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TREE SPECIES OF THE CENTRAL AMAZON AND SOIL MOISTURE ALTER STABLE ISOTOPE COMPOSITION OF NITROGEN AND OXYGEN IN NITROUS OXIDE EVOLVED FROM SOIL

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The use of stable isotopes of N and O in N_2O has been proposed as a way to better constrain the global budget of atmospheric N_2O and to better understand the relative contributions of the main microbial processes (nitrification and denitrification) responsible for N_2O formation in soil. This study compared the isotopic composition of N_2O emitted from soils under different tree species in the Brazilian Amazon. We also compared the effect of tree species with that of soil moisture, as we expected the latter to be the main factor regulating the proportion of nitrifier- and denitrifier-derived N_2O and, consequently, isotopic signatures of N_2O.

Tree species significantly affected $\delta^{15}N$ in nitrous oxide. However, there was no evidence that the observed variation in $\delta^{15}N$ in N_2O was determined by varying proportions of nitrifier- *vs.* denitrifier-derived N_2O. We submit that the large variation in $\delta^{15}N$-N_2O is the result of competition between denitrifying and immobilizing microorganisms for NO_3^-. In addition to altering $\delta^{15}N$-N_2O, tree species affected net rates of N_2O emission from soil in laboratory incubations. These results suggest that tree species contribute to the large isotopic variation in N_2O observed in a range tropical forest soils. We found that soil water affects both ^{15}N and ^{18}O in N_2O, with wetter soils leading to more depleted N_2O in both ^{15}N and ^{18}O. This is likely caused by a shift in biological processes for ^{15}N and possible direct exchange of ^{18}O between H_2O and N_2O.

Keywords: Denitrification; Natural variations; Nitrification; Nitrogen 15; Nitrous oxide; Oxygen 18; Tree species; Tropical soils

INTRODUCTION

The increase in the atmospheric concentration of nitrous oxide (N_2O) has attracted considerable scientific attention from different disciplines during the last 10–15 years, because N_2O is one of the major greenhouse gases and also participates in the destruction of the ozone layer [1]. Denitrification and nitrification are the main biological processes leading to N_2O formation and emission from the soil. Understanding the relative contributions

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of each process to total N_2O emission is critical for modeling and predicting changes in N_2O fluxes under varying environmental conditions, including altered temperature and precipitation patterns. Denitrification is known to be favored when soils are moist and anaerobic, whereas nitrification is favored under more mesic to xeric conditions. The sources of N_2O can be identified using selective inhibitors, sterilization, or by adding substrates [2], but all of these methods are destructive and intrusive, and thus may not accurately reflect the sources of N_2O [3]. Another possible way to identify the processes producing N_2O is to study fluctuations in the isotopic composition of N_2O around natural abundance [4, 5]. The isotopic composition provides a window through which we may understand biological processes underlying N_2O emission from the soil, since the isotopic composition (*i.e.* ratios of $^{15}N/^{14}N$ and $^{18}O/^{16}O$) of denitrifier-derived N_2O differs from that of nitrifier-derived N_2O [6, 7]. Despite the potentially high value of the knowledge of isotopic composition, there are very few published data and a general lack of understanding of the mechanisms and extent of isotopic fractionation of N_2O. For example, a large variation of stable isotopes in N_2O is currently interpreted as a different contribution of nitrification and denitrification without any information on activities of these processes. N_2O produced by nitrification is often more depleted in ^{15}N than that produced by denitrification, leading to the suggestion that the $\delta^{15}N$ composition of N_2O emitted from the soil is thought to reflect the relative contributions of nitrification and denitrification to the total N_2O flux [4, 7–10]. The picture is complicated by N_2O-reductase, which also selects light N_2O for reduction to N_2, enriching the remaining un-reacted N_2O in ^{15}N, and thereby further contributing to the more positive $\delta^{15}N$ values associated with denitrification [8]. The scarcity of the available information is also explained by methodological difficulties and the large air sample volume ($\sim$100 L) necessary for ^{15}N and ^{18}O measurements. Recent advances in sample preparation procedure [11] promise rapid growth of the data and deeper understanding of N_2O fluxes and underlying processes.

Tropical forest soils are known to be one of the main natural sources of N_2O [12]. In recent years, large tracts of tropical rain forests have been subjected to conversion to grasslands or tree-based agroforestry systems. Tree species altering soil microbial processes would also be expected to affect the isotopic composition of N_2O. Pérez *et al.* [13] measured the stable isotopic composition of N_2O evolved from soils in tropical rain forests, and observed large spatial heterogeneity in $\delta^{15}N$ and $\delta^{18}O$ values in N_2O. Here, we evaluate whether the influence of individual tree species on soil processes contributes to this observed variation in the stable isotopes of N_2O evolved from soil. We also compared the effects of tree species with that of soil moisture, as moisture is likely to influence the proportion of nitrifier- and denitrifier-derived N_2O and, consequently, the isotopic signatures of N_2O. We expected that, at low soil moisture, nitrification would be the main source of N_2O, leading to depleted $\delta^{15}N$ values, and that denitrification, in contrast, would prevail at high soil moisture, leading to enrichment of $\delta^{15}N$. Variation in soil moisture was used to alter the relative contribution of denitrification and nitrification to N_2O efflux. Because of potential influence of N_2O-reductase on stable isotopes in N_2O, its activity was measured in all soils. For this, C_2H_2 inhibition technique in anaerobic incubation experiments was used. We assumed that soils with the highest potential N_2O reduction capacity would also have the strongest effect of N_2O-reductase on stable isotopes in N_2O under anaerobic conditions.

MATERIALS AND METHODS

Research Site

The experimental plots of the Empresa Brasileira de Pesquisa Agropecuaria (Embrapa – Amazonia Ocidental) are located 40 km north of Manaus (Central Amazon). The soil type

is a Xanthic Ferralsol [14], which is typical for the "terra firme" ecosystems of the Central Amazon, Brazil. The climate is humid tropical with an average annual precipitation of 2503 mm. This soil is characterized by a high clay content (80%), low nutrient content and an acidic reaction (pH < 4.5).

The experiment was set up in a completely randomized block design with three blocks, each consisting of four plots. The plots were established on an abandoned rubber plantation after slash and burn for the tree cropping systems (one plot with timber and one with non timber species) and the secondary forest (one plot). Adjacent to the three plots was another plot within the natural forest; together these four plots constituted one block. We analyzed the data using a completely randomized design, where species was the only treatment, assuming thus that there is no block effect.

The studied trees were two non-timber tree species – *Bixa orellana* L. (annatto; used as natural dye), *Theobroma grandiflorum* Willd. (ex Spreng.) K. Schum. (cupuaçu; for ice-cream and juice production), growing together on the one plot, and two species planted for wood production – *Carapa guianensis* Aubl (andiroba) and *Ceiba pentandra* (growing together on second plot). Plots with secondary forests consisted of 1 species – *Vismia sp.* In the plots of primary forest two species were chosen – *Oenocarpus bacaba* and *Eschweilera* spp. Both trees are typical of the terra firme forest.

Bixa was planted in rows with 4 m distance in the row and *Theobroma* with 7 m alternating with Brazil nut (*Bertholletia excelsa* Humb. Bonpl.). Total planting density was 93.3 and 156.3 trees ha^{-1} for *Theobroma* and *Bixa*, respectively. At the natural forest sites no other tree species were closer than 4 m from the stem of the tested species, so neighboring trees likely had little effect on the microbial activities or soil chemistry compared to *Oenocarpus* and *Eschweilera.* The tree plantations and the secondary forest were 7 years old at the time of the experiment.

In February 1999, soil cores at 0–5 cm depth were taken at 50 cm distance from tree stems using a soil auger. We took 4 soil samples under two trees in each plot and these 8 samples were mixed. All the 21 soil samples (3 aggregated samples for each of the 7 species) were air-dried, sieved (2 mm) and transported to the laboratory.

Incubation Experiments

As N_2O-reductase may significantly affect $\delta^{15}N$ values in N_2O [13], its activity was measured according to [15]. In short, soil samples were moistened with distilled water and pre-incubated at 28 °C during 3 days. 1 ml of water solution with KNO_3 and glucose was added to 5 g of soil in 20 ml flask. The resulting concentration of nitrate was 40 mg NO_3-N $kg\ soil^{-1}$, that of glucose, 100 mg C kg^{-1} soil. Anaerobiosis was achieved by replacing the headspace of each flask with He and sealing with airtight rubber stoppers and clamps. Half of the sub-samples received 2.5 ml (10% v/v) of C_2H_2 as an inhibitor of N_2O-reductase. Soil samples were incubated for 96 hours at 28 °C. After 6, 12, 24, 48, 72 and 96 h, the headspace of each flask (1 ml) was sampled for the analysis of N_2O using gas chromatography (GC) – Shimadzu 14A, equipped with electron capture detector (ECD ^{63}Ni). Results were recorded as mg N_2O-N kg^{-1}. Activity of N_2O reduction to N_2 was calculated as a difference between N_2O production without and with C_2H_2.

A separate aerobic incubation experiment was conducted to study the isotopic composition of N_2O. For this, 20 g of each soil sample was placed in 100 ml glass flasks, moistened with distilled water and pre-incubated at 28 °C during 3 days to initiate microbial activities. After that, a distilled water solution with NH_4NO_3 was added to each flask to increase the concentration of inorganic N to 500 mg NH_4NO_3-N kg^{-1}. Soil moisture contents were adjusted to be 30%, 60% and 90% of the water holding capacity (WHC). The flasks were closed with

airtight rubber stoppers, fixed with clamps, and than incubated for 72 h. During the incubation the headspace of each flask was sampled at 24 h, 48 h and 72 h (last time point was not measured in soils under *Ceiba, Oenocarpus* and *Vismia*). At each sampling, we removed 1 ml of the headspace to determine N_2O concentrations using GC as described above and 5 ml of headspace to determine the isotopic composition of N_2O using a coupled gas chromatograph/isotope-ratio mass spectrometer (GC-IRMS). The 5 ml samples were injected in 100 ml glass containers filled with He, stored (1–2 days), and after that measured on the GC-IRMS. In contrast to the first experiment, this second incubation experiment involved: i) aerobic conditions and neither glucose nor C_2H_2 application to allow nitrification to take place, ii) addition of NH_4NO_3 instead of KNO_3 to promote both nitrification and denitrification, and iii) three moisture levels to obtain a range of proportions of nitrifier- and denitrifier-derived N_2O. We expected that increasing soil moisture should increase the proportion of N_2O derived from denitrification and decrease N_2O from nitrification.

Isotopic Measurements in N_2O

The ratios of the stable isotopes $^{15}N/^{14}N$ and $^{18}O/^{16}O$ in N_2O emitted from soils were determined using an on-line GC-IRMS system, consisting of a trace gas cryogenic preconcentration device (PreCon, FINNIGAN MAT, Bremen), gas chromatograph (Hewlett-Packard 5890, Series II), and an isotope-ratio mass spectrometer (Delta S, FINNIGAN MAT, Bremen).

Ion currents corresponding to m/z 44, 45 and 46 were used to obtain the ratios of masses 45:44 and 46:44 in N_2O samples were measured which were then used to estimate ratios of $^{15}N^{14}N^{16}O/^{14}N_2{}^{16}O$ and $^{14}N_2{}^{18}O/^{14}N_2{}^{16}O$. Other combinations of the isotopes of N and O were discounted because they are so rare that their contributions to the 45 and 46 mass peaks are negligible [11]. The isotopic composition was expressed using the δ notation, in which increasingly larger δ values (less negative) indicate enrichment of the heavier isotope (^{15}N or ^{18}O) in the sample N_2O relative to that in the reference:

$$\delta = \frac{R_{\text{sample}} - R_{\text{reference}}}{R_{\text{reference}}} \times 1000‰$$

where R is the ratio $^{15}N/^{14}N$ or $^{18}O/^{16}O$.

We used N_2O as a working reference gas (99.9990%, Linde), with $\delta^{15}N$ determined via reduction to N_2 under copper at 1150 °C. This signature was then compared to an IAEA-calibrated N_2 reference gas. The $\delta^{18}O$ signature of the reference N_2O was determined by comparison with CO_2 of known $\delta^{18}O$.

Statistical Data Analysis

The first incubation experiment was performed with two replications for each sample. The effects of species, time and C_2H_2 treatment and their interactions were determined by three-way ANOVA (repeated measures design) with 3 replicates (means of two measurements for each of three soil samples under each species). The second incubation experiment was done without replicates because determination of isotopes in N_2O is still very time-consuming process (20 min for one measurement), thus yielding 3 replicates (3 soil samples for 1 tree species). Because of an incomplete design (some isotope data were missing and under three species the 72 h point was not measured), and because our primary interest was effect of species and moisture, we considered time as replicate and not as a factor. The effects of species and moisture on ^{15}N and ^{18}O in N_2O were calculated with two-way

ANOVA. Where the main effect was significant, the post hoc comparison with Turkey test was performed to identify which species and/or what moisture level differed significantly. We considered the effect significant at $P < 0.05$. All statistics were carried out with the statistical package STATISTICA [16].

RESULTS

N_2O-reductase Activity

Cumulative N_2O production measured in the N_2O-reductase incubation experiment (with and without C_2H_2) and calculated N_2O consumption are presented in Figure 1(A). All three factors (species, time, presence of C_2H_2) strongly affected the amount of N_2O produced (for all, $P < 0.001$).

Over the entire 96 h incubation with the C_2H_2 treatment, soils under *Theobroma* and *Carapa* produced 43–85 mg N_2O-N kg^{-1}, significantly more than soils from under the other species. Soils from under *Bixa*, *Ceiba*, *Eshweilera*, *Oenocarpus*, and *Vismia*, did not differ in N_2O production with added C_2H_2, accumulating between 28 and 54 mg N_2O-N kg^{-1} during the 96 h incubation period.

In all soils, except under *Carapa*, net N_2O accumulation (without C_2H_2) was positive for all measurement times, indicating that N_2O-reductase activity was lower than the activity of N_2O-producing enzymes. Only under *Carapa* did N_2O concentration begin to decline after 72 h, at which point the rate of N_2O reduction exceeded the rate of N_2O production. Low but detectable N_2O reduction occurred in all soils after 24 hours, except from *Oenocarpus*, where N_2O reduction was not detected until after 48 hours. The rate of N_2O reduction increased with time under *Theobroma* and *Carapa*, but not for the other species. By the end of the incubation period, soils under *Theobroma* and *Carapa* had consumed about 50% of the total amount of N_2O produced during the 96 h incubation. Thus, isotopic discrimination by N_2O-reductase is most likely to be apparent in soils beneath *Theobroma* and *Carapa.*

Net N_2O Production

Net N_2O production during the aerobic incubation experiment is presented in Figure 1(B). Species ($P < 0.001$) and moisture ($P = 0.025$) affected N_2O production, but the effects of species depended on moisture content (interaction, $P = 0.050$). For example, soils under *Ceiba* and *Oenocarpus* formed the highest amount of N_2O at 30% and 60% WHC, respectively, while soils under other species produced higher amount of N_2O at the highest moisture content (90% WHC).

The slight decrease in net N_2O accumulated with time was observed only under *Carapa* at 72 h and 90% WHC. This might be attributed to N_2O-reductase activity, given that the highest potential N_2O-reductase activity was also observed under *Carapa.*

Net CO_2 Production

The rate of CO_2 production during the second aerobic incubation experiment with varying moisture levels is presented in Figure 2. The CO_2 production was strongly affected by tree species ($P < 0.001$), but not by soil moisture. *Ceiba, Oenocarpus* and *Vismia* produced more CO_2 than all other species ($P < 0.001$).

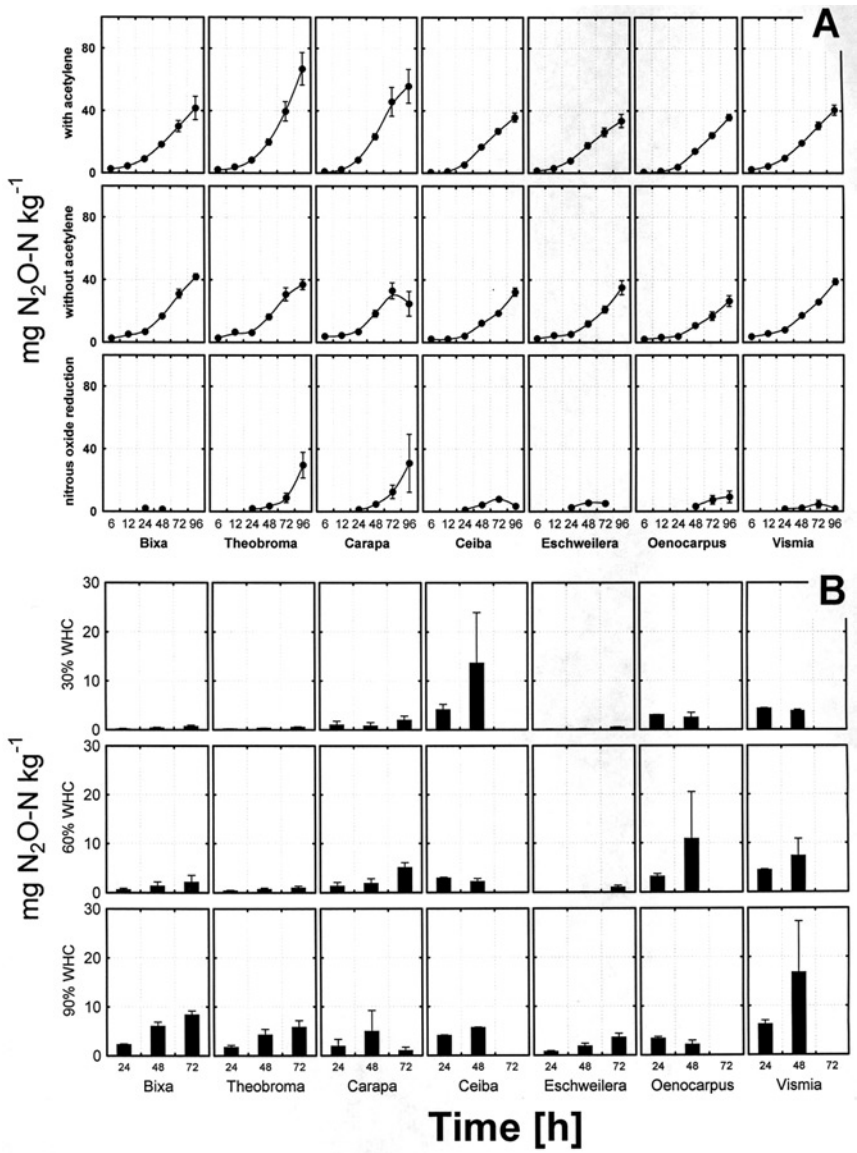

FIGURE 1 (A) Net N_2O production (mg N_2O-N kg^{-1}) during anaerobic incubation in the presence and absence of C_2H_2, and N_2O reduction activity (calculated as the difference between N_2O concentration with and without C_2H_2). The symbols indicate means ± standard errors ($n = 3$). (B) Net N_2O production (mg N_2O-N kg^{-1}) of soils beneath different tree species in Brazilian Amazon during aerobic incubation under three soil moisture levels (means and standard errors; $n = 3$).

δ^{15}N Signature in N_2O

Measured δ^{15}N-N_2O values varied in our soils from −37.8 to 2.7‰ (Fig. 3(A)). The δ^{15}N values were significantly affected by tree species ($P < 0.001$) and by soil moisture ($P = 0.030$) with significant interaction between these factors ($P = 0.033$), indicating that the tree species had a different effect on the isotopic composition of N-N_2O at different levels of soil moisture content. Species affected δ^{15}N-N_2O only at 30 and 60% of WHC. At 30% WHC, *Ceiba* had a significantly higher δ^{15}N-N_2O value than *Carapa* ($P = 0.012$), while at

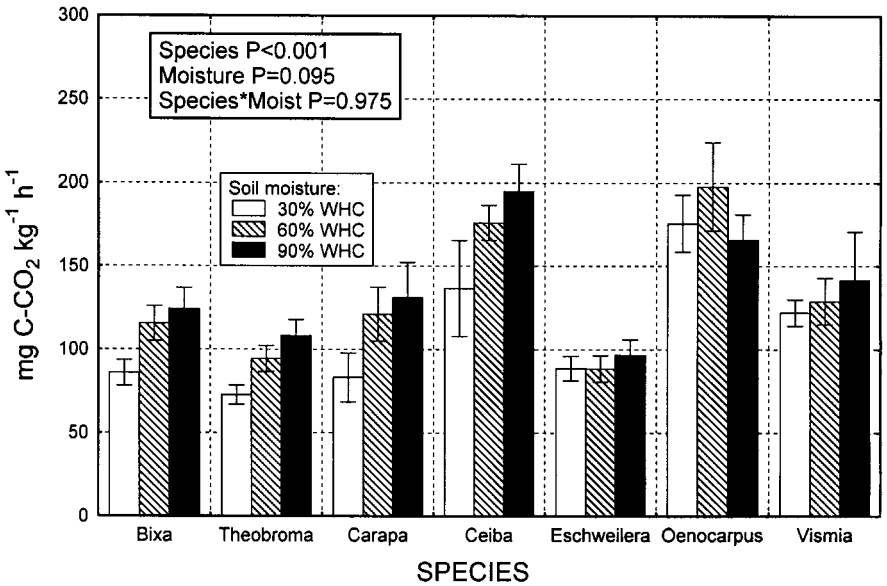

FIGURE 2 The rate of CO_2 production (mg CO_2-C kg^{-1} h^{-1}) in soils beneath different tree species in the Brazilian Amazon during aerobic incubation under three soil moisture levels. Values are means ± standard errors ($n = 3$)

60% WHC, *Ceiba* and *Oenocarpus* had higher δ^{15}N-N_2O values than all other species except *Eschweilera* ($P = 0.058$).

Overall, considering all moisture levels together, the heaviest nitrogen in N_2O was under *Ceiba* (-9.3 ± 2.6‰, $N = 10$) and *Oenocarpus* (-9.8 ± 2.4‰, $N = 9$). The values significantly differed from all other species ($P < 0.001$). The most depleted δ^{15}N-N_2O was under *Carapa* (-23 ± 2‰, $N = 15$) and *Theobroma* (-20 ± 1.4‰, $N = 9$); these values however did not significantly differ from those found under *Bixa*, *Eschweilera* and *Vismia.*

As stated above, water contents strongly affected nitrogen isotopes in N_2O (Fig. 4), with decreasing δ^{15}N values as soil water content increased. The significant difference in δ^{15}N was between 30% and 90% of WHC ($P = 0.010$).

δ^{18}O Signature in N_2O

Measurements in our soils showed larger variation in δ^{18}O-N_2O (from -21.2 to 45.8‰) than in δ^{15}N-N_2O (Fig. 3(B)). Tree species did not affect δ^{18}O in N_2O ($P = 0.222$), whereas soil moisture had a highly significant effect ($P < 0.001$). In contrast to δ^{15}N, no interaction between tree species and soil moisture was found ($P = 0.227$), suggesting that the effects of soil moisture on δ^{18}O in N_2O are similar under all tree species. Overall, δ^{18}O decreased with increasing soil water content, the same pattern as that found for δ^{15}N (Fig. 4). The δ^{18}O-N_2O values at 90% WHC were lower than those at 30 and 60% WHC (for both, $P < 0.001$).

DISCUSSION

Species Effect on δ^{15}N-N_2O

Measured δ^{15}N-N_2O values varied from -37.8 to 2.7‰, nearly in the same range as recently reported for tropical forest soils in Costa Rica and Brazil – from -34 to 2‰ [13]. We found a

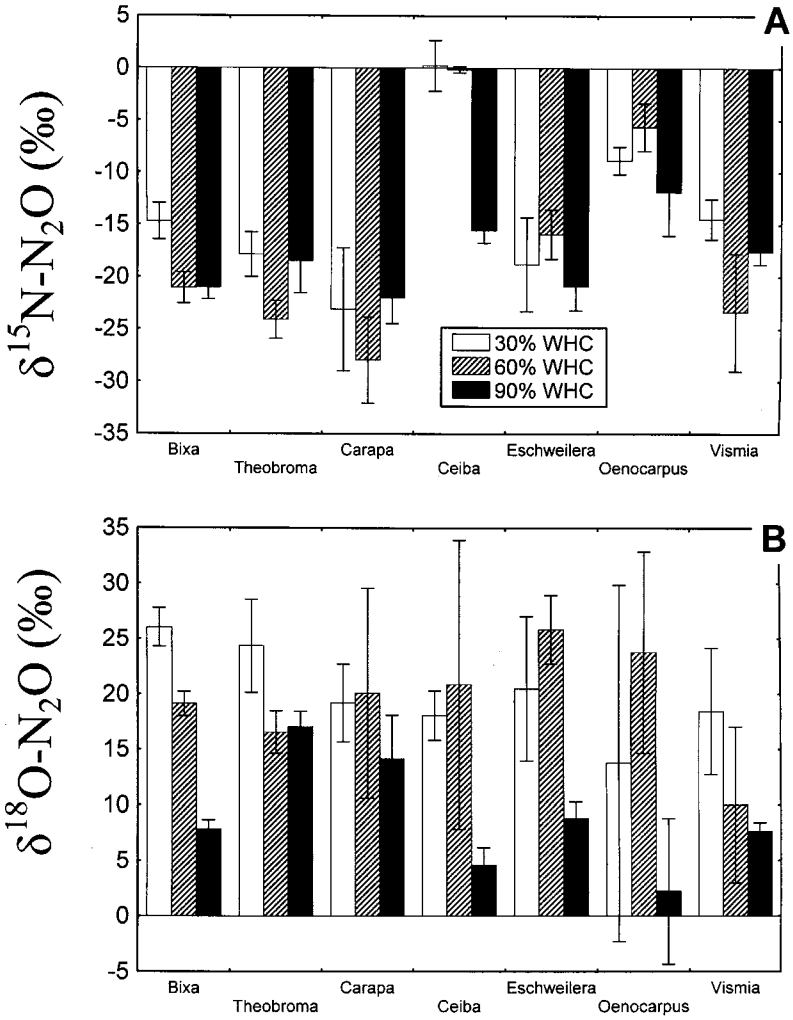

FIGURE 3 δ^{15}N (A) and δ^{18}O (B) in N_2O (‰) produced by soils beneath different tree species in Brazilian Amazon during aerobic incubation at three soil moisture levels. Values are means ± standard errors ($n = 3$)

strong effect of tree species: soils under *Ceiba* and *Oenocarpus* produced 10–25‰ heavier δ^{15}N-N_2O than the other species. The significant differences in the isotopic composition of δ^{15}N-N_2O suggest different microbiological processes responsible for N_2O production under different species.

We found that *Ceiba* and *Oenocarpus* had the lowest values of denitrification potential and the highest rates of CO_2 production. However, the higher relative contribution of nitrification to N_2O flux under these species seems unlikely because nitrification should discriminate more against the heavy isotope of N in N_2O, producing N_2O with δ^{15}N that is approximately 30‰ more negative than those associated with denitrification [4, 13, 17]. In contrast, the δ^{15}N values of N_2O produced under *Ceiba* and *Oenocarpus* were more positive than the other species. N_2O-reductase was also unlikely to have contributed to δ^{15}N discrimination, as its activity was low under these species.

We suspect that NO_3^- immobilization affected δ^{15}N-N_2O by leading to substrate limitation of denitrification. Isotope discrimination is strongest when substrates are non-limiting [18].

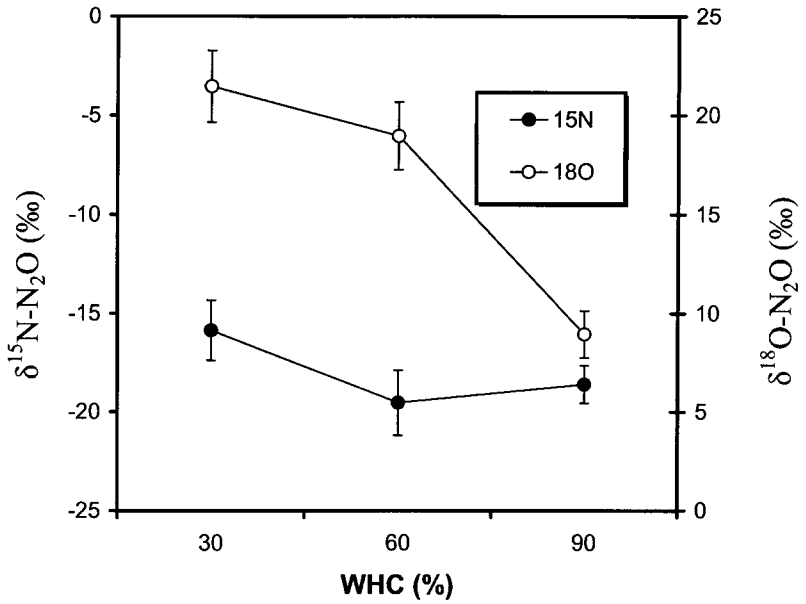

FIGURE 4 Effects of soil moisture content on $\delta^{15}N$ and $\delta^{18}O$ (‰).

If denitrification was the main source of N_2O produced under *Ceiba* and *Oenocarpus* even at 30 and 60% WHC, and in these soils denitrification was limited by NO_3^- availability due to the high activity of heterotrophic C-mineralizing microorganisms, one would expect the isotope effects during denitrification to be relatively small and the $\delta^{15}N$-N_2O values to be more positive. Another possible explanation is that discrimination against ^{15}N during microbial immobilization of NO_3^- enriched the remaining NO_3^- in $\delta^{15}N$ values, leading to the more positive $\delta^{15}N$-N_2O observed, which is likely high under *Ceiba* and *Oenocarpus.* Fractionation during NO_3^- immobilization remains poorly understood and will require further investigation. However, published values for isotopic discrimination against ^{15}N during nitrate and ammonium assimilation are near 10‰ [19–21]. Thus, fractionation during NO_3^- immobilization may partly explain the difference in $\delta^{15}N$-N_2O values under *Ceiba* and *Oenocarpus*.

Based on published data for isotopic effects during denitrification, the differences between $\delta^{15}N$-N_2O and $\delta^{15}N$-NO_3^- should be 13–28‰ [8, 22]. The isotope effect measured in our study for 5 species were thus in the reported range (18–25‰), if we assume that $\delta^{15}N$ values of NO_3^- applied were around 0‰ as typically found for man-made fertilizers. The difference in $\delta^{15}N$-N_2O between *Ceiba* and *Oenocarpus* and all other species were 10–25‰, a slightly smaller difference possibly due to NO_3^- limitation of denitrification resulting in a smaller isotope effect.

Thus, the observed effect of the species on $\delta^{15}N$-N_2O was likely caused not by differences in the proportion of nitrifier- *vs.* denitrifier-derived N_2O, but rather by differences among plant species in their effects on soil NO_3^- immobilization.

Soil Moisture Effect on Stable Isotopes in N_2O

We expected to observe an increase in $\delta^{15}N$-N_2O values with increasing soil moisture due to increase in denitrifier-derived N_2O. However, we observed the opposite pattern, with increasing

soil moisture further depleting $\delta^{15}N\text{-}N_2O$ values. Hence, this pattern is very unlikely due to a shift in nitrification/denitrification ratio. Even if most N_2O formed at 90% of WHC was derived from denitrification, the observed depletion in $\delta^{15}N\text{-}N_2O$ could be due to a greater isotope effect due to reduced substrate limitation by nitrate-immobilizing microorganisms. However, the data on CO_2 production do not directly support this conclusion, as soil moisture had no effect on CO_2 production. The possible explanation could be that microorganisms change the preference for assimilation of inorganic N forms from NO_4^+ to NO_3^- as soil moisture increases. This would result in higher NO_3^- limitation to denitrification at higher soil moisture. However, testing this hypothesis requires further research.

Whereas tree species did not alter $\delta^{18}O\text{-}N_2O$, increasing soil moisture caused a decline in $\delta^{18}O\text{-}N_2O$. Compared to $\delta^{15}N$ values, less data are available in the literature on $\delta^{18}O$ variation in N_2O. The $\delta^{18}O$ in N_2O formed by nitrification may reflect the isotopic signatures hydroxylamine, molecular oxygen and soil water [13]. The $\delta^{18}O$ of denitrifier-derived N_2O should reflect the isotopic composition of NO_3^-, the substrate for denitrification. The reported values for oxygen fractionation during denitrification differ significantly. For example, Barford [23] estimated an ε of 105‰, assuming no interaction of oxygen isotopes between N_2O and H_2O. Wahlen and Yoshinari [9] determined that, during N_2O reduction by denitrifying organisms, ^{18}O gets heavier by a range of 37 to 42‰.

Measurements in our soils revealed a larger variation in $\delta^{18}O\text{-}N_2O$ (from -21.2 to 45.8‰) than in $\delta^{15}N\text{-}N_2O$. These values covered also a wider range than reported for $\delta^{18}O$ by Pérez *et al.* [13]: from -4 to 18‰. One of the possible explanations is the wider range of soil moisture contents in our incubation experiments (30–90% WHC) than in the fields where Pérez *et al.* conducted their measurements (57–95%, WFPS).

The reduction in $\delta^{18}O$ values with increasing soil moisture may have been caused by direct exchange of oxygen between N_2O and H_2O. In contrast to ^{15}N, the largest difference in $\delta^{18}O$ was between the mesic and highest soil water content, providing evidence that the direct effect of soil water on ^{18}O in N_2O is likely the more important factor affecting $\delta^{18}O$ in these soils. If we assume that all N_2O was derived from denitrification and that N_2O consumption did not affect the isotopic signatures in N_2O, the soil water seems to be important factor even during denitrification. This may be caused by exchange of ^{18}O of water with oxygen in N_2O and/or NO_3^-. We suggest that the effect of soil moisture on $\delta^{18}O\text{-}N_2O$ values likely results from different levels of equilibration of oxygen isotopes between N_2O and H_2O. However, the $\delta^{18}O$ values of water and nitrate used in our studies were not known, limiting our ability to understand the mechanisms causing variation in $\delta^{18}O\text{-}N_2O$ values. Nevertheless, the different patterns of response to soil moisture and tree species observed for $\delta^{15}N$ and $\delta^{18}O$ in N_2O would suggest different mechanisms affecting both isotopes, biological for ^{15}N and physical (direct exchange) for ^{18}O.

CONCLUSIONS

To our knowledge, this is the first study to show that plant species significantly affect ^{15}N in nitrous oxide evolved from soil. This is probably due to tree-mediated changes in microbiological activities. These effects of individual tree species might contribute to the variation in $\delta^{15}N\text{-}N_2O$ values observed in tropical forest soils [13]. However, there was no evidence that ^{15}N in N_2O was determined by the relative contributions of nitrification and denitrification in N_2O efflux. Based on $\delta^{15}N\text{-}N_2O$ measurements the differences among species may have been due to (a) NO_3^- limitation for denitrification caused by immobilization or (b) fractionation during nitrate immobilization. However, both explanations require experimental verification.

Nevertheless, our results suggest that the variation in $\delta^{15}N$ values in N_2O observed under field conditions may not be very useful in distinguishing the relative contributions of nitrification and denitrification to N_2O flux. Other processes, *i.e.* immobilization and related nitrate limitation, could affect isotopes in N_2O. The $\delta^{15}N$-N_2O values could indicate the strength of competition between denitrifying and nitrate immobilizing microorganisms. Finally, we demonstrated that soil water content affects the stable isotopic composition of N and O in N_2O evolved from soil, possibly due to both effects of water content on microbial activities and due to oxygen exchange between H_2O and N_2O.

Overall, analysis of stable isotopes in nitrous oxide has potential to lend insight to soil nitrogen cycling and the mechanisms of N_2O turnover. But before this method can be effectively used in the field, much work remains to be done to fully characterize the factors contributing to N_2O fractionation.

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