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The stable carbon and nitrogen isotopic composition of vegetation in tropical forests of the Amazon Basin, Brazil

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Abstract. Here we present the within-site, seasonal, and interannual variations of the carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratios of leaves, wood, bark and litter from four sites in the Amazon region, Brazil. Samples were collected in Manaus (3° 06′07″ S; 60°01′30″ W), Ji-Paraná (10°53′07″ S; 61°57′06″ W), and Santarém (2°26′35″ S; 54°42′30″ W) with mean annual precipitation of 2207, 2040 and 1909 mm respectively. The overall average for all leaf samples was-32.3 ± 2.5‰ for δ^{13}C and+5.8 ± 1.6‰ for δ^{15}N ($n = 756$). The leaf δ values at these sites were often but not always statistically distinct from each other. The δ^{13}C values varied from-37.8‰ to-25.9‰. Pronounced differences in δ^{13}C values occurred with height associated with differences in forest structure. The δ^{13}C of leaf dry matter showed seasonal variations associated with the length of the dry season, despite the fact that total annual precipitation was similar among the studied sites. Leaf δ^{15}N values ranged from+0.9‰ to a maximum value of+10.9‰, and the Santarém sites showed more enriched values than Manaus and Ji-Paraná sites. No seasonal variation was detected in the δ^{15}N of leaves, but significant differences were observed among sites and with changes in canopy height. The isotope ratio data are consistent with our current understanding of the roles of light, water availability, and recycling of soil-respired CO_2 influences on δ^{13}C and consistent with our understanding that an open nitrogen cycle can lead to high δ^{15}N values despite a significant number of legumes in the vegetation.

Introduction

Stable isotope analyses are among the approaches used to characterize components of the carbon and nitrogen cycles in the Amazon Basin (Buchmann et al. 1997; Martinelli et al. 1999; Ehleringer et al. 2002; Ometto et al. 2002). Given that tropical forests play a central role in the global carbon cycle, understanding the factors that influence net carbon gain or loss in these regions can help improve our understanding of the constraints on the global carbon cycle (Schimel 1995; Field et al. 1998; Gash et al. 2004). Recent rainforest studies within the Amazon Basin have suggested that some regions might act as a substantial carbon sink (Grace et al. 1995a, b, 1996; Malhi et al. 1999; Araújo et al. 2002), whereas other sites might have been either neutral or even a small source of carbon to the atmosphere during the past two to three years (Saleska et al. 2003; Miller et al. 2004). Part of the difference of these estimates may be attributable to the complexity and diversity of tropical forests (Cuevas and Medina 1988; Martinelli et al. 1999; Luizão et al. 2004; Vieira et al. 2004). Additionally, the Amazon region does not have a homogeneous climate with similar precipitation and temperature (Obregon and Nobre 1990; Marengo 1992; Marengo et al. 1993; Fisch et al. 1998) or forests of equivalent age and structure (Vieira et al. 2004). Thus, structural and climatic features may contribute to the different carbon sink conclusions of different studies.

Carbon isotope ratio ($\delta^{13}C$) analyses of plant materials can provide carbon-cycle insights by providing as quantitative assessement of the stomatal limitations to photosynthetic activity (Farquhar et al. 1989; Ehleringer et al. 1993). The $\delta^{13}C$ value of a leaf is in part determined by its photosynthetic pathway and in part by the gas exchange constraints associated with environmental conditions, nutrient availability, and to some extent life history. Farquhar et al. (1982) first showed the $\delta^{13}C$ values in C_3 photosynthetic pathway plants is basically controlled by the ratio between the CO_2 concentration inside the leaf intercellular space (c_i) and in the adjacent atmosphere (c_a). As c_i is affected by the photosynthetic demand for CO_2 relative to the stomatal control over CO_2 supply, environmental factors affecting carbon gain, such as water supply, light availability, nitrogen content, just to mention some, are recorded in the $\delta^{13}C$ values of plant tissues (Farquhar et al. 1989).

Variations in the $\delta^{13}C$ of leaves in tropical forests are strongly correlated with position in the canopy profile, with lighter foliar $\delta^{13}C$ (more negative) values observed in the understory vegetation as compared to the upper canopy (Medina and Minchin 1980; Ehleringer et al. 1986). This clear effect of variation in c_i/c_a ratio between the lower- and the upper-canopy leaves has been reported in rainforests in Brazil (Merwe and Medina 1989; Sternberg et al. 1989; Medina et al. 1991; Kapos et al. 1993; Kruijt et al. 1996; Martinelli et al. 1998; Ometto et al. 2002), China (Ehleringer et al. 1986), French Guiana (Buchmann et al. 1997), Panamá (Sternberg et al. 1989), Trinidad (Broadmeadow et al. 1992), and Costa Rica (Leffler and Enquist 2002). The drivers for variations in $\delta^{13}C$ values among sites are not as clear but it is hypothesized to be related to the forest stand structure affecting for instance, light availability and vapor pressure deficit (VPD) within the canopy profile.

Nitrogen isotope ratios ($\delta^{15}N$) provide information related to nitrogen cycling within ecosystems (Högberg 1990; Evans and Ehleringer 1993; Högberg and Johannisson 1993; Högberg 1997; Austin and Vitousek 1998; Roggy et al. 1999a; Martinelli et al. 1999; Amundson et al. 2003). Martinelli et al. (1999) showed evidence that foliar and soil $\delta^{15}N$ patterns in tropical forests were on average higher than values in leaves from temperate forests. One possible explanation is that gaseous nitrogen losses associated with microbial activities in tropical forests leave behind a substrate enriched in ^{15}N (Austin and Vitousek 1998). Enriched soil δ^{15}N was also observed in agricultural fields (Meints et al. 1975), temperate forests under high N fertilization (Högberg 1990; Högberg and Johannisson 1993), and in tropical regions of Hawaii, where the drier sites, with high N losses were ^{15}N-enriched in comparison with wetter sites (Austin and Vitousek 1998).

On the other hand, the abundance of legumes trees and their capability of fixing N from the atmosphere are other important characteristics of tropical forests (Moreira et al. 1992; McKey, 1994). The δ^{15}N value of leguminous trees is often near 0‰ because of symbiotic nitrogen fixation (Evans 2001; Robinson 2001). Several studies have shown that species with symbiotic N fixation occur in sites with reduced N availability (McKey 1994), often associated with disturbance (Sylvester-Bradley et al. 1980). However, Roggy et al. (1999a) found that legumes trees (comprising 7.5% of all trees) were fixing N in a primary rainforest of French Guiana without any apparent disturbance and that this amounted to approximately 7 kg N ha^{-1} year^{-1} (Roggy et al. 1999b). These activities would be expected to drive δ^{15}N values toward 0‰ in tropical ecosystems. Yet Martinelli et al. (1999) suggested that δ^{15}N of tropical species should be above 0‰ because the nitrogen cycle was open in tropical forests, with microbial activities resulting in the loss of isotopically light nitrogen gases which left isotopically heavy nitrogen available to internal cycling with tropical ecosystems.

Our objective in this paper was to investigate the variability of the carbon and nitrogen isotope ratios as a proxy for interpreting aspects of the carbon and nitrogen cycles in Amazonian rainforests. The samples were conducted to analyze the temporal variations of the isotope ratios and their relationships to the input precipitation in three locations of the Brazilian Amazônia.

Material and methods

The original and summarized datasets presented in this study were collected as part of the Large-Scale Biosphere Atmosphere Project (LBA), an international effort to better understand ecosystem processes at regional scales in the Amazon Basin. All data are available at http://www.lba.cptec.inpe.br/beija-flor/.

Study area

The study sites were official sites of LBA program and located near the cities of Manaus (AM), Ji-Paraná (RO), and Santarém (PA) in Brazil. The mean annual precipitation for these sites are 2285, 2040, 1909 mm year^{-1} respectively. The forests consisted of dense evergreen terra-firme tropical vegetation in Manaus and Santarém, and open evergreen terra-firme forest in Ji-Paraná

(Higuchi et al. 1998; Alder and Silva 2000). The Manaus forest site was located 70 km north of the city (2.59° S; 60.11° W), in a reserve controlled by the Instituto Nacional de Pesquisas da Amazônia. The designation for this site is ZF2, referring to the road allowing access to the sites C14 and K34 where eddy covariance towers are installed (Araújo et al. 2002). The "Rebio" primary forest site is located 80 km north of Ji-Paraná in a Federal Biological Reserve known as Reserva Biológica do Jaru (10.08° S; 61.92° W), and is controlled by the Brazilian Environment Protection Agency (IBAMA). In Santarém there were two sites located at the Floresta National do Tapajós (Flona Tapajós) 67 km south of the city, which was established as a federal reserve in 1974. One site, "Flona-1", at 2.86° S; 54.96° W, and the second site, "Flona-2", located approximately 3 km from the Flona-1 site. "Flona 2" was the control plot of a rain exclusion experiment (Seca Floresta) conducted by the Instituto de Pesquisas Amazônicas (IPAM) and the Woods Hole Research Institute (Davidson et al. 2004).

The three locations contrasted in the length of the dry season, which is defined as the number of months with total precipitation less than 100 mm. Manaus had a 3-month dry season from July to September. At Ji-Paraná the dry season, extended for 4 months from May to August, and Santarém had the longest dry season (5 months) extending from July through November.

The soils at the Manaus and Santarém sites were deeply weathered oxisols (Hapludox) with high clay content (60–80%), low pH (4.0–4.3) and low nutrient content (Telles et al. 2003). The soil in Ji-Paraná site have been classified by Hodnett et al. (1996) as an orthic acrisol, with 85%, or more, of sand at the surface layer.

Sampling

Field samples were collected from May 1999 to June 2004. The sampling at the ZF-2 sites (C14 and K34) were from May 1999 to November 2002; at Flona-1 from June 2000 to June 2004, at Flona-2 from May 1999 to June 2000, February and September 2001, and March 2002; at Rebio in February and September 2000, and February 2001. Leaf samples were collected from plants along a height profile through the canopy, using a scaffold tower in all sites as well from catwalks in the Flona-2. Each sample from a single individual consisted of mature, healthy leaves combined to form a simple sample. A total of 756 tree leaves samples were collected. At ZF2 site, from a total of 176 leaf samples 83 were collected from trees with botanical identification. In Santarém area, 284 and 165 tree leaf samples were collected in the Flona-1 and in the Flona-2, respectively. From these totals, 207 and 125 samples were collected from trees classified to species level, respectively. Finally, at the Rebio site 48 tree leaf samples were collected without botanical classification. Litter, bark and wood samples were randomly collected from some study sites. At total 129 samples of litter were collected, mostly from Flona-1 (80 samples) and at ZF2

(42 samples) sites. Only 8 litter samples were collected at Rebio. Twenty-three samples of living wood and thirteen of bark were collected, at ZF2 (14, 7) and at Flona-1 (10, 6), for wood and bark respectively. Additionally, 11 samples of decaying wood were collected at Flona-1 and 16 were collected at Flona-2.

Analyzes

Soil, litter and plant material were dried at 65° C until a constant weight was obtained and then ground to a fine powder. A 1–2 mg sub sample was placed in a tin capsule. Samples were combusted in an elemental analyzer (Carlo Erba) coupled with an isotope ratio mass spectrometer (IRMS Delta Plus, Finnigan Mat, San Jose, CA, USA) operating in a continuous flow mode. From these analyses, we obtained both isotope ratio (δ^{13} C; δ^{15} N) and elemental content (%C; %N) for carbon and nitrogen.

Data are expressed in "delta" notation (δ^{13} C and δ^{15} N) as:

$$\delta = (\mathrm{R_{sample}}/\mathrm{R_{standard}} - 1) \times 1000 \tag{1}$$

where, $\mathrm{R_{sample}}$ and $\mathrm{R_{standard}}$ are the ratio ^{13}C:^{12}C or the ratio ^{15}N:^{14}N of the sample and standard, respectively. and the standards for carbon and nitrogen are PDB and AIR, respectively.

Statistical analysis

An unequal "n" HSD post hoc test and a Tukey HSD test were used to determine significant differences between the group means of the performed analysis of variance of the sites. The "Statistica 5.0" package was used for the analysis.

Results

Overall tendencies

With 756 separate leaf observations, the overall average δ^{13}C and δ^{15}N leaf values were-32.2 ± 2.5‰ and $+5.8 \pm 1.6$‰, respectively (Table 1). We will come back to these values in the discussion, comparing the range presented in this study with ranges of leaf values in different terrestrial tropic ecosystems. There were no significant relationships between δ^{13}C and δ^{15}N leaf values (Figure 1). The δ^{13}C values of leaf materials were depleted in ^{13}C relative to litter, bark, wood, and decaying wood and with statistically significant differences among some of these components (see Table 1). By comparison, the

Table 1. The average and standard deviations for the carbon isotope ratios (δ^{13}C), nitrogen isotope ratios (δ^{15}N), % tissue carbon, % tissue nitrogen, and C:N ratios of terra-firme forests studied in the Amazon Basin.

	δ^{13}C(‰)	δ^{15}N (‰)	%C	%N	C:N
Leaf (756)	−32.2 ± 2.5a	5.8 ± 1.6a	46.9 ± 4.6a	2.29 ± 0.7a	22.6 ± 7.7a
Litter (129)	−30.3 ± 1.6b	6.0 ± 1.6a	45.8 ± 5.9b	1.93 ± 0.7b	25.4 ± 6.0a
Decaying Wood (27)	−29.5 ± 1.6b	5.8 ± 1.4b	43.6 ± 4.0c	1.58 ± 0.6c	31.1 ± 11.2b
Wood (24)	−28.3 ± 1.5c	4.6 ± 2.0c	47.4 ± 2.6a	0.60 ± 0.6d	145.4 ± 82.1c
Bark (13)	−29.4 ± 2.4b	3.5 ± 2.1d	51.5 ± 7.9d	0.78 ± 0.3d	70.4 ± 26.0d

The number in parentheses indicates the total number of samples). Different letters indicate significant statistical difference at the $p < 0.05$ or less level.

leaf δ^{15}N values were statistically similar to those observed for both litter and decaying wood. Yet leaf δ^{15}N values were statistically heavier than observed in either undecayed wood or bark, implying that when plant materials were intact on standing trees there were pronounced spatial δ^{15}N different among components that disappeared once plant materials hit the ground and began to decay (Table 1).

Undecayed wood, in its δ^{13}C values, was statistically heavier than decaying wood, bark, or litter. Consistent with the expectation that would had not been impacted by soil microbes and that these other decay components had been, we

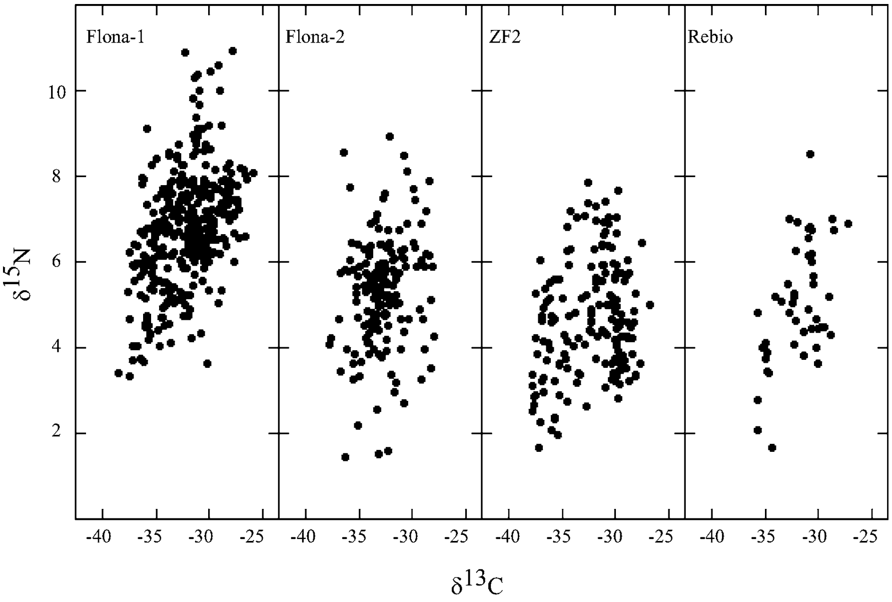

Figure 1. Plots of the nitrogen isotope ratio (δ^{15}N) versus carbon isotope ratio (δ^{13}C) for leaves from all study sites.

detected the highest %C, lowest %N, and highest C:N ratio in undecayed wood.

As expected, the highest %N contents were observed in leaves and these concentrations were statistically higher than in all other tissue components analyzed (Table 1). Undecayed wood and bark had the lowest %N contents, roughly 3–4 fold lower than in leaves. Yet surprisingly the litter and decaying wood components was roughly 2–3 folds higher in %N than undecayed wood.

Difference among study sites

The Flona-1 site stood out as distinctive from the other three forest site in two ways (Figure 1). First, although δ^{15}N leaf values for all locations were generally clustered between + 3 to +8‰, the very highest δ^{15}N leaf values were measured at Flona-1 with many species having values exceeding +9‰. Second, while the lowest range of δ^{15}N leaf values at other sites were + 1 to +3‰, these lowest values were absent at Flona-1. As a consequence, the δ^{15}N average value was significantly higher at Flona-1 in relation to all other sites (Table 2). Interestingly, Flona-1 and Flona-2 are only 3 km apart and yet had statistically different δ^{15}N values. The basis of these differences was not clear once not all species were represented in both sites. Looking at differences between trees families occurring in Flona-1 and Flona-2, *Caesalpinaceae* (legume), *Burseraceae, Rubiaceae, Sapotaceae, Lecythidaceae*, showed the same pattern discussed above. Only *Lecythidaceae* had the δ^{15}N values higher at Flona-2, however the species within this family were not the same.

When a comparison is made of the δ^{13}C values of leaves at all canopy heights across these study sites, there was no significant difference across sites (Table 2). The very isotopically light ^{13}C leaf values reflected a strong contribution from partly-to-fully shaded vegetation elements of the canopy. For an alternative comparison, we considered vegetation samples up to a height of 26 m, which is where each of the canopies opened up. If we considered these

Table 2. The average and standard deviations of the carbon isotope ratios (δ^{13}C), nitrogen isotope ratios (δ^{15}N), % tissue carbon, % tissue nitrogen, and C:N ratios of terra-firme forests studied in the Amazon Basin.

	δ^{13}C(‰)	δ^{15}N (‰)	%C	%N	C:N
ZF2 C14 – 133 (133)	−32.1 ± 2.9a (−32.2 ± 3.0)a	4.6 ± 1.3a	49.7 ± 3.7a	2.07 ± 0.7a	26.8 ± 8.3a
ZF2 K34 (42)	−32.6 ± 2.7a	4.33 ± 1.7a	50.5 ± 3.3a	2.04 ± 0.6a	27.5 ± 9.5a
Flona-2 – 165 (150)	−32.8 ± 2.2a (−33.1 ± 1.9)b	5.3 ± 1.3a	45.9 ± 4.5b	2.31 ± 0.79b	22.0 ± 7.6b
Flona-1 – 366 (280)	−32.1 ± 2.6a (−32.9 ± 2.1)b	6.8 ± 1.3b	46.5 ± 5.3b	2.45 ± 0.76b	20.9 ± 7.6b
Rebio – 48 (40)	−31.9 ± 2.2a (−32.3 ± 2.0)ab	5.0 ± 1.4a	45.9 ± 3.5b	2.48 ± 0.65b	19.8 ± 5.6b

Different letters indicate significant statistical difference at the $p < 0.05$ or less level. Numbers following the sites names indicate the number of samples. Average δ^{13}C values between brackets were obtained considering a maximum canopy height of 26 m (see text for further explanation).

"shaded" conditions, then the average $\delta^{13}C$ values at Flona-1 and Flona-2 were significantly lower than the ZF2 site (Table 2). As no statistical differences in carbon or nitrogen isotope ratios were found between the two tower sites in Manaus, they will be considered hereafter only as ZF2. Latter in the discussions we will consider the implication of the foliar isotopic signature in these sites in relation to different carbon assimilation by the vegetation proposed by Araujo et al. (2002).

The average N concentration was significantly lower and the C average concentration was significantly greater at the ZF2 site when compared with other sites (Table 2). As a consequence, the C:N ratio was significantly higher in the ZF2 site than in the other sites (Table 2).

Nitrogen isotope ratios and nitrogen contents of leguminous species

From the 756 tree leaf samples, 353 had botanical identifications, allowing enough samples to evaluate distinctions between legume and non-legume plants. We expected lower leaf $\delta^{15}N$ values if these plants were fixing N from the atmospheric air; generally legume leaves have been found richer in N than non-legume plants (McKey 1994; Evans 2001). The Rebio site had no species-level identifications at all and could not be considered in these evaluations. Considering the other three forest sites, the average foliar N concentration was statistically higher in legume leaves than in non-legume leaves at each of these forest sites ($p < 0.01$. Table 3). Considering all samples, the total average foliar N concentration among 95 legume trees was 2.77 ± 0.68%, which was statistically higher ($p < 0.01$) than the total average foliar concentration of 258 non-legume trees (2.06 ± 0.66%.). The foliar N concentration of non-legume trees was not significantly different among sites; nor was the foliar N concentration of legume trees significantly different between sites (Table 3).

The foliar $\delta^{15}N$ values of a fixing legume tree should reflect the atmosphere N source (ca. 0‰) and therefore should contrast with the $\delta^{15}N$ values of non-fixing species. However, at the ZF2 and Flona-1 sites there were no significant differences in the foliar $\delta^{15}N$ among legume and non-legume trees (Table 3).

Table 3. Foliar average and standard deviations of the N concentration and $\delta^{15}N$ among the study sites.

	Legume (%N)	Non-legume (%N)	Legume $\delta^{15}N$(‰)	Non-legume $\delta^{15}N$(‰)
ZF2	2.45 ± 0.74Ab (19)	1.94 ± 0.62Ba (61)	5.5 ± 1.5Ab (19)	4.8 ± 1.3Ab (61)
Flona-2	3.27 ± 0.67Aa (20)	2.07 ± 0.67Ba (105)	6.3 ± 1.3Aa (20)	5.1 ± 1.1Bb (105)
Flona-1	2.67 ± 0.54Ab (37)	2.16 ± 0.67Ba (66)	7.0 ± 0.9Aa (37)	6.9 ± 1.0Aa (66)

The number in parentheses indicates the number of values. Different upper case letters indicate difference between columns, and different lower case letters indicate significant difference between rows at the $p < 0.05$ or less level.

The total percentage of legumes samples to the total leaf samples at Flona-1 was 21%, while at Flona-2 and ZF2 were 15 to 16%, respectively.

Soil organic matter

The δ^{15}N of soil organic matter at Flona 1 and 2 ranged from +7.5 to+11.5‰ in 50-cm soil profiles (Figure 2). In a broader survey (Telles et al. 2003) have shown δ^{15}N values from +8 to +10‰ at surface and from +10 to +12‰ at depth within the Manaus and Santarem regions. Soil organic nitrogen content mirrored δ^{15}N values, with δ^{15}N becoming increasingly more positive with soil depth.

At the same time, the soil δ^{13}C values increased with depth, ranging almost 2‰ from−28‰ to −26‰ (Figure 2), and the organic matter content decrease in depth, with most of the carbon located in the upper soil layer (0–10 cm). Interestingly the soil δ^{13}C values spanned a much smaller range than did the canopy leaf δ^{13}C values, suggesting that the leaves with very negative δ^{13}C values were likely not contributing significantly to the soil carbon pool.

Temporal variability

The sampling at ZF2 (Manaus) and Flona-1 (Santarém) allowed us to investigate the temporal variability of the δ^{13}C and δ^{15}N values of tree leaves over a 5-year period. For ZF2 there was slight response in δ^{13}C values of ca. 2‰ in the understory vegetation to variations in precipitation, with higher δ^{13}C values occurring drier periods and lower values occurring during the wet seasons (Figure 3). A statistical analysis indicated a significant, negative correlation between understory δ^{13}C values of vegetation and the monthly mean precipitation ($p < 0.05$). In the mid-canopy heights ($h = 5$–20 m), the changes in δ^{13}C

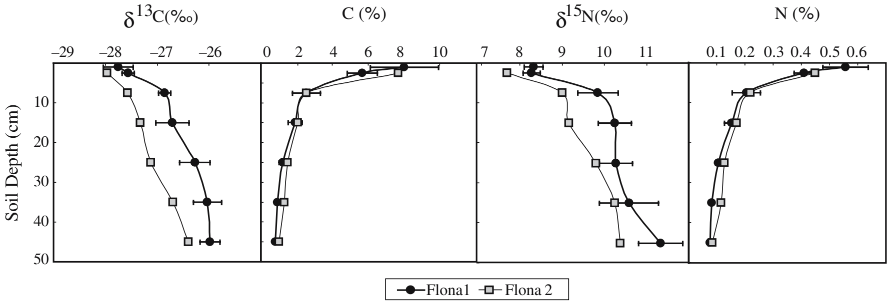

Figure 2. Plots of the variations in carbon isotope ratio (δ^{13}C), nitrogen isotope ratio (δ^{15}N), % soil carbon content, and % soil nitrogen content of soil organic matter as a function of soil depth at the Flona 1 and Flona 2 study sites in Santarém. Data are averages and standard deviations.

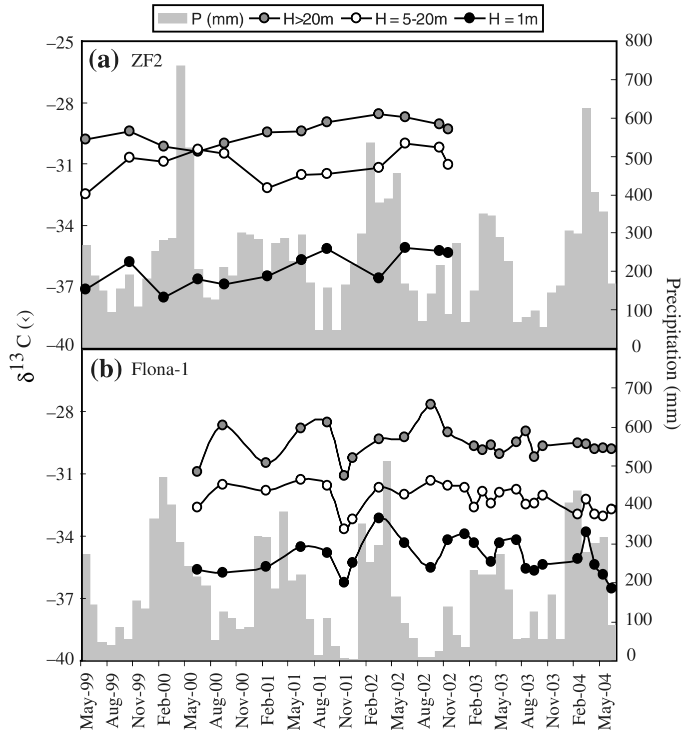

Figure 3. Temporal variations in the leaf carbon isotope ratios ($\delta^{13}C$) of tree leaves at three different heights and average monthly precipitation at (a) Manaus (ZF2) and (b) Santarém (Flona-1) sites. $H = 1.0$ m represents the average $\delta^{13}C$ of all leaves collected in the understory at a height of 1.0 m. $H = 15 - 20$ m represents the average $\delta^{13}C$ of all leaves collected between a height of 15 and 20 m in the canopy. $H > 20$ m represents the average $\delta^{13}C$ of all leaves collected in the canopy at a height higher than 20 m.

values were muted showing no significant temporal variability, suggesting a damped response of the vegetation to changes in the seasonal moisture inputs (Figure 3a). At Flona-1 in Santarém, the $\delta^{13}C$ values showed a positive correlation with monthly precipitation although with a lag of 5 months, for both upper canopy and understory leaves (Figure 3b). The increase of water availability in the system would change the $\delta^{13}C$ of the leaves by changes in the c_i/c_a ratio, although the patterns observed indicated a difference between the expected isotopic signature of a recent carbon assimilated and the bulk leaf carbon. An important aspect of Figure 3a and 3b), is the different species specific responses to seasonal-scale environment variability, in this case precipitation. Two trees (*Copaifera multijuga* and *Manilkara huberi*) at the top of the canopy had distinct foliar $\delta^{13}C$ values and distinct response to precipitation that seemed to follow a multi-year cycle (Figure 4a). Similarly, the lianas at the top of the canopy (*Priomostemma aff. aspera and Tetrapterrys* sp.) at Flona 1 site also showed long-term $\delta^{13}C$ changes that were not annual (Figure 4b).

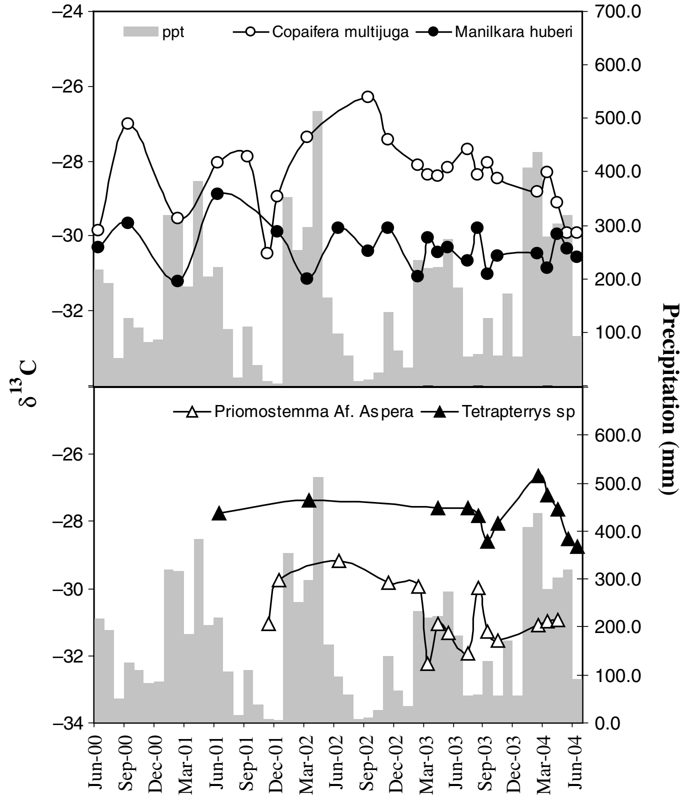

Figure 4. Temporal variations in the leaf carbon isotope ratios (δ^{13}C) of two canopy trees occurring at the highest canopy strata at Flona 1: *Copaifera Multijuga* (open circles) *and Manilkara huberi* (close circles), and lianas: *Priomostemma Af. Aspera* (open triangles), *and Tretrapterrys* sp. (close triangles).

A 2‰ temporal variation of the foliar δ^{15}N values was seen in the leaves of the understory vegetation at the ZF2 site; this pattern was positively correlated to variations in precipitation in the understory vegetation, but not significantly correlated with precipitation in the mid-canopy and top-canopy layers (Figure 5a).The seasonal variation of the foliar δ^{15}N at the Flona-1 showed significant negative correlations between δ^{15}N of both the understory and mid-canopy vegetation and the average monthly precipitation ($p < 0.01$); for the mid canopy vegetation there was a 5-month lag correlation with the monthly precipitation (Figure 5b).

Height variability of the foliar δ^{13}C values

The foliar δ^{13}C values were significantly correlated with canopy height at all forest sites (Figure 6). Linear equations between canopy height and δ^{13}C were

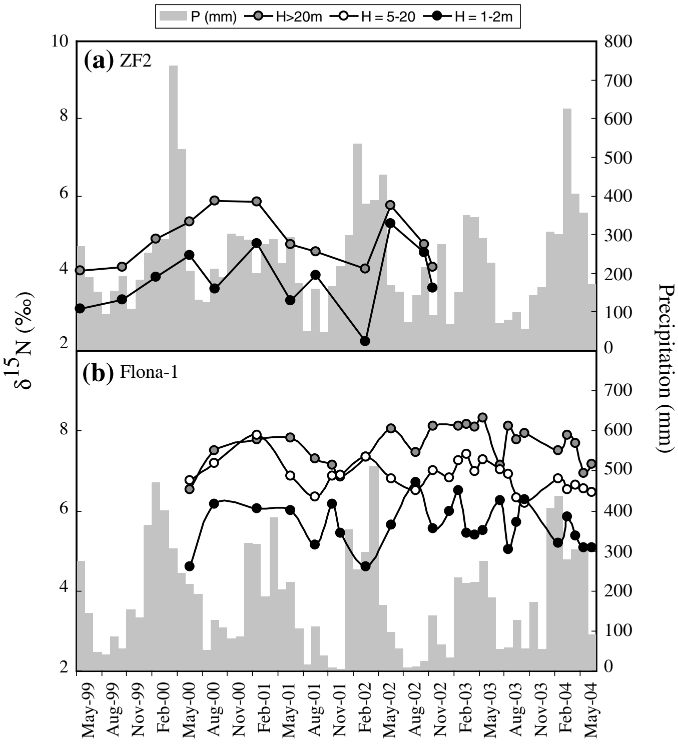

Figure 5. Temporal variations in the nitrogen isotope ratios ($\delta^{15}N$) of tree leaves from different canopy heights at (a) Manaus (ZF2) and (b) Santarém (Flona-1) sites. $H = 1.0$ m represents the average $\delta^{15}N$ of all leaves collected in the understory at a height of 1.0 m. $H = 15$–20 m represents the average $\delta^{15}N$ of all leaves collected between a height of 15 and 20 m in the canopy. $H > 20$ m represents the average $\delta^{13}C$ of all leaves collected in the canopy at a height higher than 20 m.

determined and the intercepts and slopes of these height relationships at Flona-1 and Flona-2 were not distinguishable statistically (Table 4). The equation describing $\delta^{13}C$ variation with canopy height at the Rebio site had a similar slope to the Flona observations, but a higher intercept. Finally, the equation describing $\delta^{13}C$ variation with canopy height at Manaus had a smaller intercept but a higher slope than the equations for the other sites (Table 4).

Height variability of the foliar $\delta^{15}N$ values

We evaluated correlations between $\delta^{15}N$ and canopy height for each of these forest sites. At the ZF-2 and Flona-1 sites, there were significant, positive correlations between $\delta^{15}N$ and canopy height, however the proportion of the variance in the $\delta^{15}N$ values explained by canopy height was small at both sites (Table 5, Figure 7). We separated the $\delta^{15}N$ values of tree leaves into four

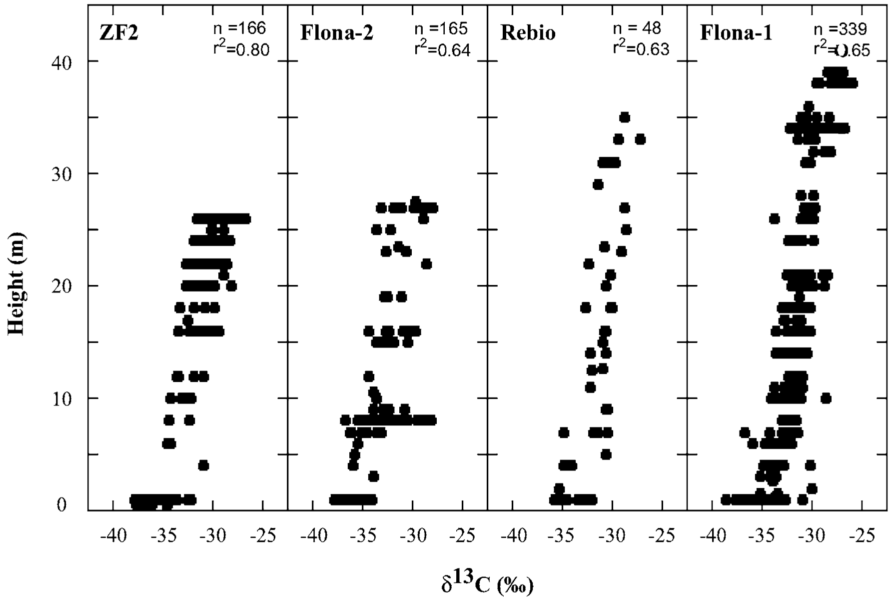

Figure 6. Variations in the carbon isotope ratios (δ^{13}C) of leaves with height for each of the four study sites (regression lines found in Table 4).

height categories at all sites (Table 6). Significant differences in δ^{15}N values were detected among understory vegetation and the upper canopy layers for ZF2 and Flona-1, but none of these relationships were statistically significant at Rebio or Flona-2. For all regions no statistical difference on the δ^{15}N of tree leaves were detected among the upper canopy vegetation.

Discussion

The use of stable isotope analysis of plant material can provide important information about the ecology and dynamic of the vegetation and its interaction with the surrounding environment. In the Amazon region of Brazil,

Table 4. Equations describing the statistical relationships between δ^{13}C and height (*H*), the R^2 value for that relationship, and number of data (*N*) for the four forest sites.

Site	Equation	R^2	*N*
Manaus-ZF2	$\delta^{13}C = -35.9 + 0.25^{*}H$	0.80**	166
Santarém-Flona-2	$\delta^{13}C = -34.8 + 0.19^{*}H$	0.64**	165
Santarém-Flona-1	$\delta^{13}C = -34.9 + 0.17^{*}H$	0.75**	339
Ji-Paraná-Rebio	$\delta^{13}C = -34.0 + 0.16^{*}H$	0.63**	48

All regressions were statistically significant at the $p < 0.05$ or less level.

Table 5. Equations describing the relationships between foliar $\delta^{15}N$ and height (H), the R^2 value for that relationship, and number of data (N) for the four forest sites.

Site	Equation	R^2	N
Manaus-ZF2	$\delta^{15}N = 4.3 + 0.030^{*}H$	0.09**	166
Santarém-Flona-2	$\delta^{15}N = 5.05 + 0.025^{*}H$	0.01ns	165
Santarém-Flona-1	$\delta^{15}N = 5.95 + 0.053^{*}H$	0.24**	340
Ji-Paraná-Rebio	$\delta^{15}N = 4.63 + 0.030^{*}H$	0.03ns	48

All regressions were statistically significant at the $p < 0.05$ or less level.

despite being the largest contiguous tropical forest of the world, few studies have been carried on the past looking at the carbon and nitrogen isotopic variation of the vegetation (Martinelli et al. 1998).

The data present here indicate a variability of the $\delta^{13}C$ among species and sites, but with most of the variation associated with canopy height. Similar patterns have been noted in other tropical forest sites (Kruijt et al. 1996; Buchmann et al. 1997; Martinelli et al. 1998; Guehl et al. 1998a, b, and Bonal et al. 2000a, b). The overall average $\delta^{13}C$ value for all our samples ($-32.2 \pm 2.5‰$; $n = 756$) was similar to average values reported for other tropical forests by Buchmann et al. (1997) and consistent with an expected high photosynthetic carbon isotopic discrimination (Farquhar et al. 1989). Between 63% and 80% of the variance of $\delta^{13}C$ values was explained by the canopy

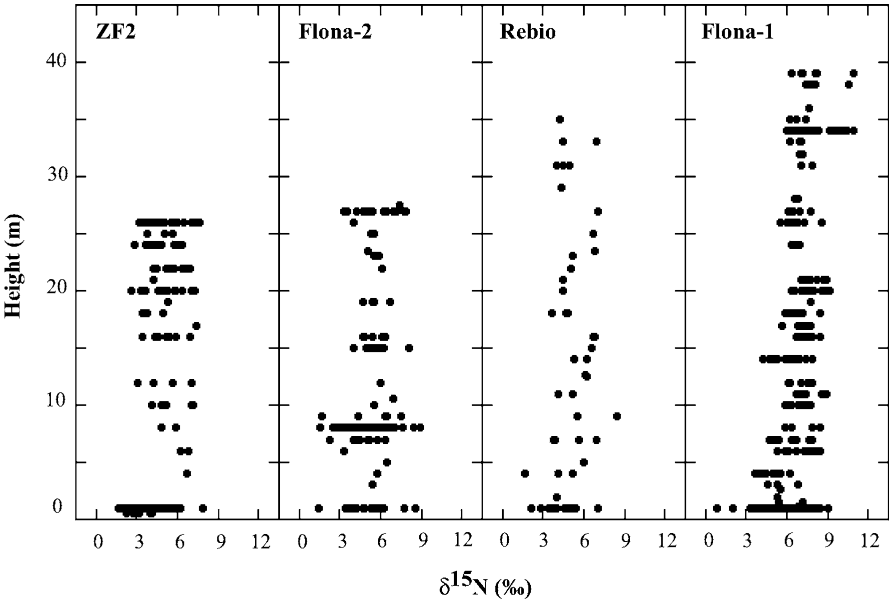

Figure 7. Variations in the nitrogen isotope ratios ($\delta^{15}N$) of leaves with height for each of the four study sites study sites (regression lines found in Table 5).

Table 6. Foliar average $\delta^{15}N$ and standard-deviation at different canopy heights at the different study sites.

Canopy height	ZF2	Flona-1	Flona-2	Rebio
≤ 1 m	4.1 ± 1.3 (57)a	5.7 ± 1.3 (100)a	5.0 ± 1.3 (18)a	4.2 ± 1.4 (12)a
5 to 10 m	5.6 ± 1.4 (13)b	7.1 ± 1.0 (62)b	5.2 ± 1.3 (101)a	5.1 ± 1.5 (10)a
15 to 20 m	4.9 ± 1.2 (33)b	7.0 ± 1.1 (78)b	5.7 ± 1.2 (24)a	5.5 ± 1.4 (12)a
≥ 20 m	4.9 ± 1.2 (63)b	7.4 ± 1.2 (100)b	5.6 ± 1.3 (22)a	5.2 ± 1.3 (14)a

Numbers in parentheses are the number of samples for each canopy height. Different letters indicate statistical significant differences between averages of the different heights at the $p < 0.05$ or less level.

height (Table 4). These data agree with findings by Martinelli et al. (1998) in a *terra-firme* forest at the western Brazilian Amazon. Difference in the foliar carbon isotope ratios of forest canopies profiles have also been detected in the past (Ehleringer et al. 1986; Medina et al. 1986, 1991, Jackson et al. 1993; Lloyd et al. 1996; Bassow and Bazzaz 1997; Berry et al. 1997; Buchmann et al. 1997; Bonal et al. 2000b; Fessenden and Ehleringer 2002; Ometto et al. 2002). Upon further examination, the observations in Table 4 suggest a canopy that is more closed in the Manaus forest when compared to tropical forests in Santarém and Ji-Paraná. These patterns are consistent with known differences in the lengths of the dry season among these sites, and are predicted given that the Manuas forests had the shortest dry season. The $\delta^{13}C$ data can also be interpreted as indicating that some portion of recycled respired CO_2 as source to the photosynthesis of understory leaves, contributing to the leaf $\delta^{13}C$ values (Sternberg et al. 1989). At an extremely high c_i/c_a ratio of 0.9, we would expect that leaf $\delta^{13}C$ values would approach $-33‰$ under typical atmospheric $[CO_2]$ values today. Yet many of the leaf $\delta^{13}C$ observations were less than $-33‰$ (e.g., Figure 1), strongly inferring a more negative atmospheric $\delta^{13}C$ value than $-8‰$. Ometto et al. (2002) did provide evidence that the $\delta^{13}C$ values of atmospheric CO_2 in the bottom of these forest canopies were more negative than at the top of the canopy. In the past, variations in $\delta^{13}C$ values have been correlated with other parameters not immediately related to canopy height, including water-use efficiency (Guehl et al. 1998a, b; Bonal et al. 2000a), branch morphology and hydraulic conductivity (Walcroft et al. 1996; Panek 1996), structural and compositional differences between leaves (Broadmeadow and Giffiths 1993; Bonal et al. 2000a, b; Leffler and Enquist 2002), and leaf age and position in the crown (Gebauer and Schulze 1991; Donovan and Ehleringer 1992). Just how much additional these factors contributed to variations in $\delta^{13}C$ values is unknown.

Ehleringer et al. (2000) noted that soils tend to become ^{13}C enriched with depth as soil carbon became older and more processed by soil microbes. The $\delta^{13}C$ of the soil organic matter for Flona-1 and Flona-2 reflected this decay process being ca.2‰ heavier than the ground surface litter component (Table 1 and Figure 7), and becoming ^{13}C enriched in the deeper soil layers as soil carbon decreased. These observations support previous observations by Camargo et al. (1999) for other rainforest regions within the Amazon basin. The soil-depth profiles in tropical regions are distinct from temperate regions because of the more rapid decline in soil organic carbon with soil depth. Similar to our observations, Natelhoffer and Fry (1988) had shown that soil $\delta^{15}N$ values increased with soil depth. The results from these tropical forest profiles are again distinct in that most of the changes occur in the initial 10-cm depth into the soil.

The $\delta^{15}N$ of leaves can reflect the nutrient pools that the vegetation is exploiting, nitrogen fixation, soil pools, re-translocation within the plant, among others. The average $\delta^{15}N$ value for non-leguminous trees in our data ($5.3 \pm 1.2‰$, Table 3) was similar to the value presented by Martinelli et al. (1999) for several tropical forests around the world. These high $\delta^{15}N$ values have been interpreted as an indication of relatively high nitrogen abundance in these ecosystems. However, our dataset contrasts with the Roggy et al. (1999a) observations for $\delta^{15}N$ foliar values for French Guiana. In that study, they found 82% of the foliar $\delta^{15}N$ values falling between$-1‰$ to$+3‰$. In our study only ca. 5% of the values were lower than3‰, which agrees with global model predictions by Amundson et al. (2003). Another aspect to point out was the slight, but significantly lower foliar $\delta^{15}N$ values observed in the understory vegetation when compared with the upper canopy strata at the ZF2 and Flona-1 forests (Table 5). The contrasting $\delta^{15}N$ values within a site might be interpreted as indicating that these plants were obtaining different nitrogen sources in the soil. Here the form of nitrogen uptake could differ among species or perhaps nitrogen losses by volatilization at upper canopy leaves might leave behind enriched ^{15}N in relation to understory leaves (J.Berry, personal communication). Finally, a preferential uptake of NH_4 by larger trees and a preferential uptake of NO_3 by plants of the understory would also contribute to increase the $\delta^{15}N$ in the upper canopy, since in the soil the $\delta^{15}N$–NH_4 is generally higher than $\delta^{15}N$–NO_3 (Högberg 1997). The high average $\delta^{15}N$ value found in this study confirms that tropical forests are more ^{15}N- enriched than temperate forests (Martinelli et al. 1999). The $\delta^{15}N$ values were also more enriched relative to the vegetation in savannas to the south of Brazil (Cerrado, Bustamante et al. 2004).

Precipitation gradient

The response of photosynthesis and respiration to precipitation or water availability will be reflected in the carbon isotope ratios of the vegetation (Farquhar et al. 1989). The carbon isotope ratios of foliar tissue represent a balance between stomatal conductance to supply CO_2 and photosynthetic demand consuming CO_2, integrated in the c_i/c_a ratio. A recent study of ecosystem gas flux in Santarém showed that the gross ecosystem production (GEP) responded weakly to seasonal changes in precipitation while respiration had a much stronger response (Saleska et al. 2003). Our data for Santarém Flona-1 site (Figure 3b) showed that the seasonal distribution of precipitation was correlated with the $\delta^{13}C$ variability of tree leaves. Even though an expected trend would indicate smaller $\delta^{13}C$ signal with increase of water availability and c_i/c_a ratio (e.g. Walcroft et al. 1997), our observations suggested a time lag between the carbon structurally allocated on the leaves tissues and the carbon fixed by photosynthesis, being correlated, therefore, to previous rain events. The eddy-covariance studies at the Flona-1 site predict carbon fixation that is more ^{13}C enriched associated with less woody growth in the dry season and with a significant increase at the beginning of the rainy season (Saleska et al. 2003; Goulden et al. 2004; Miller et al. 2004). The mechanism suggested by Saleska et al. (2003) for the increase of wood increment "just before" the returning of the rain could be related to the specific carbon re-allocation by the plant and then reflected in the lag of the $\delta^{13}C$ values as shown in our temporal data.

In Manaus, the seasonal variation of foliar $\delta^{13}C$ values were small (Figure 3a), with the exception of changes in the $\delta^{13}C$ values of understory vegetation. In this strata there were ca. 2‰ variations between seasons. Consistent with the smaller interseasonal variations in ^{13}C values, NEE (Net Ecosystem Exchange) observations using eddy-covariance approaches at ZF2 in Manaus have also indicated a smaller seasonal variation (Grace et al. 1996; Araújo et al. 2002), especially when compared to Flona-1 in Santarém (Saleska et al. 2003). The basis for these differences is likely to be the differences in the lengths of the dry season, with Manaus experiencing a much shorter dry season even though the total annual precipitation values are similar.

The $\delta^{15}N$ of leaves were quite positive and generally did not show significant variations within sites. A possible explanation for this pattern would be a more open nitrogen cycle in which N gas losses by microbes resulted in the preferential loss of light N and the residual accumulation of ^{15}N components in the soil. Here observations and the predictions of $\delta^{15}N$ with the Amundsen et al. (2003) global model are in disagreement. In their model soils should become ^{15}N-depleted with the high precipitation amounts that characterize rainforests. In fact, differences in the annual amount of rainfall are not big between Manaus and Santarém regions, but the length of the dry season is quite different among these sites (see also Figure 2 a and b). The biological processing of the soil organic nitrogen might present local peculiarity between the tropical regions, causing changes in the soil nitrogen mineral form available to plants. Nardoto et al. (in preparation) suggest that in Santarém the major nitrogen form available to plants in the soil is ^{15}N-enriched ammonium (NH_4^+). In Figure 1 one can observe the spatial variation of the leaf nitrogen isotope ratios at our study sites, especially at Flona-1. The basis for this difference remains unclear.

Leguminous trees in a non limiting nitrogen environment

The foliar N content was especially high in legume trees if compared with non-legumes (Table 3), in agreement with earlier findings by McKey (1994). The N enrichment in leaves of legume trees was already noted for tropical forests (Roggy et al. 1999a; Vitousek et al. 2002). One of the possible explanations is that a fraction of the N present in the legumes is derived from the biological fixation from the atmosphere. Although we observed an average foliar δ^{15}N for legume trees close to +5‰ at ZF2 and Flona-2 and close to +7‰ at Flona-1 (Table 3), suggesting that most of the legume trees were not fixing nitrogen in the terra-firme forests of the study regions of the Amazon. A similar conclusion was proposed by others authors looking at nitrogen fixation in tropical forests (Sylvester-Bradley 1980; Yoneyama et al. 1993; Vitousek et al. 2002; Gehring 2003). Based on these studies Vitousek et al. 2002 suggested that symbiotic N fixation in tropical forests might occur only during temporary N shortage. However, in contrast, Roggy et al. (1999a and 1999b) found a significant contribution of legume atmospheric fixed-N_2 to the nitrogen budget of a tropical forest on Oxisol soils in French Guiana.

Most of the legume tree species in the Flona-1 and Flona-2 sites belong to the sub-family Caesalpinoideae, which is known by having few N-fixing species (Souza et al. 1994). N-fixing species are much more common in the sub-families Mimosoideae and Papilionoideae (Sprent 1995). At Flona-1, from 37 samples of Caesalpinoideae, 35% belongs to the genus *Sclerolobium* (a nitrogen fixing genus). However, none of them had δ^{15}N values significantly lower than the average of non-legume trees. Only one species (*Ormosia* sp.) belonged to the sub-family Papilinoideae. This specie had a δ^{15}N value of +4.5‰, which is lower than +6.9‰, the average found for non-legume trees in this site. Five species of the sub-family Mimosoideae were found at Flona-2 and most of the Caesalpinoideae trees belong to the genus *Tachigali* and *Sclerolobium* (both nodulating trees with potential for fixing nitrogen, Sprent 1995). However, from this total, only two species of *Inga* (Mimosoideae) had δ^{15}N values significantly lower than the average δ^{15}N value found for non-legume trees. Finally, at ZF-2 site most samples belonged to the Mimosoideae family, but one tree (*Inga* sp.) had a δ^{15}N value (+3.0‰) significantly lower than the average δ^{15}N value of non-legume trees (+4.8‰) (Table 3).

A significant difference on the δ^{15}N of tree leaves was found between Flona-1 and Flona-2 sites, despite the proximity of the sites (Table 6). A similar result was not detected in the understory vegetation, although in all other canopy heights the δ^{15}N values at Flona-1 were significantly higher than at Flona-2 (Table 6 and Figure 1). We also compared the foliar δ^{15}N of legume and non-legume trees at both sites. For non-legume trees the difference between these two sites was still significant, but there was no statistical difference for legume trees (Table 6). Therefore, it seems that non-legume trees were responsible for the observed difference in δ^{15}N values between these two sites. Based on this fact, we selected two species of non-legume trees that occurred in the two sites.

This procedure rules-out any potential difference in the foliar $\delta^{15}N$ values caused by physiological differences among species. Both plants, *Coussarea racemosa* and *Manilkara huberi* had higher foliar $\delta^{15}N$ values at Flona-1 (6.10 ± 0.77a, $n = 14$; 6.60 ± 0.48a, $n = 16$) than at Flona-2 (4.76 ± 0.46b, $n = 9$; 4.73 ± 0.70b, $n = 6$) for each species at the sites (same letters indicate statistical significance). As there is a small but significant correlation between foliar $\delta^{15}N$ and tree height, we tested for differences between the two tree species using an ANOVA analysis with a co-variance for tree height and the different sampling heights in the two sites did not produce the differences the $\delta^{15}N$ of both species.

It is not easy to explain the differences in $\delta^{15}N$ values observed in non-legume trees of Flona-1 and Flona-2, since the sites are quite close to each other, with similar vegetation, and the same precipitation and temperature regimes. In a general sense the $\delta^{15}N$ value may vary with the type of vegetation (Handley et al. 1999), dominant nitrogen form assimilated by the different plant species (Stewart et al. 1992; Roggy et al. 1999a), or climatic patterns (Handley et al. 1999; Amundson et al. 2003). Martinelli et al. (1999) working with white sand-soils ("campinarana" forest) and savannas within the Amazon forest observed that the foliar $\delta^{15}N$ of tree species have much lower values of $\delta^{15}N$ than the more extensive terra-firme forests in similar climatic pattern. The $\delta^{15}N$ value of total-N in the soil is not always similar to the nitrogen available-N to plants, especially when nitrate is formed by nitrification (Hogberg 1997). Our results show a $\delta^{15}N$ of soil organic matter that was on average 0.7‰ heavier at the Flona-1 forest site when compared to the Flona-2 forest site (Figure 2). The foliar $\delta^{15}N$ values of Flona-1 were ca. 1.5‰ heavier in this site when compared to Flona-2 (Table 8). Thus, it is reasonable to hypothesize that part of the difference between the two sites may be explained by differences in the $\delta^{15}N$ of the soils. Although, further investigations on the dominant form of inorganic nitrogen available to the plants (NH_4^+ or NO_3^-), on the organic N pool and on the isotopic fractionation during the uptake and assimilation process (Hogberg 1997) would be very helpful for further understanding of the nitrogen isotope variation between these two sites.

The differences in forest structure are important in the ecological understanding of the system and the $\delta^{13}C$ values obtained in this study showed a consistent response changing it value with the canopy height, reflecting the forest structure. Changes in canopy heights also observed for the nitrogen probably indicate different pools been explored in the soil, once the nitrogen availability in the Amazon primary forest is high. Isotopic variations associated with the length of the dry season, observed in carbon indicate an interesting patterns and the importance of recent fixed carbon to the integrated isotopic signature of the leaf organic carbon. Our current ecophysiological knowledge concerning light and water availability, nutrients and recycling of soil-respired CO_2 show the isotopic data here presented consistent with an open carbon and nitrogen cycle by an highly diverse vegetation dealing with high energy and water input and nitrogen availability.

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