the field. However, to make these interpretations more robust, additional site preference measurements on soils is required, particularly that for nitrifier denitrification.

4.3. Quantifying N$_2$O Consumption in the Soils Based on the Correlations Between Isotopologues

[41] The ratio of N$_2$O to N$_2$ has been used previously to investigate the importance of N$_2$O consumption via reduction to N$_2$. However, simultaneous measurements of N$_2$O and N$_2$ emitted from in situ soil emission samples have been limited due mainly to the problem of making direct measurements of N$_2$ emitted from the soil against the large background of N$_2$ in air [e.g., *Bol et al.*, 2003; *Scholefield et al.*, 1997; *Swerts et al.*, 1996]. In spite of the great usefulness of soil microcosm experiments, their results are limited for extrapolation purposes due to the fact that natural soil conditions are drastically changed. Alternatively, we suggest that correlations (or lack thereof) between $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, and $\delta^{18}O$ measured on in situ soil air samples can be used to examine the role of N$_2$O reduction to N$_2$ in these soils. Since the reduction of N$_2$O to N$_2$ primarily involves breaking the N$^{\alpha}$-O bond of N$_2$O, the remaining N$_2$O will become enriched in ^{18}O and in ^{15}N at the central (α) position due to a primary kinetic isotope effect in the breaking of this bond (i.e., the bonds of the light N$_2$O isotopologues break faster than those containing heavy isotopes). While a secondary kinetic isotope effect might be relevant in affecting the terminal nitrogen isotopic composition, it should be a much smaller effect than the primary effect for the isotopic composition at the central nitrogen atom position. Thus, simultaneous enrichments in ^{18}O and in ^{15}N at the central nitrogen of N$_2$O should occur if N$_2$O consumption in soils is occurring to a significant degree, resulting in a positive correlation between $\delta^{18}O$ and $\delta^{15}N^{\alpha}$ and little to no correlation between $\delta^{18}O$ and $\delta^{15}N^{\beta}$. Such correlations could therefore serve as evidence of microbial reduction of N$_2$O to N$_2$ in some environments.

[42] Using our soil air profile data, we can demonstrate how such isotopic correlations can be applied. Plots of $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ versus $\delta^{18}O$ are given in Figure 5. For the Brazilian Amazon the correlation between $\delta^{15}N^{\alpha}$ and $\delta^{18}O$ is statistically significant (R^2 = 0.66, p < 0.0001), while the correlation between $\delta^{15}N^{\beta}$ and $\delta^{18}O$ is not (R^2 = 0.00, p = 0.98). Furthermore, no statistically significant correlation

was observed between $\delta^{15}N^{bulk}$ and $\delta^{18}O$ (R^2 = 0.46, p = 0.0002; not shown), which is also consistent with our kinetic isotope effects argument given above since a positive correlation with $\delta^{18}O$ for $\delta^{15}N^{\alpha}$ and no correlation for $\delta^{15}N^{\beta}$ would result in essentially no correlation with $\delta^{18}O$ for $\delta^{15}N^{bulk}$.

[43] In contrast, for the Venezuelan soil air profile samples, the correlations between $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ versus $\delta^{18}O$ are both statistically significant (see Figure 5). This difference between the Venezuelan and the Brazilian Amazon soils could be explained by the fact that the Venezuelan cornfields are not in steady state since the sources and sinks of N$_2$O change drastically over a very short period of time due to the effects associated with the agricultural practice (e.g., soil plowing, nitrogen fertilization, N$_2$O consumption, and precipitation). Also these soils are more porous than the Brazilian soils due to the history of plowing and land use, which could lead to more rapid escape of N$_2$O to the atmosphere before it can be reduced, and therefore to less of an influence of N$_2$O-to-N$_2$ reduction on $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$. In contrast, for the Brazilian TNF forest soils, which are in steady state and have relatively stable soil nitrogen concentrations, N$_2$O microbial production is unlikely to shift dramatically during a short period of time. This implies that N$_2$O consumption to produce N$_2$ might be more noticeable under natural environmental conditions without disturbing the existing nitrogen cycling (e.g., the Brazilian forest soils) rather than a non steady state system where N$_2$O production is changing on a daily basis due to agricultural activities (e.g., the Venezuelan cornfield). Overall, the correlations among $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, and $\delta^{18}O$ provide further evidence, indeed stronger evidence than the individual ^{18}O and $^{15}N^{bulk}$ enrichments alone, that N$_2$O consumption is playing a significant role in soils prior to emission and that the relatively large enrichments in ^{15}N and ^{18}O in N$_2$O emitted from Brazilian Amazon rainforest natural soils compared to those for the agricultural fields could be a result of a more prominent role for N$_2$O consumption in the soils.

[44] Given the isotopic evidence that N$_2$O emissions in the Brazilian Amazon samples have been affected by the reduction of N$_2$O and, thus, the possibility that the amount of N$_2$O originally produced there may be larger than observed, we can attempt to estimate the fraction of the amount of N$_2$O originally produced that was consumed based solely on isotopic compositions. First, since the reduction of N$_2$O is an irreversible process, the $^{15}N^{\alpha}$ and ^{18}O isotopic compositions of the remaining N$_2$O can be described with a Rayleigh fractionation model

$$\delta^x - \delta^x_0 = \varepsilon^x \times \ln\left([N_2O]/[N_2O]_0\right), \qquad (1)$$

where x = ^{18}O or $^{15}N^{\alpha}$, the subscript o corresponds to initial values, and ε^x is the enrichment factor in ‰. We assume that the observed N$_2$O mixing ratio corresponds to the remaining N$_2$O. Thus, the quantity $(1 - [N_2O]/[N_2O]_o)$ gives the fraction of N$_2$O consumed. In using equation (1) to solve for the quantity $(1 - [N_2O]/[N_2O]_o)$, note that the initial $\delta^{15}N^{\alpha}$ and $\delta^{18}O$ values (i.e., the source N$_2$O isotope compositions for the N$_2$O initially produced) and the enrichment factors (ε^x) are also unknown and we must estimate them.
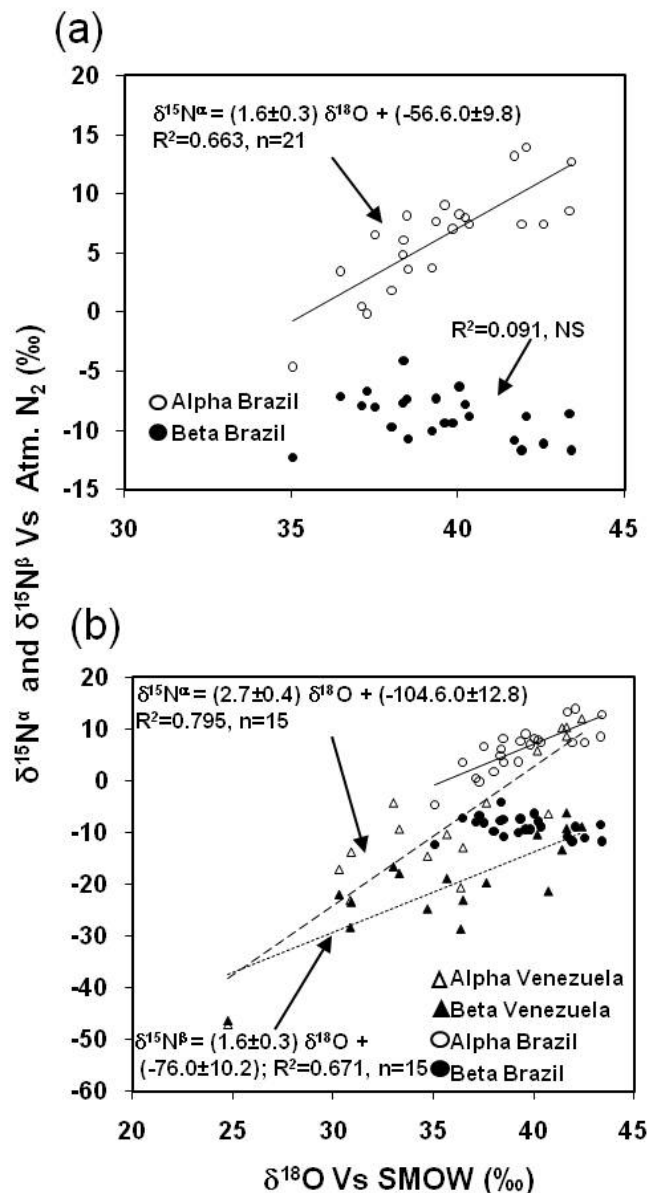
Figure 5. The $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ versus $\delta^{18}O$ of N₂O in soil air depth samples from (a) the Brazilian Amazon Forest soils and (b) the Venezuelan agricultural cornfield and Brazilian Amazon Forest soils (for comparison). Linear regressions for Brazilian $\delta^{15}N^{\alpha}$ versus $\delta^{18}O$ (solid line), Venezuelan $\delta^{15}N^{\alpha}$ versus $\delta^{18}O$ (dashed line), and Venezuelan $\delta^{15}N^{\beta}$ versus $\delta^{18}O$ (dotted line) are given.

The initial values for the isotopic compositions can be inferred from the y-intercepts of Keeling plots, which are plots of the isotopic composition versus the inverse of the mixing ratio of the species of interest and are based on a two end-member mixing model; the y-intercept of $\delta^{15}N^{\alpha}$ versus $1/[N_2O]$ is $-1.0‰ \pm 1.7‰$ ($R = 0.71$, $n = 28$, $p < 0.0001$) and the y-intercept of $\delta^{18}O$ versus $1/[N_2O]$ is $+36.9‰ \pm 0.9‰$ ($R = 0.57$, $n = 28$, $p < 0.001$). For the remaining

unknowns, ε^{α} and ε^{18}, note that, if we know either of the two, the other can be estimated from the slope of the $\delta^{15}N^{\alpha}$ versus $\delta^{18}O$ plot in Figure 5, which is equal to $\varepsilon^{\alpha}/\varepsilon^{18}$. Thus, $\varepsilon^{\alpha}/\varepsilon^{18} = +1.6 \pm 0.3$. We must next make the assumption that an average value for ε^{18} of $\sim-15‰$ for the reduction of N₂O [*Menyailo and Hungate*, 2006; *Ostrom et al.*, 2007; *Vieten et al.*, 2007] is relevant for the soils of this study (at least for the sake of our back of the envelope calculation here). Thus, we derive a value for ε^{α} of $-24‰$. Using these estimates for $\delta^{15}N^{\alpha}{}_o$, $\delta^{18}O_o$, ε^{α}, and ε^{18}, equation (1) can be used to give a rough estimate of the fraction of N₂O consumed $(1 - [N_2O]/[N_2O]_o)$. Depending on the "final" (i.e., measured) values for $\delta^{15}N^{\alpha}$, $\delta^{18}O$, and N₂O mixing ratio (which in turn depended on soil depth), the fraction of N₂O consumed ranged from 0.3% to 48% for $\delta^{15}N^{\alpha}$ and from 1% to 35% for $\delta^{18}O$ varying with N₂O mixing ratio. We find an inverse correlation (non significant) between the values of percentage of N₂O consumption to N₂ and concentration ($R^2 = 0.20$ and $R^2 = 0.34$ for the estimates calculated with $\delta^{18}O$ and $\delta^{15}N^{\alpha}$, respectively). Although non significant, this correlation suggests that higher N₂O concentrations leads to more production processes in the soils than consumption. These percentage estimates of N₂O to N₂ consumption suggest that a considerable fraction of N₂O produced in these tropical forest soils during the rainy season may have been consumed under the surface before the remainder diffused out to the atmosphere. Although this calculation is very approximate, it is a new approach based solely on observed isotopic compositions.

5. Conclusions

[45] We presented measurements of $\delta^{15}N^{bulk}$, $^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, and $\delta^{18}O$ of N₂O on in situ soil surface emissions and soil air samples collected during the rainy season in a tropical forest (Tapajos National Forest, TNF, Para, Brazil) and in a tropical agricultural cornfield ("Fundo Tierra Nueva," Guárico State, Venezuela), and used them to test the utility of various isotopic tracers for deciphering the microbial pathways of the production and consumption of N₂O in soils.

[46] N₂O produced in Brazilian Amazon tropical forest soils was more enriched in $^{15}N^{bulk}$ and ^{18}O than that produced in the Venezuelan agricultural cornfield, reflecting different isotopic signatures associated with "natural" versus "anthropogenic" N₂O sources (i.e., background forest soils versus N-fertilized agricultural soils). Similarities in the emission-weighted averages of the site-specific ^{15}N between the Brazilian Amazon soils and the Venezuelan agricultural cornfield seem to imply that the site-specific values may be less useful as tracers for distinguishing and quantifying natural and anthropogenic N₂O sources compared to the bulk ^{15}N isotopic composition. However, the in situ site-specific $\delta^{15}N$ results for N₂O showed a potential for identifying soil microbial processes of N₂O production (e.g., nitrification or nitrifier denitrification versus denitrification), particularly in combination with the results of soil incubation experiments of *Pérez et al.* [2006]. The measurements from the Brazilian Amazon soils and the Venezuelan agri-

cultural field revealed that most of the N_2O emitted to the atmosphere at both sites is produced by denitrification. This appears to be consistent with the currently accepted idea that denitrification is responsible for a larger fraction of global N_2O production than nitrification [e.g., *Müller et al.*, 1997; *Williams et al.*, 1998]. We also found that the correlations among $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, and $\delta^{18}O$ can be used as a new tracer of N_2O consumption (i.e., reduction of N_2O to N_2 via denitrification) in soils prior to emission. The measurements and our analyses performed in this study demonstrate the possible applications of site-specific isotope compositions of N_2O measured to interpreting the sources and sinks of N_2O in natural and agricultural soils, and suggest that combining these measurements with $\delta^{15}N^{bulk}$ measurements and N_2O concentrations should ultimately make it easier to understand and quantify the large spatial and temporal variations in N_2O isotopic compositions so far observed in the atmosphere.

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