

Understanding the Influences of Spatial Patterns on N Availability Within the Brazilian Amazon Forest

Gabriela Bielefeld Nardoto,^{1*} Jean Pierre Henry Balbaud Ometto,¹ James R. Ehleringer,² Niro Higuchi,³ Mercedes Maria da Cunha Bustamante,⁴ and Luiz Antonio Martinelli¹

¹Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Avenida Centenário, 303, 13416-000 Piracicaba, SP, Brazil; ²SIRFER, Biology Department, University of Utah, Salt Lake City, Utah 84112, USA; ³Instituto Nacional de Pesquisas da Amazônia, Manaus 69083-000, AM, Brazil; ⁴Departamento de Ecologia, Universidade de Brasília, Brasília 70919-970, DF, Brazil

ABSTRACT

Nitrogen variations at different spatial scales and integrated across functional groups were addressed for lowland tropical forests in the Brazilian Amazon as follows: (1) how does N availability vary across the region over different spatial scales (regional × landscape scale); (2) how are these variations in N availability integrated across plant functional groups (legume × non-legume trees). Leaf N, P, and Ca concentrations as well the leaf N isotope ratios ($\delta^{15}\text{N}$) from a large set of legume and non-legume tree species were measured. Legumes had higher foliar N/Ca ratios than non-legumes, consistent with the high energetic costs in plant growth associated with higher foliar P/Ca ratios found in legumes than in non-legumes. At the regional level, foliar $\delta^{15}\text{N}$ decreased with increasing rainfall.

At the landscape level, N availability was higher in the forests on clayey soils on the plateau than in forests on sandier soils. The isotope as well as the non-isotope data relationships here documented, explain to a large extent the variation in $\delta^{15}\text{N}$ signatures across gradients of rainfall and soil. Although at the regional level, the precipitation regime is a major determinant of differences in N availability, at the landscape level, under the same precipitation regime, soil type seems to be a major factor influencing the availability of N in the Brazilian Amazon forest.

Key words: nitrogen; lowland tropical forest; nitrogen stable isotopes; Amazonia; foliar phosphorus; terra-firme forest; campinarana forest.

INTRODUCTION

Studying the potential influence of climate on nitrogen and organic matter content of the soil, Jenny and others (1948) showed that for a given pair of temperature and moisture values, the nitrogen content of the soil was much higher in tropical soils than in the temperate soils of the Americas. In two other studies (Jenny and others

1949; Jenny 1950), it was hypothesized that the cycling of nitrogen in tropical soils was faster than in temperate soils, and that the higher gains of nitrogen in tropical soils were due to nitrogen additions via rainfall and symbiotic and non-symbiotic nitrogen fixation, mainly by the large numbers of legume trees in tropical forests.

The richness of nitrogen in tropical forests was confirmed by Greenland and Kowal (1960) working in Ghana, but their observations did not support the hypothesis of the importance of symbiotic

nitrogen fixation as the main source of nitrogen to these forests, because only 8% of the trees belonged to the Legume family. Nye (1961) examined these same forests and found higher litterfall rates in tropical forests than in temperate forests, and that the litter in tropical forests was more N enriched compared to temperate forests. Enriched nitrogen in tropical forest litter was fully confirmed later by Vitousek (1984) and Vitousek and Sanford (1986). Other authors have reached similar conclusions: typically nitrogen circulates at higher rates annually through lowland tropical forests (rainforests on flatter terrain in low altitudes) and does so at high concentrations (Cuevas and Medina 1986; Keller and others 1986; Proctor 1987; Matson and Vitousek 1987; Piccolo and others 1994; Neill and others 1995; Martinelli and others 1999; Vitousek 2004; Davidson and others 2007).

The Amazon forest covers extensive areas of Brazil and surrounding portions of South America, constituting one of the most important ecosystems of the Earth. This basin accounts for 45% of the world's tropical forest, and is largely known for its high species diversity (Prance 1990; Prance 1994) with legumes representing one of the most diverse and abundant families of higher plants in this ecosystem (Moreira and others 1992; Steege and others 2006). A high percentage of Amazonia is covered by non-flooded lowland evergreen rain forests (Whitmore 1984), often referred to in Brazil as terra-firme forest (Daly and Prance 1989), and characterized by high species diversity (Pires and Prance 1985; Steege and others 2006). Soils of the Amazon Basin are often highly weathered and nutrient poor (Sanchez and others 1982), exhibiting considerable spatial heterogeneity in soil texture at both the local and regional level (Sombroek 2000). Biogeochemical and hydrological characteristics of Amazonian forest ecosystems differ throughout the soil textural mosaic in this region (Silver and others 2000), but substantial evidence suggests an apparent abundance of nitrogen across the Brazilian Amazonian terra-firme forests (Martinelli and others 1999; Ometto and others 2006). Rates of net N mineralization and leaching losses are elevated in these Amazonian terra-firme forests (Silver and others 2000; Luizão and others 2004; Silver and others 2005) and emissions of N-containing trace gases from soil to the atmosphere are also generally high (Davidson and others 2004; Keller and others 2005). In central Amazonian Oxisols, relatively high concentrations of organic N in the topsoil and a large accumulation of nitrate in the subsoil have been demonstrated (Renck and Lehmann 2004). Therefore, pathways of N losses

seem likely to be as losses through leaching of nitrate into the subsoil (Salati and Vose 1984) and gaseous loss through nitrification/denitrification (Silver and others 2000).

Much is known about the nitrogen status in lowland tropical forests; however, much less is known concerning nitrogen variations at different spatial scales and integrated across functional groups. Given that soil types and precipitation regime affect N cycling, these issues were addressed for lowland tropical forests in the Brazilian Amazon as follows: (1) how does N availability vary across the region over different spatial scales (regional \times landscape scale); (2) how are these variations in N availability integrated across plant functional groups (legume \times non-legume trees).

Because foliar nutrient concentrations have long been used to indicate plant nutrient status and even likelihood of limitation by particular nutrients (Medina and Cuevas 1994), we measured foliage from the upper canopy of a significant number of tree species as well as soil concentrations of N, P, and Ca to address the issues outlined above. Additionally, nitrogen isotope ratios in leaves and soil samples were used to infer about N cycling under different conditions (Handley and others 1999; Martinelli and others 1999; Amundson and others 2003). Clearly, the nitrogen stable isotopic composition of a plant is not solely an integration of its N source, but it is an integration of the whole nitrogen balance of a plant (Evans 2001). Mechanistic explanations for local or regional patterns of $\delta^{15}\text{N}$ values would require, at least, the use of Rayleigh equations to determine variations in the product (for example, plants) and the N source (for example, source) (Robinson 2001) but due to the complexity of the N dynamics in a soil system, it is very difficult to apply a Rayleigh model approach (Högberg 1997). Several previous studies have been focused on qualitative patterns of $\delta^{15}\text{N}$ values in ecosystems to derive information about the N cycle. Substantial $\delta^{15}\text{N}$ variations within and among ecosystems on a local (Austin and Vitousek 1998; Schuur and Matson 2001) or large scale (Handley and others 1999; Martinelli and others 1999; Amundson and others 2003) have been documented. Variations in $\delta^{15}\text{N}$ values reflect both nitrogen isotope fractionations as well as differences in substrate values. In biological systems, the leaves usually become ^{15}N depleted relative to their substrate (Högberg 1997). Recently, Houlton and others (2006) used an isotopic approach to examine a series of tropical forests in Hawai'i and to identify the mechanisms responsible for variations in the elevation of soil $\delta^{15}\text{N}$ values relative to atmospheric

N_2 , where the isotopic discrimination by microbial denitrification appeared to be the major determinant of N isotopic variations across a rainfall gradient. If N functions as an excess nutrient in tropical forests, then large losses of N by fractionation pathways would result in increased $\delta^{15}N$ values in these forests.

METHODS

Study Sites

Comparison at the Regional Level

The sites studied in the Brazilian Amazon basin were: (1) in the eastern Amazon, the Tapajós National Forest situated 50 km south of the city of Santarém, Pará, between the Tapajós river and the road BR 163 (2.85 S; 54.95 W); (2) in the central Amazon, the ZF-2 Experimental Station of Tropical Forestry administered by the National Institute for Research in the Amazon (INPA), located 70 km north of Manaus, in the state of Amazonas (02.37 S; 60.09 W), and (3) in the western Amazon, the National Park of Pico da Neblina, located 70 km northeastern from the city of São Gabriel da Cachoeira, Amazonas state (0.14 N; 66.46 W).

Two additional locations with large foliar isotope records collected during previous assessments were used here. The details about these two sites, Rebio and Samuel, both located in the south-western Amazon in the Rondônia state, can be found in Ometto and others (2006) and Martinelli and others (2000), respectively.

The annual precipitation at the study sites varied between 1800 and 2000 mm in Santarém, 2000 and 2200 mm in both Rebio and Samuel, 2200 and 2400 mm in Manaus, and 3200 and 3600 mm in São Gabriel da Cachoeira (Sombroek 2001). The number of consecutive months with less than 100 mm of rainfall (defined as the dry season) is 5 months for Santarém, 4–5 months for Rebio and Samuel, 2–3 months for Manaus, whereas there is no well-defined seasonality in São Gabriel da Cachoeira (Sombroek 2001).

In all the study sites used for this regional comparison, dense terra-firme forests (closed canopy; flat terrain, upland non-flooded tropical forests (Whitmore 1984; Pires and Prance 1985) were sampled and soils are classified as Oxisols (Dubroeuq and Volkoff 1998; Telles and others 2003). In Santarém, soils are deeply weathered with high clay content (600–800 mg kg⁻¹), low pH (3.6–4.0), low effective cation exchange capacity (CEC), and high aluminum saturation (Silver and others 2000; Telles and others 2003) (Table 1). This region is

Table 1. Physico-Chemical Characterization of the Superficial Soils (0–5 cm Depth) Sampled at the Studied Sites

Site	Clay (%)	pH	Organic C (g kg ⁻¹)	Total N (g kg ⁻¹)	C:N	Total P (mg dm ⁻³)	Available P (mg kg ⁻¹)	Ca ²⁺ (mmol dm ⁻³)	SB ^a (mmol dm ⁻³)	CEC ^b (mmol dm ⁻³)	BS ^c (%)	Fe ^d (mmol dm ⁻³)	Al ^d (mmol dm ⁻³)
Santarém	82 ^c	3.2 ^c	57	4.1	13.8	10.8 ^f	17 ^e	12 ^f	18 ^f	110.4 ^e	9 ^c	0.99 ^h	22.4 ^h
Manaus-plateau	65 ^g	3.9 ^g	30	2.1	13.9		2.7 ^h	1 ^h	2 ^h	110 ^h	2 ^h	0.99 ^h	22.4 ^h
Manaus-baixio	5 ^g	4.4 ^g	33	1.8	19.2		8.8 ^h	0 ^h	2 ^h	115 ^h	2 ^h	0.22 ^h	14.8 ^h
Manaus-campinarana	15	3.6	18	1.2	16.1	5		1	1	61.1	5		
São Gabriel	30	3.7	18	1.3	14.9	8.5		2.5	4.6	90	6		

^aSB = sum of exchangeable bases (Ca²⁺ + Mg²⁺ + K⁺ + Na⁺); ^bCEC = SB + potential acidity at pH 7; ^cBS = base saturation; ^dSoil at 20-cm depth; ^eData from Telles and others (2003); ^fData from RC Oliveira Junior (personal communication); ^gData from Luizão and others (2004); ^hData from V Neu (personal communication).

situated on a large plateau with a deep water table (up to 100 m depth). In Manaus, the dense terra-firme forest site is located on a plateau that is dissected by streams (Telles and others 2003; Luizão and others 2004). The soils have high clay content, low pH, and low effective CEC (Table 1). In São Gabriel da Cachoeira, the terrain is gently rolling with altitudes varying between 80 and 160 m of height. The soils presented low pH (3.6–3.9) and low CEC values and contain an intermediate percent of clay (Table 1).

Comparison at Landscape Level (Manaus Toposequence)

In the Central Amazon, near the city of Manaus (in the state of Amazonas), different forests occupying different positions along a toposequence were sampled to assess forest sites differing in their soil type. One was the dense terra-firme forest site located on a plateau (same site used for the comparison at regional level), and the other terra-firme forest site was located on a valley bottom (locally known as baixio), which is an area partially flooded during the rainy season (Telles and others 2003; Luizão and others 2004). This undulating relief has a maximum peak-to-trough height difference of 120 m. The dominant soil type on the plateaus is well-drained Oxisols rich in kaolinite clay, whereas in the baixios, a quartz sand mix with organic matter predominated (Spodosols). The dense terra-firme forest on the plateau had higher aboveground biomass and canopy height when compared to the terra-firme forest on the baixio, which had a dense sub-canopy with several palms. These terra-firme forests were evergreen with mean canopy height of 23.3 ± 8.4 m and 17.5 ± 6.1 m and maximum canopy height of 54.5 m and 32.2 m in plateau areas and baixio areas in Manaus, respectively (M. Keller, personal communication). We also sampled an intermediate forest along the toposequence, locally called as campinarana forest (official site of the BIONTE Project—INPA/ODA, Manaus, Brazil), which had a dense root mat above the mineral soil, distinctive vegetation features including scleromorphism, trees with small diameters and heights, and a dense sub-canopy with high abundance of epiphytes (Anderson 1981; Ribeiro and others 1999). The soils of this area are classified as Ultisols (Ranzani 1980) (Table 1).

Field Sampling

With the exception of São Gabriel da Cachoeira, field sampling of vegetation was conducted in areas that already had detailed vegetation surveys. In the Santarém forest site (hereafter referred to as San-

tarém), sampling was conducted along four transects (50×1000 m) of a permanent forest inventory belonging to the LBA international research project (Large-Scale Biosphere-Atmosphere Experiment in Amazonia) (Rice and others 2004; Vieira and others 2004). In the Manaus region, sampling was conducted along a single transect (20×2500 m) established by the long-term project JACARANDA (INPA, Manaus). This transect is situated at km 34 of the unpaved road ZF-2, and included a dense terra-firme forest site on the plateau (hereafter referred to as Manaus-plateau) and a terra-firme forest site on the baixio (hereafter referred to as Manaus-baixio). One permanent plot (100×100 m) belonging to the long-term project BIONTE (INPA, Manaus) was chosen in the campinarana site (hereafter called Manaus-campinarana), situated at km 24 of the unpaved road ZF-2. In São Gabriel da Cachoeira (hereafter referred to as São Gabriel) the study was carried out along a transect of 20×700 m located west of km 55 of the unpaved road (BR 307) that crosses the National Park of Pico da Neblina.

In Santarém and Manaus the species were selected based on the important value index (IVI), which calculates the importance of each species according to their abundance, frequency, and dominance. IVI values were taken from previous inventories carried out at these sites, where trees were already tagged, mapped, and most identified at the species level. Sampling was conducted according to the putative N_2 -fixing ability (individuals belonging to the Leguminosae family and hereafter called legumes) or inability (individuals belonging to families other than legumes and hereafter called non-legumes) according to previous reports (Allen and Allen 1981; Faria and others 1989; Moreira and others 1992; Gehring and others 2005).

Plant and woody materials from the trees sampled in São Gabriel were submitted to the Department of Forest Management, INPA (Manaus, AM) for identification at the species level. Subsequently, we separated species into legumes and non-legume groupings. Leaf collections from all sites were obtained during the rainy season.

The number of samples of legumes and non-legumes collected in each site is shown in Table 2. Trees with a diameter greater than 10 cm were used for sampling in Santarém and Manaus sites; trees with a diameter greater than 5 cm at breast height (DBH) were selected for sampling in São Gabriel. A composite sample of healthy, fully expanded leaves from the upper canopy was harvested from each sampled individual (150–200 g). Most leaves were sampled by climbing to reach the

Table 2. Foliar $\delta^{15}\text{N}$ and Foliar N, P, and Ca Concentrations (Mean and 1 Standard Deviation) for Legumes (L) and Non-Legumes (NL) Sampled at Each Dense Terra-Firme Forest in Oxisols Under Different Precipitation Regimes and Each Forest Site of the Manaus Toposequence

	Santarém		Manaus-plateau		São Gabriel		Manaus-campinarana		Manaus-baixio	
	L	NL	L	NL	L	NL	L	NL	L	NL
No. samples	142	155	91	120	26	74	56	57	59	36
$\delta^{15}\text{N}$ (‰)	8.0 ^a (1.5)	7.9 ^d (1.5)	4.5 ^{b,g} (1.1)	4.3 ^{e,j} (1.5)	3.6 ^c (1.1)	3.4 ^f (1.2)	2.2 ^h (1.7)	0.7 ^k (2.5)	0.2 ⁱ (1.0)	0.6 ^k (1.6)
N (g kg ⁻¹)	30.3 ^{*,a} (6.0)	24.4 ^d (6.2)	23.3 ^{*,b,g} (5.2)	20.3 ^{*,d,j} (5.1)	27.0 ^{*,b} (6.5)	21.9 ^d (6.8)	26.1 ^{*,g} (8.2)	16.5 ^j (4.6)	18.9 ^{*,h} (4.8)	14.9 ^j (3.1)
P (g kg ⁻¹)	0.9 ^{*,a} (0.2)	0.8 ^d (0.1)	0.7 ^{*,b,g} (0.2)	0.6 ^{e,j} (0.1)	0.9 ^{*,a} (0.2)	0.8 ^d (0.3)	0.8 ^{*,g} (0.4)	0.6 ^j (0.6)	0.7 ^g (0.1)	0.6 ^j (0.1)
Ca (g kg ⁻¹)	6.9 ^a (3.3)	8.9 ^d (4.3)	3.4 ^{b,g} (1.9)	3.2 ^{e,j} (1.9)	2.7 ^b (1.0)	3.4 ^c (1.2)	1.2 ^h (0.8)	1.3 ^k (1.3)	4.0 ^g (2.3)	4.9 ^j (3.3)

Statistical differences in the paired tests (L vs NL) for every site (*t* test; $P < 0.05$) are indicated by the asterisk (*). Statistical differences among sites (ANOVA followed by post hoc Tukey; $P < 0.05$) are represented by different letters as follows: "a", "b", "c" for the legumes (L), and "d", "e", "f" for the non-legumes (NL) of the Santarém, Manaus-plateau, and São Gabriel and by "g", "h", "i", "j", "k", "l" for the legumes (L), and "m", "n", "o", "p", "q", "r" for the non-legumes (NL) of Manaus-plateau, Manaus-campinarana, and Manaus-baixio.

upper canopy. The litter-layer on the floor was sampled at random locations using a 50 × 50 m wooden frame (10 samples per site). Soil pits were excavated to a depth of 50 cm in all studied sites. Samples from three to six walls in every studied site were taken at each depth interval (0–5, 5–10, 10–20, 20–30, 30–40, and 40–50 cm).

Laboratory Analyses

All leaves and litter were oven-dried at 65°C until constant weight and then ground to a fine powder. Soil samples were air-dried, sieved using a 2-mm mesh, and homogenized. A smaller sub-sample was taken, handpicked to remove fine roots and other debris, and then ground to a fine powder.

A 1.5–2 mg sub-sample of ground organic material (leaf and litter) or 15–30 mg sub-sample of pounded soil was placed in a tin capsule and loaded into a ThermoQuest-Finnigan Delta Plus isotope ratio mass spectrometer (Finnigan-MAT; CA, USA) interfaced with an Elemental Analyzer (Carla Erba model 1110; Milan, Italy) at the Laboratory of Isotope Ecology (CENA-USP, Brazil) to obtain both the nitrogen isotope ratio ($\delta^{15}\text{N}$) and the carbon and nitrogen contents of these samples. Stable isotope ratios of nitrogen were measured relative to internationally recognized standards. Internal reference materials (atropine, yeast and soil standard no. 502–308 from LECO Corporation) were included in every analytical run. Stable isotope values are reported in "delta" notation as δ values in parts per thousand (‰), so that $\delta\text{‰} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000$, where R is the molar ratio of the rare to abundant isotope ($^{15}\text{N}/^{14}\text{N}$) in the sample and the standard. Atmospheric air was used as nitrogen standard.

Extra soil samples collected in São Gabriel and Manaus-campinarana were used to determine soil texture, pH, total P, and the concentrations of major cations. The analyses followed the procedures of EMBRAPA (1999). Sub-samples (0.4 g dry weight) of the organic material (leaf and litter) from all studied sites were digested in 6 ml of concentrated perchloric acid and hydrogen peroxide. Phosphorus concentration was determined by colorimetry (EMBRAPA 1999) and an atomic absorption spectrophotometer (Varian, Inc. model SpectrAA) was used to determine Ca concentrations (Malavolta and others 1997).

Statistical Analyses

The data were normally distributed (Kolmogorov-Smirnov test). Therefore, the analyses were performed using parametric tests and Pearson

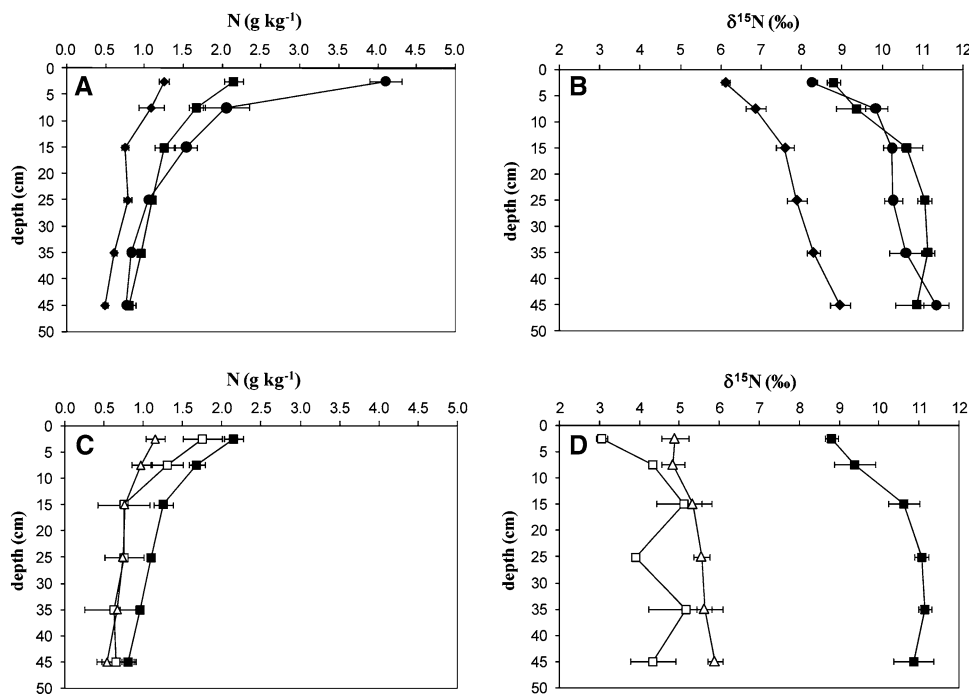


Figure 1. Variations in total N concentration (g kg⁻¹) (mean ± standard errors) throughout soil profiles in (A) Santarém (●), Manaus-plateau (■), and São Gabriel (◆) and (C) Manaus-plateau (■), Manaus-campinarana (Δ), and Manaus-baixio (□); and variations in δ¹⁵N signatures (‰) (mean ± standard errors) throughout the soil profiles in (B) Santarém (●), Manaus-plateau (■), and São Gabriel (◆) and (D) Manaus-plateau (■), Manaus-campinarana (Δ), and Manaus-baixio (□).

correlations. Student's *t*-test was used to assess differences between legumes and non-legumes within a site, between two sites or for seasonal comparisons in a same site. ANOVA followed by a post hoc Tukey HSD test was used to determine differences when more than two sites were compared. All statistical analyses were performed using the software STATISTICA, version 6.1 for Windows (StatSoft, Inc. 2004). Differences at the 0.05 level were reported as significant.

The dense terra-firme forests in Oxisols (Santarém, Manaus-plateau, Rebio, Samuel, and São Gabriel) were used to compare sites under different precipitation conditions. Throughout the Manaus topequence, the dense terra-firme forest in Oxisol (Manaus-plateau) was compared with the terra-firme forest on Spodosol (Manaus-baixio) and with the campinarana forest on Ultisol (Manaus-campinarana). A complete list of the species sampled in each forest site, as well as the average and standard deviation values for foliar δ¹⁵N, foliar N, C:N ratios, and P and Ca concentrations, can be accessed online at <http://www.juruti.cena.usp.br/geoprocessamento/phpBB2/dload.php>

RESULTS

Soil Characterization

Comparison at the Regional Level

The percent of clay of the surface soils (0–5 cm depth) from the dense terra-firme forest sites in

Oxisols, that is, Santarém, Manaus-plateau and São Gabriel, ranged from 30 to 80% (Table 1). The Santarém Oxisol had the highest N content ($P < 0.05$), highest available, and total P values as well as the highest concentrations of base cations when compared to each of the other studied soils.

Total N concentration decreased with soil depth at each site. Nitrogen concentration through the soil profile was higher in Santarém when compared to the other dense terra-firme forests ($P < 0.05$) (Table 1; Figure 1A). At the same time, soil N concentrations did not differ significantly between the Manaus-plateau and São Gabriel, except at the surface soil (0–5 cm depth), which was higher in the Manaus-plateau ($P < 0.05$) (Figure 1A).

The soil profiles from Manaus-plateau and Santarém had nearly identical δ¹⁵N values, but were more enriched in ¹⁵N than the soils from São Gabriel ($P < 0.05$) (Figure 1B).

Comparison at the Landscape Level (Manaus Toposequence)

Throughout the Manaus topequence, the percentage of sand and clay varied significantly, ranging from 95% sand (baixio) to 65% clay (plateau) (Table 1). There was a tendency for higher total N soil concentrations along the profile at the Manaus-plateau site compared to the other two forest sites, but the differences were not statistically significant, except at the surface soil (0–5 cm depth) (Figure 1C).

Soil $\delta^{15}\text{N}$ values in Manaus-baixio and Manaus-campinarana did not differ with depth, but were less enriched in ^{15}N when compared to Manaus-plateau site ($P < 0.05$) (Figure 1D).

Nutrient and Isotopic Composition of Leaves

Comparison Between Legumes and Non-Legumes

The average foliar N concentration was statistically higher in legume leaves than in non-legume leaves at each study site ($P < 0.05$) (Table 2). Averaging over all sample collections, the total average foliar N concentration was $26.0 \pm 7.6 \text{ g kg}^{-1}$ (mean \pm 1 standard deviation; SD) for legumes, which was significantly higher than the $21.2 \pm 6.5 \text{ g kg}^{-1}$ (mean \pm 1 SD) for the non-legumes trees ($P < 0.05$).

Mean foliar $\delta^{15}\text{N}$ values did not differ significantly between legumes and non-legume trees sampled within a site, but varied among sites (Table 2). Some of the legumes appeared to be fixing N_2 (with foliar $\delta^{15}\text{N}$ values between 0–2‰) in the dense terra-firme forests in Oxisols. Although the average foliar $\delta^{15}\text{N}$ value for non-legume trees was $4.3 \pm 1.5\text{‰}$ (mean \pm 1 SD) on the Manaus-plateau, the values measured in leaves of some potentially N_2 -fixing legumes were significantly more depleted in ^{15}N : *Swartzia tomentifera* ($0.9 \pm 0.7\text{‰}$; $n = 4$), *Swartzia ulei* ($1.6 \pm 0.4\text{‰}$; $n = 4$), *Andira micrantha* (0.6‰ ; $n = 1$), *Ormosia paraensis* (1.6‰ ; $n = 1$), and *Ormosia grossa* ($1.7 \pm 0.7\text{‰}$; $n = 3$). In the São Gabriel aseasonal forest, the average foliar $\delta^{15}\text{N}$ signature for non-legumes was $3.4 \pm 1.2\text{‰}$ and two potentially N_2 -fixing legumes had markedly different and depleted $\delta^{15}\text{N}$ values: *Swartzia corrugata* (0.7‰ ; $n = 1$) and an unidentified Fabaceae (0.6‰ ; $n = 1$). These apparent N_2 -fixing legumes were excluded from subsequent analyses.

At all but the Manaus-baixio study site, legumes had significantly higher foliar P concentrations than the non-legumes ($P < 0.05$) (Table 2). In Santarém, Manaus-plateau, and Manaus-baixio, there were no differences in the foliar N:P ratios between legumes and non-legumes, whereas at the other sites, legumes had higher foliar N:P ratios than non-legumes ($P < 0.05$). No significant differences were found in the foliar Ca concentration between legumes and non-legumes (Table 2).

Melding both legumes and non-legumes observations into the analysis, foliar N concentrations and foliar $\delta^{15}\text{N}$ values were positively correlated ($r = 0.49$). Additionally, foliar P concentrations and

foliar $\delta^{15}\text{N}$ values were positively correlated ($r = 0.43$). Foliar N and P concentrations were also positively correlated with each other ($r = 0.52$) ($P < 0.05$), but foliar N:P ratio and $\delta^{15}\text{N}$ was not significantly correlated ($r = 0.10$) with each other.

Comparisons at the Regional Level

Aggregating all trees sampled across sites, the foliar N concentrations were not significantly different among the dense terra-firme forests in Oxisols under contrasting precipitation regimes (Table 2). Foliar P concentrations at the Santarém and São Gabriel sites were similar, and were greater than values measured in the Manaus-plateau site ($P < 0.05$). On the other hand, leaves from trees collected in Santarém generally had twice as much foliar Ca as leaves collected from trees in the Manaus-plateau and São Gabriel forests ($P < 0.05$) (Table 2).

For both the non-legume and legume trees the dense terra-firme forest in Santarém had the highest foliar $\delta^{15}\text{N}$ values (Table 2). The foliar $\delta^{15}\text{N}$ in the dense terra-firme forests increased with increasing length of the dry season and with decreasing rainfall, as follows: São Gabriel < Manaus-plateau = Rebio < Samuel < Santarém ($P < 0.05$) (Figure 2).

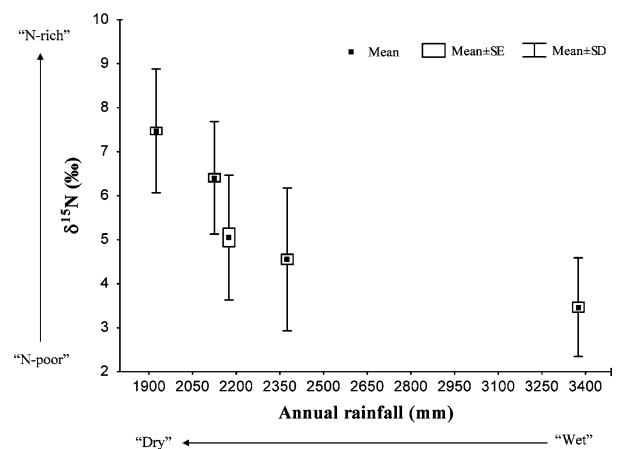


Figure 2. Isotopic expression of N availability for dense terra-firme forests in Oxisols across the Brazilian Amazon Basin. The values on the “Y” axis represent the foliar $\delta^{15}\text{N}$ including both the legumes and non-legumes sampled at each site. The values on the “X” axis are the annual precipitation for the studied sites: 1800–2000 mm for Santarém, PA; 2000–2200 mm for Rebio and Samuel, RO; 2200–2400 mm for Manaus, AM; and 3200–3600 mm for São Gabriel da Cachoeira, AM.

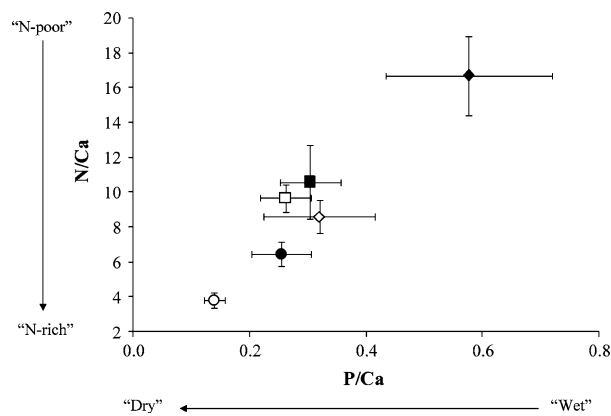


Figure 3. Non-isotopic expression of N availability for dense terra-firme forests in Oxisols across the Brazilian Amazon Basin. The values in the axes represent the foliar N/Ca and P/Ca (mean \pm standard errors) for (●) legumes from Santarém, (○) non-legumes from Santarém; (■) legumes from Manaus-plateau, (□) non-legumes from Manaus-plateau; (◆) legumes from São Gabriel and (◇) non-legumes from São Gabriel.

Calcium tends to be mainly found as a structural element in the leaves, often reflecting leaf thickness, although in Ca richer sites this element may be accumulated in vacuoles as calcium oxalate. However, in the Ca poor studied sites (see Table 1) most Ca in foliar tissues is probably found as a structural element. Leaves of the legume and non-legume trees did not differ in their foliar Ca concentrations. Based on that, we plotted foliar N:Ca versus P:Ca ratio (Figure 3). Together with the foliar $\delta^{15}\text{N}$ values versus precipitation (Figure 2), we developed a matrix of conditions that depicted how we expected N availability to manifest itself in these different forests.

Comparisons at the Landscape Level (Manaus Toposequence)

Foliar N and P concentrations did not differ significantly at the three different forest sites (Table 2).

However foliar Ca concentrations were lower in leaves from Manaus-campinarana ($P < 0.05$) when compared with the leaves produced at the other two sites (Manaus-plateau and Manaus-baixio).

Foliar $\delta^{15}\text{N}$ values from Manaus-baixio and Manaus-campinarana did not differ significantly (Table 2). However, woody plants from these two sites had lower foliar $\delta^{15}\text{N}$ values when compared to leaves on plants growing at the Manaus-plateau site ($P < 0.05$). For instance, the $\delta^{15}\text{N}$ values of some legumes and non-legumes at these sites revealed that in a single common taxa, leaves had different N isotope values depending on their position along the Manaus toposequence (Table 3).

DISCUSSION

Spatial Scales Variation in N Availability Across the Brazilian Amazon Forest

Regional Scale

A decrease in foliar $\delta^{15}\text{N}$ with increasing rainfall and decreasing length of dry season was observed for terra-firme forests in Oxisols across the immense geographical area of the Brazilian Amazon Basin. This trend is consistent with other tropical forests worldwide (Austin and Vitousek 1998; Handley and others 1999; Amundson and others 2003; Houlton and others 2006).

Recently, Houlton and others (2006) pointed out that gaseous N loss through denitrification is the major determinant of N isotope variations across differences in rainfall. Although in the drier sites, incomplete nitrate consumption by denitrification causes an elevation of the bulk soil $\delta^{15}\text{N}$ values, in the wetter sites, complete denitrification prevents this process from occurring.

Annual N-inputs via litterfall were considerably lower in the Manaus-plateau on Oxisol and in a forest site on Oxisol near the São Gabriel terra-firme forest, in the region of San Carlos del Rio Negro (see

Table 3. Mean (and Standard Errors) Foliar $\delta^{15}\text{N}$ Signatures (‰) for Some Legumes and Non-Legumes Co-occurring Along the Manaus Toposequence (Located in the Amazonas State, Brazil)

Family	Species	Site		
		Manaus-plateau	Manaus-campinarana	Manaus-baixio
Fabaceae	<i>Dipteryx odorata</i> Willd.	3.96 (0.6)	1.25 (1.1)	1.56
Fabaceae	<i>Swartzia reticulata</i> Ducke	3.20 (0.4)	1.39 (0.5)	-1.65
Mimosaceae	<i>Inga</i> sp.	6.85 (0.6)	3.84 (0.3)	-0.48
Bombacaceae	<i>Scleronema micranthum</i> Ducke	4.08 (0.3)	0.90 (0.2)	0.90 (0.8)
Chrysobalanaceae	<i>Licania</i> sp.	4.91 (0.6)	-0.11 (0.7)	-0.84
Sapotaceae	<i>Micropholis guyanensis</i> T.D.Penn.	3.76 (0.3)	0.89 (1.7)	1.09

Table 4. Litter Production and Nitrogen Fluxes, Soil Inorganic-N and Nutrient Use Efficiency (NUE) Assessed for Each Studied Site

Site	Mass* (Mg ha ⁻¹ y ⁻¹)	N ^a (Kg ha ⁻¹ y ⁻¹)	P ^a (Kg ha ⁻¹ y ⁻¹)	Ca ^a (Kg ha ⁻¹ y ⁻¹)	NUE (N) ^b (µg g ⁻¹)	NUE (P) ^b (µg g ⁻¹)	NH ₄ -N (µg g ⁻¹)	NO ₃ -N (µg g ⁻¹)	Net N mineralization (µg Ng ⁻¹ d ⁻¹)	Net nitrification (µg Ng ⁻¹ d ⁻¹)	NO (mg N cm ⁻² h ⁻¹)	N ₂ O (ng N cm ⁻² h ⁻¹)
Santarém	12.0 ^c	176	4.6	102	48	1830	3–8 ^{g,h}	7–14 ^{g,h}	1–2 ^g –0.3–0.5 ⁱ	1.9–2.3 ^g 2.9–4.3 ⁱ	0.2–2.0 ^j 8.8–9.0 ^k 2.9 (0.5) ^l	0.2–3.5 ^j 7.0–7.9 ^k 13.4 (1.2) ^l
Manaus-plateau	8.9 ^d	109	1.9	34	64	3650	0.2–20 ^d	0.2–22 ^d	0.15 ^d	0.33 ^d		
Manaus-baixio	6.6 ^d	59	1.4	12.7	83	3490	0–12 ^d	0.2–12 ^d	–0.70 ^d	–1.35 ^d		
Manaus-campinarana	6.3 ^e	69	1.1	10.5	63	3960	29.6 (21.4) ⁱ	0.51 (0.42) ⁱ	–2.0–0.05 ⁱ	0.05–0.1 ⁱ		1.5–3.5 ^m
São Gabriel	10.3 ^f	122	3.1	30.4	62	2450						

*fine litterfall; ^aleaf litterfall x nutrient concentration in leaf litter; ^bleaf litterfall/nutrient flux via litterfall (according to Vitousek 1984); ^cRice and others (2004); ^dLuizão and others (2004) (0–10 cm depth); ^eData from F Luizão (personal communication); ^fCuevas and Medina (1986) data were used because it is the closest terra-firme forest site to Sao Gabriel that has been sampled; ^gSilver and others (2000) (0–10 cm depth); ^hPerez and others (2006); ⁱData from RCC Luizão (personal communication); ^jDavidson and others (2004); ^kKeller and others (2005); ^lSilver and others (2005); ^mLuizão and others (1989). The values are reported as means (and 1 standard deviation) or the minimum and maximum values.

Table 4). As a consequence, the nutrient use efficiency (NUE) indices [as defined by Vitousek (1984)] for both N and P were lower in the Santarém site (Table 4), denoting more efficient nutrient cycling at the wetter rainforest sites. Additionally, vegetation on Oxisol soils at Santarém has greater rates of potential net N mineralization and nitrification (Silver and others 2000; Silver and others 2005; Perez and others 2006) and high denitrification enzyme activity (Silver and others 2000).

Gaseous N losses may be the basis of the high N isotope ratios observed in this study. Perez and others (2006) reported high δ¹⁵N values of the inorganic N form in Santarém forest soil (δ¹⁵N–NH₄⁺ and δ¹⁵N–NO₃⁻ of 31.6‰ and 4.2‰, respectively), whereas the calculated enrichment factors for the δ¹⁵N–N₂O product of nitrification and denitrification were –80.1 and –26.7‰, respectively. These high isotopic values suggest that trees in Santarém were taking up relatively enriched ¹⁵N–NH₄⁺ and ¹⁵N–NO₃⁻ that were the net result of losses of N from the ecosystem, mainly by nitrification and denitrification (Silver and others 2000; Perez and others 2006). High soil-atmosphere NO and N₂O emissions from Santarém forest soil (Table 4) and N₂O fluxes of 6–7 kg N ha⁻¹ y⁻¹ have been measured in Santarém and are among the highest flux rates measured for any old-growth tropical forest ecosystem (Keller and others 2005).

These ¹⁵N differences among sites suggest that ecosystem N losses are higher where ecosystem N availability is higher. The mechanism proposed by Houlton and others (2006) based on high N losses from the soil leaving behind a ¹⁵N enriched substrate may explain the occurrence of higher δ¹⁵N in Santarém (drier site). Non-isotopic independent data on N availability pointed toward a similar trend, with higher N availability in the drier sites.

Landscape Scale

Both litterfall and the annual flux of N via litterfall were considerably lower in the campinarana and the lowland terra-firme forest in the baixio compared to the dense terra-firme forest on the plateau. In addition, soil microbial processes differ along this toposequence with nitrification as the dominant process in the soil of the dense terra-firme forest on the plateau and immobilization of N predominating at terra-firme forest on the baixio (Luizão and others 2004). Vitousek and Matson (1988) reported that sandy soils of the lower topographic positions near Manaus exhibited low potential net NO₃ production and relatively low levels of recovery of added ¹⁵NO₃. Lower net

Table 5. Mean $\delta^{15}\text{N}$ Values and N Concentration of Leaves (and Standard Errors) from Some Sites in the Amazon Basin

Site	Vegetation	Soil Type	MAP ^a (mm)	MAT ^b (°C)	Legume $\delta^{15}\text{N}$ (‰)	Non-legume $\delta^{15}\text{N}$ (‰)	Legume N (g kg ⁻¹)	Non-legume N (g kg ⁻¹)
Santarém - FLONA 1 ^c	terra-firme forest	Oxisol	1900	24	7.0 (0.15)	6.9 (0.09)	26.7 (0.8)	21.6 (1.2)
Santarém - FLONA 2 ^c	terra-firme forest	Oxisol	1900	24	6.3 (0.29)	5.1 (0.07)	32.7 (1.5)	20.7 (1.0)
Manaus-ZF2c	terra-firme forest	Oxisol	2300	26.5	5.5 (0.35)	4.8 (0.16)	24.5 (1.7)	19.4 (0.8)
Near Manaus-AM ^d	terra-firme forest	Oxisol	2300	26.5	4.3	4.0	29.5	22.7
REBIO-RO ^c	terra-firme forest	Oxisol	2100	25		5.0 (0.22)		24.8 (1.0)
Samuel-RO ^c	terra-firme forest	Oxisol	2100	25	6.1 (0.16)	6.4 (0.2)	25.6 (0.2)	19.6 (0.3)
Piste de St. Eli-Guiana ^f	terra-firme forest	Oxisol	3250	26	1.9 (0.24)	5.0 (0.13)	24.4 (0.8)	21.8 (0.5)
Piste de St. Eli-Guiana ^f	terra-firme forest	Spodosol	3250	26	0.25 (0.33)	1.8 (0.21)	22.1 (1.6)	20.1 (0.9)
Piste de St. Eli-Guiana ^g	terra-firme forest	Oxisol	3250	26	1.7 (0.19)	4.9 (0.10)	23.6 (0.67)	21.7 (0.5)

^aMean annual precipitation; ^bMean annual temperature; ^cOmetto and others (2006); ^dGehring and Vlek (2004); ^eMartinelli and Medina (unpublished data); ^fRoggy and others (1999a); ^gRoggy and others (1999b).

nitrification rates and NO_3 concentrations are likely to reduce denitrifier activity (Livingston and others 1988).

The decrease of foliar $\delta^{15}\text{N}$ throughout the Manaus topequence as well as the differences found in $\delta^{15}\text{N}$ values of the same taxa depending on their position throughout this topequence, suggest that differences in soil type (differing in sand and clay content) were linked to the changes in N cycling processes. Silver and others (2000) reported that greater N inputs raised productivity and soil organic matter (SOM) content substantially both in sand and clay soils, but less SOM is stabilized in sands than in clays. Consequently less N may be available for mineralization.

Annual N_2O fluxes from old-growth forests were four times greater on clayey Oxisol soils compared to sandy loam Ultisol soils (Keller and others 2005), whereas potential denitrification was four times greater in clayey Oxisol than in sandy loam Ultisol soils (Silver and others 2000). Perez and others (2006) reported that the ^{15}N enrichment factors for nitrification (NH_4^+ to N_2O) and for the “first” step of denitrification (NO_3^- to N_2O) differ with soil texture and site, where higher ^{15}N -enrichment was found in soils with higher clay content. Based on these characteristics of N cycling, it appears that the remaining N pool was more enriched in ^{15}N in the dense terra-firme forest on the plateau when compared to the forests on sandier soils. Given that forests on sandier soils had more similarities both in terms of isotopic and nutritional composition of leaves (except Ca) and soil when compared to the dense terra-firme forest on the plateau, these data suggest that under the same precipitation condition, differing sand and clay content of the soil is a

major factor influencing the N availability. In the case of the Central Amazon reported here, N availability is higher in the dense terra-firme forests on clayey soils on the plateau than in forests on sandier soils. The relationships here documented explain to a large extent the variation in $\delta^{15}\text{N}$ signatures across gradients of rainfall and soil. Although at the regional level, the precipitation regime is a major determinant of differences in N availability, at the landscape level, under the same precipitation regime, soil type seems to be a major factor influencing availability of N in the Brazilian Amazon Basin.

Nitrogen Variation Across Plant Functional Groups (Legume × Non-Legume Trees)

Independent of the studied site, legume trees had consistently higher foliar N concentrations than non-legume trees. This same trend has been reported for other tropical rainforests (Roggy and others 1999a, b; Vitousek and others 2002; Ometto and others 2006) (see Table 5), and may be related to a more efficient N assimilation process in legumes especially those characterized by high-N-demand patterns (McKey 1994). Such a pattern is consistent with the high energetic costs in plant growth associated with higher foliar P/Ca ratios in legumes when compared to non-legumes trees.

Högberg (1997) predicted that when plants derived a substantial portion of their N inputs from atmospheric N_2 , then leaf $\delta^{15}\text{N}$ values would be about 0–2‰ and, the ^{15}N natural abundance method requires a minimum 5‰ difference

between the mean $\delta^{15}\text{N}$ signals of potentially N_2 -fixing and of reference plants, to estimate symbiotic biological N fixation (symbiotic BNF) with some degree of certainty. However, many problems that affect the ^{15}N natural abundance method are bound to vary between study sites or vegetation types in heterogeneous forests (Gehring and Vlek 2004). The lack of a straightforward method for measuring the $\delta^{15}\text{N}$ -signature of 'soil-derived' N, poorly understood interactions between differing plant and soil N pools, and resulting small-scale spatial $\delta^{15}\text{N}$ -patterns are some of the factors responsible for the failure of the ^{15}N natural abundance method for symbiotic BNF estimation (Gehring and Vlek 2004).

Given the above-outlined limitations of quantitative symbiotic BNF estimation, searches for indirect evidence on the role of symbiotic BNF across the gradients of soil and rainfall in the Brazilian Amazon forest were addressed. At the campinarana forest and the terra-firme on the baixio in Manaus, foliar $\delta^{15}\text{N}$ values of potentially N_2 -fixing trees overlapped with the depleted foliar $\delta^{15}\text{N}$ of the non-legume trees.

Across the terra-firme forests in Oxisol soils, N isotope ratios were high and the enriched foliar $\delta^{15}\text{N}$ values were not significantly different between legumes and non-legume species; these results suggest that most of these woody legumes were not fixing atmospheric N. In these wetter dense terra-firme forest sites, only 8–14% of the legume species sampled were apparently fixing atmospheric N_2 at Manaus and São Gabriel, respectively, based on N isotope ratio values. Gehring and others (2005) considered significant symbiotic BNF to be a more important N input in secondary re-growth areas (where N-limiting conditions tend to be higher) than in mature rainforests of the Central Amazon.

Although Roggy and others (1999a, b) reported that a substantial N contribution in N_2 -fixing species from a drier rain forest on Oxisol soils in French Guiana was 'nitrogen derived from the atmosphere', our evidence is consistent with most of the previous studies indicating low symbiotic BNF rates in mature tropical forests (Vitousek and others 2002; Gehring and others 2005; Ometto and others 2006), especially those in which P rather than N is apparently the limiting primary production (Vitousek 2004; Davidson and others 2007). In the setting of tropical forests here studied, plant-soil interactions at smaller scales and involving different N-pools are further great challenges for future ^{15}N -based research in such heterogeneous ecosystems.

ACKNOWLEDGEMENTS

We are grateful to Vilany Carneiro and Everaldo Pereira (INPA, Manaus) for their valuable help with plant identification in São Gabriel and Manaus sites. We thank R. Delfini for field and lab assistance and M.A. Perez, F. Fracassi (CENA/USP), and C. Cook (University of Utah) for lab support. We acknowledge the technical support from Dr. P.B. de Camargo, Dr. M.Z. Moreira, and Dr. Takashi Muraoka (CENA/USP). We thank the Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus and the office of LBA project for logistical support. We thank IBAMA for access to sites in the FLONA Tapajós in Santarém and in São Gabriel da Cachoeira. This study was supported by grants from FAPESP (project no. 01/09984–6) and NASA to project CD-02 in the LBA Terrestrial Ecology program.

REFERENCES

- Allen ON, Allen EK. 1981. The Leguminosae—a source book of characteristics, uses, and nodulation. Madison: The University of Wisconsin Press, p 812.
- Amundson R, Austin AT, Schuur EAG, Yoo K, Matzer V, Kendall C, Uebersax A, Brenner D, Baisden WT. 2003. Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochemical Cycles* 17(1): Art. No. 1031.
- Anderson AB. 1981. White-sand vegetation of Brazilian Amazonia. *Biotropica* 13:199–210.
- Austin AT, Vitousek PM. 1998. Nutrient dynamics on a precipitation gradient in Hawai'i. *Oecologia* 113(4):519–29.
- Cuevas E, Medina E. 1986. Nutrient dynamics within Amazonian forest ecosystems. I nutrient flux in fine litter fall and efficiency of nutrient utilization. *Oecologia* 68:466–72.
- Daly DC. 1989. Brazilian Amazon. In: Campbell DG, Ed. *Floristic inventory of tropical countries*. New York: The New York Botanical Garden. p 401–26.
- Davidson EA, Ishida FY, Nepstad DC. 2004. Effects of an experimental drought on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Glob Chang Biol* 10(5):718–30.
- Davidson EA, Carvalho CJR, Figueira AMS, Ishida FY, Ometto JPHB, Nardoto GB, Saba RT, Hayashi SN, Leal EC, Vieira ICG, Martinelli LA. 2007. Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. *Nature* 447:995–8.
- Dubroeuq D, Volkoff B. 1998. From oxisols to spodosols and histosols: evolution of the soil mantles in the Rio Negro basin (Amazonia). *Catena* 32:245–80.
- EMBRAPA. 1999. Manual de análises químicas de solos, plantas e fertilizantes. Brasília: EMBRAPA, p 370.
- Evans RD. 2001. Physiological mechanisms influencing plant nitrogen isotope composition. *Trends Plant Sci* 6(3):121–6.
- Faria SM, Lewis GP, Sprent JI, Sutherland JM. 1989. Occurrence of nodulation in the Leguminosae. *New Phytol* 111:607–19.
- Gehring C, Vlek PLG. 2004. Limitations of the N-15 natural abundance method for estimating biological nitrogen fixation in Amazonian forest legumes. *Basic Appl Ecol* 5(6):567–80.

- Gehring C, Vlek PLG, Souza LAG, Denich M. 2005. Biological nitrogen fixation in secondary regrowth and mature rainforest of central Amazonia. *Agric Ecosyst Environ* 111:237–52.
- Greenland DJ, Kowal JM. 1960. Nutrient content of the moist tropical forest of Ghana. *Plant Soil* 12(2):154–74.
- Handley LL, Austin AT, Robinson D, Scrimgeour CM, Raven JA, Heaton THE, Schmidt S, Stewart GR. 1999. The N-15 natural abundance (delta N-15) of ecosystem samples reflects measures of water availability. *Aust J Plant Physiol* 26(2):185–99.
- Högberg P. 1997. Tansley review No 95—N-15 natural abundance in soil-plant systems. *New Phytol* 137(2):179–203.
- Houlton BZ, Sigman DM, Hedin LO. 2006. Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. *Proceedings of the National Academy of Sciences of the United States of America* 103:8745–50.
- Jenny H. 1950. Causes of the high nitrogen and organic matter content of certain tropical forest soils. *Soil Sci* 69:63–9.
- Jenny H, Bingham FT, Padilla-Saravia B. 1948. Nitrogen and organic matter contents of equatorial soils of Colombia, South America. *Soil Sci* 66:173–86.
- Jenny H, Gessel SP, Bingham FT. 1949. Comparative study of decomposition rates of organic matter in temperate and tropical regions. *Soil Sci* 68:419–32.
- Keller M, Kaplan WA, Wofsy SC. 1986. Emissions of N₂O, CH₄ and CO₂ from tropical soils. *J. Geophys Res* 91:11791–802.
- Keller M, Varner R, Dias JD, Silva H, Crill P, de Oliveira RC Jr, Asner GP. 2005. Soil-atmosphere exchange of nitrous oxide, nitric oxide, methane, and carbon dioxide in logged and undisturbed forest in the Tapajos National Forest, Brazil. *Earth Interact* 9:23.
- Livingston GP, Vitousek PM, Matson PA. 1988. Nitrous oxide flux and nitrogen transformations across a landscape gradient in Amazonia. *J Geophys Res* 93:1593–9.
- Luizão FJ, Matson P, Livingston G, Luizão R, Vitousek P. 1989. Nitrous oxide flux following tropical land clearing. *Global Biogeochem Cycles* 3(3):281–5.
- Luizão RCC, Luizão FJ, Paiva RQ, Monteiro TF, Sousa LS, Kruijt B. 2004. Variation of carbon and nitrogen cycling processes along a topographic gradient in a central Amazonian forest. *Glob Chang Biol* 10(5):592–600.
- Malavolta E, Vitti GC, Oliveira AS. 1997. Avaliação do estado nutricional das plantas. Potafo: Princípios e aplicações. Piracicaba, p 319.
- Martinelli LA, Piccolo MC, Townsend AR, Vitousek PM, Cuevas E, McDowell W, Robertson GP, Santos OC, Treseder K. 1999. Nitrogen stable isotopic composition of leaves and soil: tropical versus temperate forests. *Biogeochemistry* 46(1–3):45–65.
- Martinelli LA, Almeida S, Brown IF, Moreira MZ, Victoria RL, Filoso S, Ferreira CAC, Thomas WW. 2000. Variation in nutrient distribution and potential nutrient losses by selective logging in a humid tropical forest of Rondonia, Brazil. *Biotropica* 32(4):597–613.
- Matson PA, Vitousek PM. 1987. Cross-system comparisons of soil nitrogen transformations and nitrous oxide flux in tropical forest ecosystems. *Global Biogeochem Cycles* 1:163–70.
- McKey D. 1994. Legumes and nitrogen: the evolutionary ecology of a nitrogen-demanding lifestyle. In: Sprent JL, McKey D, Eds. *Advances in legume systematics: Part 5—the nitrogen factor*. Richmond: Kew Publishing. p 211–28.
- Medina E, Cuevas E. 1994. Mineral nutrition: humid tropical forests. *Prog Bot* 55:115–27.
- Moreira FMD, da Silva MF, de Faria SM. 1992. Occurrence of nodulation in legume species in the Amazon region of Brazil. *New Phytol* 121(4):563–70.
- Neill C, Piccolo MC, Steudler PA, Melillo JM, Feigl BJ, Cerri CC. 1995. Nitrogen dynamics in soils of forests and active pastures in the Western Brazilian Amazon Basin. *Soil Biol Biochem* 27(9):1167–75.
- Nye PH. 1961. Organic matter and nutrient cycles under moist tropical forest. *Plant Soil* 13(4):333–46.
- Ometto JPHB, Ehleringer JR, Domingues TF, Berry JA, Ishida FY, Mazzi E, Higuchi N, Flanagan LB, Nardoto GB, Martinelli LA. 2006. The stable carbon and nitrogen isotopic composition of vegetation in tropical forests of the Amazon region, Brazil. *Biogeochemistry* 79:251–74.
- Perez T, Garcia-Montiel D, Trumbore S, Tyler S, De Camargo P, Moreira M, Piccolo M, Cerri C. 2006. Nitrous oxide nitrification and denitrification N-15 enrichment factors from Amazon forest soils. *Ecol Appl* 16(6):2153–67.
- Piccolo MC, Neill C, Cerri CC. 1994. Net nitrogen mineralization and net nitrification along a tropical forest-to-pasture chronosequence. *Plant Soil* 162:61–70.
- Pires JM, Prance GT. 1985. The vegetation types of the Brazilian Amazon. In: Prance GT, Lovejoy TE, Eds. *Amazonia: key environment*. London: Pergamon Press. p 109–45.
- Prance GT. 1990. The floristic composition of the forests of Central Amazonian Brazil. In: Gentry AH, Ed. *Four neotropical rainforests*. New Haven: Yale University Press. p 112–40.
- Prance GT. 1994. A comparison of the efficacy of higher taxa and species numbers in the assessment of biodiversity in the neotropics. *Philos Trans R Soc Lond B Biol Sci* 345:89–99.
- Proctor J. 1987. Nutrient cycling in primary and old secondary rain forests. *Appl Geogr* 7:135–52.
- Ranzani G. 1980. Identificação e caracterização de alguns solos da Estação Experimental de Silvicultura Tropical do INPA. *Acta Amazônica* 10(1):7–41.
- Renck A, Lehmann J. 2004. Rapid water flow and transport of inorganic and organic nitrogen in a highly aggregated tropical soil. *Soil Sci* 169(5):330–41.
- Ribeiro JELS, Hopkins MJG, Vicentini A, Sothers CA, Costa MAS, Brito JM, Souza MAD, Martins LHP, Lohmann LG, Assunção PACL, Pereira EC, Silva CF, Mesquita MR, Procópio LC. 1999. Flora da Reserva Ducke. Guia de Identificação das Plantas Vasculares de uma Floresta de Terra-firme na Amazônia Central. Manaus: INPA-DFID, p 816.
- Rice AH, Pyle EH, Saleska SR, Hutyrá L, Palace M, Keller M, Camargo PB, Portilho K, Marques DF, Wofsy SC. 2004. Carbon balance and vegetation dynamics in an old-growth Amazonian forest. *Ecol Appl* 14(4):55–71.
- Robinson D. 2001. Delta N-15 as an integrator of the nitrogen cycle. *Trends Ecol Evol* 16(3):153–62.
- Roggy JC, Prevost MF, Garbaye J, Domenach AM. 1999. Nitrogen cycling in the tropical rain forest of French Guiana: comparison of two sites with contrasting soil types using delta N-15. *J Trop Ecol* 15:1–22.
- Roggy JC, Prevost MF, Gourbiere F, Casabianca H, Garbaye J, Domenach AM. 1999. Leaf natural N-15 abundance and total N concentration as potential indicators of plant N nutrition in legumes and pioneer species in a rain forest of French Guiana. *Oecologia* 120(2):171–82.
- Salati E, Vose PB. 1984. Amazon Basin: a system in equilibrium. *Science* 225:127–38.

- Sanchez PA, Bandy DE, Villachica JH, Nicholaides JJ. 1982. Amazon Basin soils: management for continuous crop production. *Science* 216:821–7.
- Schuur EAG, Matson PA. 2001. Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest. *Oecologia* 128(3):431–42.
- Silver WL, Neff J, McGroddy M, Veldkamp E, Keller M, Cosme R. 2000. Effects of soil texture on belowground carbon and nutrient storage in a lowland Amazonian forest ecosystem. *Ecosystems* 3(2):193–209.
- Silver WL, Thompson AW, McGroddy ME, Varner RK, Dias JD, Silva H, Crill PM, Keller M. 2005. Fine root dynamics and trace gas fluxes in two lowland tropical forest soils. *Glob Chang Biol* 11:290–306.
- Sombroek WG. 2000. Amazon land forms and soils in relation to biological diversity. *Acta Amazonica* 30:81–100.
- Sombroek WG. 2001. Spatial and temporal patterns of Amazon rainfall—consequences for the planning of agricultural occupation and the protection of primary forests. *Ambio* 30(7):388–96.
- StatSoft, Inc. 2004. STATISTICA data analysis software system, version 6. URL: <http://www.statsoft.com>.
- Steege HT, Pitman NCA, Phillips OL, Chave J, Sabatier D, Duque A, Molino JF, Prevoist MF, Spichiger R, Castellanos H, von Hildebrand P, Vasquez R. 2006. Continental-scale patterns of canopy tree composition and function across Amazonia. *Nature* 443:444–7.
- Telles EDC, de Camargo PB, Martinelli LA, Trumbore SE, da Costa ES, Santos J, Higuchi N, Oliveira RC. 2003. Influence of soil texture on carbon dynamics and storage potential in tropical forest soils of Amazonia. *Global Biogeochemical Cycles* 17(2): Art. No.1040.
- Vieira S, de Camargo PB, Selhorst D, da Silva R, Hutyrá L, Chambers JQ, Brown IF, Higuchi N, Dos Santos J, Wofsy SC, Trumbore SE, Martinelli LA. 2004. Forest structure and carbon dynamics in Amazonian tropical rain forests. *Oecologia* 140:468–79.
- Vitousek PM. 1984. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology* 65(1):285–98.
- Vitousek PM, Sanford RL. 1986. Nutrient cycling in moist tropical forest. *Annu Rev Ecol Syst* 17:137–67.
- Vitousek PM, Matson PA. 1988. Nitrogen transformations in a range of tropical forest soils. *Soil Biol Biochem* 20(3):361–7.
- Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli LA, Rastetter EB, Sprent JI. 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* 57(1):1–45.
- Vitousek PM. 2004. Nutrient cycling and limitation—Hawai'i as a model system. New Jersey: Princeton University Press, p 223.
- Whitmore TC. 1984. Tropical rain forests of the Far East. Oxford: Clarendon Press, p 352.