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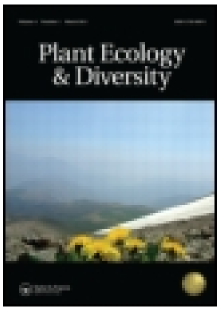
Article:

Nardoto, GB, Quesada, CA, Patiño, S et al. (14 more authors) (2014) Basin-wide variations in Amazon forest nitrogen-cycling characteristics as inferred from plant and soil ^{15}N:^{14}N measurements. Plant Ecology and Diversity, 7 (1-2). pp. 173-187. ISSN 1755-0874

https://doi.org/10.1080/17550874.2013.807524



Plant Ecology & Diversity
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tped20

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To cite this article: Gabriela B. Nardoto , Carlos A. Quesada , Sandra Patiño , Gustavo Saiz , Tim R. Baker , Michael Schwarz , Franziska Schrodt , Ted R. Feldpausch , Tomas F. Domingues , Beatriz S. Marimon , Ben-Hur Marimon Junior , Ima C. G. Vieira , Marcos Silveira , Michael I. Bird , Oliver L. Phillips , Jon Lloyd & Luiz A. Martinelli (2013): Basin-wide variations in Amazon forest nitrogen-cycling characteristics as inferred from plant and soil $^{15}N/^{14}N$ measurements, Plant Ecology & Diversity, DOI:10.1080/17550874.2013.807524

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Basin-wide variations in Amazon forest nitrogen-cycling characteristics as inferred from plant and soil $^{15}N/^{14}N$ measurements

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Abstract

***Background*:** Patterns in tropical forest nitrogen cycling are poorly understood. In particular, the extent to which leguminous trees in these forests fix nitrogen is unclear.

Aims: Determine factors that explain variation in foliar $\delta^{15}N$ ($\delta^{15}N_F$) for Amazon forest trees. Evaluate extent to which putatively N_2-fixing Fabaceae acquire nitrogen from the atmosphere.

Methods: Upper-canopy $\delta^{15}N_F$ were determined for 1255 trees sampled across 65 Amazon forest plots. Along with plot inventory data, differences in $\delta^{15}N_F$ between nodule-forming Fabaceae and other trees were used to estimate the extent of N_2-fixation.

Results: $\delta^{15}N_F$ ranged from –12.1‰ to +9.3‰. Most of this variation was attributable to site-specific conditions with extractable soil phosphorus and dry season precipitation having strong influences, suggesting a restricted availability of nitrogen on both young and old soils and/or at low precipitation. Fabaceae constituted fewer than 10% of the sampled trees, and only 36% were expressed fixers. We estimated an average Amazon forest symbiotic fixation rate of 3 kg N ha^{-1}year^{-1}.

Conclusion: Plant $\delta^{15}N$ indicate that low levels of nitrogen availability are only likely to influence Amazon forest function on immature or old weathered soils and/or where dry season precipitation is low. Most Fabaceae species that are capable of nodulating do not fix nitrogen in Amazonia.

Key words: ^{15}N natural abundance; Amazon, Fabaceae, nitrogen; nitrogen fixation, phosphorus; tropical forest

Introduction

Tropical forests play a key role in the global nitrogen cycle with their vegetation typically rapidly cycling nitrogen through growth and litterfall processes (Nye 1960; Vitousek 1984; Vitousek and Sanford 1986; Lloyd et al. 2009). Tropical forest soils are also characterised by relatively high rates of mineralisation and nitrification as compared to their temperate counterparts (Silver et al. 2000; 2005; Pardo et al. 2006). They can also sustain relatively high rates of loss of N_2O to the atmosphere (Davidson et al. 2004; Keller et al. 2005; Houlton et al. 2006) as well as release substantial amounts of nitrogen through riverine export (Lewis et al. 1999; Hedin et al. 2003; 2009).

Tropical forests are often characterised by a high abundance of members of the family Fabaceae (Gentry 1988), principally among large to medium-sized trees and lianas. Some species of this family live in symbiosis with rhizobia that are able to fix nitrogen from the air and provide it to the host plant in exchange for carbohydrates (Sylvester-Bradley et al. 1980; Salati et al. 1982; De Faria et al. 1989; Moreira et al. 1992; McKey 1994; Sprent 1994, 1995, 1996; Vitousek et al. 2002; Pons et al. 2007; Hedin et al. 2009; Barron et al. 2011). Several authors have hypothesised that an apparent nitrogen-richness of tropical forests can be attributed to these N_2-fixing trees (Jenny et al. 1948, 1949; Roggy, Prevost, Garbaye, et al. 1999; Pons et al. 2007). Nevertheless, some studies have shown Fabaceae in undisturbed mature tropical forests to not fix nitrogen, even though the genera involved clearly had the ability to do so. It has thus been argued that N_2-fixation may only occur in cases of transient nitrogen limitation (Gehring et al. 2005; Ometto et al. 2006; Barron et al. 2011). Hedin et al. (2009) have termed the apparent contradiction of a high abundance of potentially N_2-fixing Fabaceae in tropical forests despite the apparent over-abundance of nitrogen in most tropical forest stands as the “nitrogen paradox” of tropical forests. Despite the potential importance of N_2-fixing Fabaceae in influencing the nitrogen cycle of tropical forests there have been surprisingly few estimates of symbiotic N_2-fixation by these forests. In the lowland tropical forests of Guiana estimates ranged from 4 to 7 kg ha^{-1}year^{-1} (Roggy, Prevost, Garbaye, et al. 1999; Pons et al. 2007). Based on a combined data/modelling exercise, Cleveland et al. (2010) found the same range of symbiotic fixation in the south-west Brazilian Amazon region. These estimates are much lower than a global average estimate of symbiotic fixation for tropical forests of 16 kg ha^{-1}year^{-1}, and ca. 20 to 25 kg ha^{-1}year^{-1} estimated by Cleveland et al. (1999) and Houlton et al. (2008), respectively. Due to this large range of estimates and their uncertainties, there is a clear need for further investigations on this topic.

From both an observational and theoretical viewpoint, it is expected that relatively high losses of nitrogen (a ‘leaky system’) should leave soils and vegetation enriched in ^{15}N in relation to ^{14}N (Högberg 1997). With δ^{15}N inputs via nitrogen biological fixation or through wet and/or dry deposition having a δ^{15}N of ~0‰ (Hoering and Ford 1960; Amarger et al. 1977; Delwiche et al. 1979; Högberg 1986; Shearer and Kohl 1986; Peoples and Craswell 1992; Sprent et al. 1996; Roggy, Prevost, Garbaye, et al. 1999; Gehring et al. 2005; Pons et al. 2007), soil and vegetation systems with relatively high nitrogen losses thus tend to have higher δ^{15}N values than those where the nitrogen cycle is more closed (i.e., with relatively low nitrogen loss). As a result of this, plant and soil δ^{15}N in nitrogen-rich tropical forests are higher and predicted to be less variable than nitrogen-poor temperate forests (Nadelhoffer et al. 1996; Martinelli et al. 1999). Measurements of the ^{15}N:^{14}N ratio of soils and vegetation can thus provide a first approximation of relative nitrogen abundance within an ecosystem and the associated relative openness of the nitrogen cycle (Vitousek et al. 1989; Martinelli et al. 1999; Houlton et al. 2006; Pardo et al. 2006; Craine et al. 2009; Posada and Schuur 2011).

Any symbiotic nitrogen fixation by Fabaceae–rhizobium associations also has the capability to influence the δ^{15}N of plant material. This is because, with a δ^{15}N of ca. 0‰ the ^{15}N:^{14}N ratio of atmospheric nitrogen is generally lower than that of the soil nitrogen pool, the latter usually being considered the most important source of nitrogen to plants (Hoering and Ford 1960; Delwiche et al. 1979). This difference in ^{15}N:^{14}N ratios between the two nitrogen sources also allows the relative reliance of the Fabaceae on N_2 fixation to be estimated by comparing the foliar stable nitrogen isotopic composition of the foliage ($\delta^{15}N_F$) of the N_2-fixing legume to a non-N_2-fixing reference plant (Shearer and Kohl 1986; Unkovich et al. 2008).

Among tropical forests, the Amazon is the largest continuous tropical biome with ca. 6 million km^2. It is of pivotal importance globally due to its high biodiversity, soil and biomass stocks of carbon and nitrogen, and capability to influence the global climate due to the huge amount of water and energy that is recycled through the vegetation of this region. Although sometimes viewed as a single tract of tropical forest, this region is encompasses distinct climates, parent material, soils and vegetation. For example, Fyllas et al. (2009) recently showed a large variability in leaf chemical composition, leaf mass:area ratios and δ^{13}C composition for trees sampled in about 60 plots across the Amazon region. They further found that some leaf nutrients were mostly linked to the taxonomic affiliation, while others were more closely associated with soil chemical composition and/or rainfall regime. Similar results were found by Patiño et al. (2009) examining Basin-wide variations in branch xylem density, as well as variations in a range of tree physical traits, such as leaf size, leaf area:sapwood area ratio and species maximum height (Patiño, Lloyd, Paiva, et al. 2012).

Investigating the physical and chemical characteristics of soils sampled across the same plots as Fyllas et al. (2009) and Patiño et al. (2009), Quesada et al. (2010) found an interesting relationship between the soil stable nitrogen isotopic composition ($\delta^{15}N_S$) and soil total extractable phosphorus concentration, both integrated from the soil surface to 0.3 m depth, the latter being denoted here as $[P]_{ex}$. They found a positive correlation of $[P]_{ex}$ with $\delta^{15}N_S$ up to about $[P]_{ex} = 30$ mg kg^{-1}. However, beyond this point $\delta^{15}N_S$ started to slowly decline with increasing $[P]_{ex}$. The lowest $\delta^{15}N_S$ values were thus found in sandy soils with low fertility and low $[P]_{ex}$ with the highest $\delta^{15}N_S$ on older soils, e.g., Ferralsols and Acrisols. Because of the decline at high $[P]_{ex}$, the youngest soils, e.g., Cambisols and Alisols, had systematically lower $\delta^{15}N_S$ than the more mature soils (see Figure S1). Thus the relationship between $[P]_{ex}$ and $\delta^{15}N_S$ was found to be distinctly non-linear.

It has also been reported that the $\delta^{15}N_S$ and $\delta^{15}N_F$ of Amazon forest may be influenced by soil texture with $\delta^{15}N$ of both leaf and soil higher in clay than in sandy soils (Silver et al. 2000; Nardoto et al. 2008; Sotta et al. 2008; Mardegan et al. 2009). Authors working in other regions have observed $\delta^{15}N_F$ to decrease with increased precipitation (Heaton 1987; Schulze et al. 1998; Austin and Vitousek 1998; Handley et al. 1999; Amundson et al. 2003; Craine et al. 2009; Posada and Schuur 2011) and it has similarly been suggested that precipitation influences $\delta^{15}N_F$ for the forests of the Brazilian Amazon region as trees growing on wetter sites tend to have lower $\delta^{15}N_F$ than those at relatively drier sites (Nardoto et al. 2008). This influence of soil texture and precipitation on the $\delta^{15}N_F$ for Amazon forest trees has, however, only been demonstrated for a few forests, mostly growing on old mature soils (Silver et al. 2000; Nardoto et al. 2008; Sotta et al. 2008). By contrast, the relationship between soil $[P]_{ex}$ and $\delta^{15}N_S$ demonstrated by Quesada et al. (2010) encompassed more than 60 sample sites across the Amazon Basin with different parent materials, soils, and precipitation regimes.

In this study we analysed $\delta^{15}N_F$ for 1255 trees across 65 Amazon forest plots, from 62 botanical families, 241 genera, and more than 400 species. Soil types of the study plots were diverse, including white sands (Arenosols or Podzols), older tropical soils, such as Ferralsols and Acrisols - often considered more typical of tropical forests soils - to younger less weathered soils such as Cambisols (generally close to the Andes), with $[P]_{ex}$ varying from a minimum of ca. 20 mg kg^{-1} in old sandy soils to ca. 400 mg kg^{-1} in some younger soils.

We were specifically interested to ascertain what factors would explain the variability in $\delta^{15}N_S$ observed. Specifically:

a) Is the relationship between $\delta^{15}N_S$ and $[P]_{ex}$ reflected in large scale $\delta^{15}N_F$ patterns and to what extent do the potentially N_2-fixing Fabaceae of the lowland tropical forests of the Amazon Basin fix atmospheric nitrogen?

b) Is there an effect of precipitation gradients as well as soil age gradient across the Amazon Basin on $\delta^{15}N_F$ as observed for other ecosystems?

Materials and methods

Study area

A total of 65 primary forest plots across the Amazon Basin (see Fyllas et al. 2009 and Quesada et al. 2010 for more details) were selected from the RAINFOR (http://www.rainfor.org) and LBA projects (http://www.lbaeco.org/). We also included seven extra plots towards the southern forest margin added from the 'Tropical Biomes in Transition' (TROBIT) project (Torello-Raventos et al. 2013) for which both soil and foliar nutrient and isotope data were introduced here for the first time (Table S1). Taken together, the plots sampled encompassed the forest vegetation of six different countries (Bolivia, Brazil, Colombia, Ecuador, Peru and Venezuela) with a wide variety of soil type and climatic conditions (see Table S2; Fyllas et al. 2009; Quesada et al. 2010; Torello-Raventos et al. 2013 for more details).

Our survey included significant areas outside the Amazon watershed, in particular, areas of the Orinoco Basin, the Guyana lowlands in Venezuela in the north and the Bolivian and Brazilian peripheral areas at the southern edge of the forest margin. These areas do, however, form a phytogeographic continuum with Amazon lowland rainforest. Hence it is reasonable to adopt the shorthand 'Amazonia' to describe this entire lowland tropical forest region. For the dataset described in Torello-Raventos et al. (2013) which encompasses the forest-savanna transition zone of central-southern Brazil and north-eastern Bolivia, only stands identified as consisting predominantly of species usually found in forest vegetation were included; this excluded stands dominated by species usually associated with savanna, even when growing in close proximity to, and/or of a similar structure to, nearby forest formations. All plots were situated in areas ranging from 20 m to 700 m above sea level, with mean annual temperature varying from approximately 23°C to 27°C, and a mean precipitation ranging from 820 mm to 4110 mm year^{-1}.

Laboratory analyses

Data for nitrogen stable isotope ratios as well as for foliar nitrogen and phosphorus concentrations were obtained at individual plant level. A total of 1255 $\delta^{15}N_F$ values were used in this analysis, representing ca. 20 randomly chosen trees per site. Data for foliar nitrogen and phosphorus concentrations were obtained for upper canopy leaves as described in Lloyd et al. (2010). The $\delta^{15}N_S$ and phosphorus availability in the surface soil (0-30 cm) were determined as described in Quesada et al. (2010). Briefly, total extractable phosphorus, $[P]_{ex}$ was obtained from a modified phosphorus fractionation scheme (Hedley et al. 1982), where different phosphorus pools are extracted sequentially with $[P]_{ex}$ considered to represent an amalgamation of the different forms of soil phosphorus potentially available to plants. An index of soil physical properties (Π_1) proposed by Quesada et al. (2010) was also utilised here (Figure S2). This represents a measure of soil physical properties that can potentially limit plant growth and establishment, such as effective soil depth, soil structure, topography and extent of anoxic conditions.

For the Brazilian, Colombian and Venezuelan samples $\delta^{15}N_S$ and $\delta^{15}N_F$ were analysed by using an isotope ratio mass spectrometer at Centro de Energia Nuclear na Agricultura (CENA) in Piracicaba, Brazil, while the Max-Planck Institute für Biogeochemie (MPI) in Jena, Germany analysed samples from Ecuador and Peru and most sites in Bolivia. Soil material from some Bolivian sites sampled as part of the TROBIT project (plots ACU-01, TUC-01, OTT-01, LFB-02) were analysed at the University of St. Andrews, Scotland, with leaf material analysed for $\delta^{15}N$ at James Cook University, Australia. Laboratories were under inter-calibration exercises and used same methodologies and sample standards and we estimate an overall precision of ±0.2‰.

Climatological data

Mean annual temperature (T_A), mean annual precipitation (P_A) and the mean monthly precipitation for the driest quarter (P_D) were derived from interpolated grid cells from the WorldClim database (http://www.worldclim.org/: see Fyllas et al. 2009 for more details).

Floristic analyses

The proportional abundance of potentially N_2-fixing Fabaceae and those considered incapable of nodulation (φ_F and φ_{NF} respectively) was evaluated for each study plot (usually 1 ha) using tree-by-tree data from the RAINFOR/ForestPlots.net database (Lopez-Gonzalez et al. 2011; Patiño, Lloyd, Loyd, et al. (2012). These calculations were made on a stem number per species basis and therefore ignore any systematic differences in tree size between the two different nodulating classes of the Fabaceae as well as any systematic differences with trees from other families in the same stand.

N_2-fixation status during survey

Recent N_2-fixation by potentially nodulating Fabaceae species was assumed to have had occurred when the $\delta^{15}N$ difference between the individual leaf $\delta^{15}N_F$ and the average $\delta^{15}N_F$ in the same plot ($\delta^{15}N_P$) was >1‰. This is clearly an arbitrary criterion that is if anything too liberal: requiring that only ca. 25% of the nitrogen in a leaf to have come from atmospheric N_2 fixation in order for the tree involved to be declared 'fixing' at the typical stand-level $\delta^{15}N_F$ of 4‰. We also checked for 'leakage' in this criterion. We therefore considered separately the percentage of the Fabaceae species in our survey known not to be capable to nodulate and would be incorrectly inferred to have recently been 'N-fixing' when subject to the same >1‰ criterion.

Statistical analysis

We first tested for normality and homogeneity of variance for $\delta^{15}N_F$ and correlated foliar nutrients (nitrogen and phosphorus on dry weight basis). As these properties did not follow a normal distribution, they were transformed using Box-Cox methods. Negative $\delta^{15}N_F$ values were transformed into positive values by incorporating into every

$\delta^{15}N_F$ value the most negative $\delta^{15}N_F$ value reported (–12.1‰). In order to partition the variance in $\delta^{15}N_S$ we used the model as proposed by Fyllas et al. (2009):

$$\delta^{15}N_F = \mu + p + f/g/s + \varepsilon, \qquad \text{(Equation 1)}$$

where μ is the overall mean $\delta^{15}N_F$ of the dataset, *p* is the plot characteristic (soil and climate*), f/g/s* represents the genetic structure of the data, i.e. that each individual belongs to a species (s), nested in a genus (g), nested in a family (f), and ε is the residual. The variance of the model was tested by Mixed ANOVA analysis assuming *p* and *f/g/s* as random variables in a hierarchically nested design. Residual Maximum Likelihood (REML) was used to estimate model parameters using the STATISTICA software package (Statsoft Inc. 2007). Differences between different Fabaceae groups (non-fixers; not N-fixing and N-fixing during survey) were tested by one-way ANOVA. The non-parametric Spearman correlation coefficient (*ρ*) was used to test correlations between leaf properties and soil and environmental variables. An adjustment of the relevant degrees of freedom and associated *P* values was carried out to account for spatial autocorrelation (Dutilleul 1993). We used the software 'Spatial Analysis in Macroecology – SAM' (Rangel et al. 2006), a non-parametric approach that allowed an evaluation of the effects of climate and soils on $\delta^{15}N_F$ without resort to predefined assumptions as to the exact nature of any simple correlative relationships occurring.

Results

From tree-by-tree census data for all 65 plots examined, we found a mean stem fraction of N_2-fixing (nodulating) Fabaceae, $\varphi_F = 0.08$ and non-nodulating Fabaceae, $\varphi_{NF} = 0.05$. Thus, according to our dataset, potential N_2-fixing Fabaceae constitute fewer than 10% of the trees of the Amazon forest; and, those members of the Fabaceae considered incapable of nodulation and hence unable to fix N_2 just slightly less abundant as putative N_2 fixers (Table S1). In several cases, such as the Podzol SCR-04 (Venezuela) or the Ferralsol VCR-01 (southern forest-savanna transition zone, Brazil) φ_F was <0.01. The highest φ_F recorded was 0.26 from a Plinthosol (DOI-02, south-east Amazon, Acre, Brazil). Only two other sites had $\varphi_F > 0.2$: a semi-deciduous forest on an Acrisol in the southern forest-savanna transition zone (OTT-01, Bolivia) and a high rainfall forest located on a poorly drained Gleysol (TIP-03, Ecuador). The proportion of non-N_2 fixing Fabaceae also varied widely, being < 0.01 on many sites, but > 0.50 at OTT-01 and for one plot in eastern Venezuela (ELD-12).

Variability in foliar $\delta^{15}N$ ($\delta^{15}N_F$)

Foliar stable nitrogen isotopic composition varied from –12.1‰ for a *Micrandra sprucei* tree growing on a Podzol at San Carlos do Rio Negro (SCR-04, Venezuela) to +9.3‰ for an unidentified *Inga* species on a Ferralsol in eastern Amazonia (TAP-123); this giving an overall range of ca. 21‰ (Figure 1). The mean $\delta^{15}N_F$ (± standard deviation) was equal to +3.1±2.3‰ (n=1255), which was similar to the median value of +3.2‰ (lower quartile = +1.9‰, upper quartile = +4.6‰) (Figure 1). The lowest plot level mean value of $\delta^{15}N_F$ was observed for SCR-04 ($\delta^{15}N_P$ = –4.3‰) with the nearby SCR-05 (–0.8‰), and another pair of Podzol plots near Manaus in the central Amazon (MAN-3 and MAN-4) located in 'baixios', areas frequently exposed to flooding also having relatively low values for $\delta^{15}N_P$ of +0.2‰ and –1.4‰ respectively (Table S1). All these soils have very high sand content (Table S1). Highest $\delta^{15}N_P$ were observed for stands growing on Ferralsols of the eastern Amazon region, in the National Forest of the Tapajós (TAP-123 and TAP-04; $\delta^{15}N_P$ = +6.5‰ and +6.2‰ respectively), and in the National Forest of Caxiuanã to the east (CAX-06; $\langle \delta^{15}N \rangle$ = +6.2‰). Ferralsols in these areas are characterised by high clay content (Table S1). A high $\delta^{15}N_P$ was also observed for a deciduous forest on a Cambisol in Bolivia, the lowest precipitation site in the dataset (TUC-01; $\delta^{15}N_P = +6.8‰$).

From the analysis of Equation 1, differences in $\delta^{15}N_F$ were mainly attributable to growing location with 0.71 of the variation attributable to plot followed by the residual term (0.22) which represents within-species variation and sampling/measurement error. Genetic characteristics were responsible for only 0.07 of the variation observed with the following distribution: family (0.03), genus (0.02), and species (0.02).

Plot climate and soil characteristics and $\delta^{15}N_P$

As bulk $\delta^{15}N_S$ does not necessarily reflect the isotopic composition of inorganic forms of nitrogen taken up by plants and with any association of plants with ectomycorrhiza fungi providing plants with nitrogen depleted in ^{15}N (Hobbie et al. 2005; Craine et al. 2009), we first tested if the stand level $\delta^{15}N_P$ reflected that of the surface soil (0.0 - 0.3 m). This showed a significant correlation between $\delta^{15}N_S$ and $\delta^{15}N_P$ with 70% of the variation in $\delta^{15}N_P$ accounted for by the soil ^{15}N:^{14}N ratio (Figure 2a). We also found an inverse relation between $\delta^{15}N_P$ and P_A, however, only 10% of the variance in $\delta^{15}N_P$ could be explained by P_A alone (Figure 2b).

Soils with high sand content (Φ_S) usually have lower mineralisation and nitrification rates that in turn lead to a lower nitrogen availability, decreasing the $\delta^{15}N_F$ at such sites. Based on the trends observed from previous studies in the Amazon region (Silver et al. 2000; Nardoto et al. 2008; Mardegan et al. 2009) we thus tested if $\delta^{15}N_F$ decreased with increasing Φ_S across our dataset. Although a significant negative correlation between $\delta^{15}N_P$ and Φ_S was found, only 9% of the variance could be explained by Φ_S alone (Figure 2c).

Using dry-season precipitation regime to broadly segregate plots into those that usually experience some sort of soil water deficit during the dry season and those which do not - $P_D < 100$ mm month^{-1} and $P_D > 100$ mm month^{-1}. This mean monthly precipitation roughly reflects the mean monthly evaporative demand of Amazon forest (Malhi and Wright 2004), we found that for plots with $P_D < 100$ mm the relationship between $[P]_{ex}$ and $\delta^{15}N_P$ was similar to that observed for $[P]_{ex}$ and $\delta^{15}N_S$ by Quesada et al. (2010; see Figure S1), with lower values at both low and high $[P]_{ex}$ than at intermediate $[P]_{ex}$. The Spearman's measure of association (which assumes a monotonic relationship) was not significant ($\rho = 0.36$, $P = 0.25$: Figure 3a). By contrast, there was a significant direct association between $\delta^{15}N_P$ and $[P]_{ex}$ for plots with $P_D > 100$ mm ($\rho = 0.45$, $P = 0.03$: Figure 3b). It was also observed that $\delta^{15}N_P$ for plots with $P_D < 100$ mm showed a significantly higher, association with Φ_S than for plots with $P_D > 100$ mm ($\rho = -0.55$ and -0.04 respectively; data not shown).

In order to further probe contrasting patterns in the relationships between $\delta^{15}N_P$ and $[P]_{ex}$ as dependent on dry-season precipitation regime, we investigated variations in stand-level average foliar dry-weight based nitrogen and phosphorus concentrations (denoted as $[N]_{DW}$ and $[P]_{DW}$ respectively). There was no difference in $[N]_{DW}$ ($F_{(1,57)} = 1.03$, $P = 0.31$), but in the plots with $P_D < 100$ mm $[P]_{DW}$ was significantly lower than in the plots with $P_D > 100$ mm ($F_{(1,57)} = 9.2$, $P < 0.004$). Although there was no significant association between $[N]_{DW}$ and $[P]_{ex}$ for plots with $P_D < 100$ mm ($\rho = 0.34$, $P = 0.17$) a strong $[N]_{DW}$ and $[P]_{ex}$ relationship was evident for plots with $P_D > 100$ mm ($\rho = 0.66$, $P = 0.002$: Figure 3d).

Irrespective of dry season precipitation amount, there was a strong relationship between $[P]_{DW}$ and $[P]_{ex}$, but with the relationship tending to saturate at high $[P]_{ex}$ for $P_D < 100$ mm. In terms of foliar nitrogen:phosphorus ratios, for plots with $P_D < 100$ mm values were, on average, significantly higher than in $P_D > 100$ mm plots ($F_{(1,71)} = 17.41$, $P < 0.0001$). There was also an inverse relationship between foliar nitrogen:phosphorus ratio and $[P]_{ex}$ evident irrespective of dry season precipitation regime (Figures 3g, h).

$\delta^{15}N_F$ values of trees of the Fabaceae family

Of a total of 118 potentially N_2-fixing Fabaceae examined, only 43 (or 36%) could be inferred as actively fixing atmospheric nitrogen according to our criterion of $\delta^{15}N_P - \delta^{15}N_F$ being 1‰ or greater (Table S3). Checking this against the percentage of non-fixing Fabaceae that would incorrectly score as actively fixing according to this '1 per mil criterion' we found a false strike rate of about 10%, this suggesting that, if anything, the real value was probably somewhat less than 36%.

Of the 48 plots with at least one putatively (nodulating) Fabaceae species having been sampled, only 29 had at least one tree inferred to be actively fixing nitrogen (Table S3). Most of these were characterised by young to intermediate age soils (21 out of 29 plots, Table S3), these often being Alisols with a low degree of weathering in comparison to more mature soils, such as Ferralsols and Acrisols (Quesada et al. 2009; 2010). However, it is important to note that in five Ferralsol plots, evidence of some N_2 fixation was indicated (Table S3). Two of these plots (SIN-01, VCR-02) were located in the State of Mato Grosso, two (TAP-123, TAP-04) in the State of Pará, and one (MAN-12) in the Amazonas State, all inside Brazil (Table S1).

Applying Equation 1 to Fabaceae trees only, about half of the variance in the observed $\delta^{15}N_F$ emerged was attributable to the plot in which the tree was growing, ca. 15% was due to genus and the remainder (35%) to the residual. With this restricted dataset, variation at the species level could not be reasonably be included for analysis at the lowest level of variation due to the generally low number of species sampled per genus and/or trees sampled per species.

Along with the $\delta^{15}N_F$ values of the putatively (nodulating) Fabaceae species ($\delta^{15}N_{Fix}$) and average $\delta^{15}N$ values of non-fixing species ($\delta^{15}N_{nF}$), we indicated the recent N_2-fixation status for the Fabaceae species using the >1‰ criterion as follow: F+, legumes which were assumed to fix nitrogen during the survey; and F-, legumes whose leaf $\delta^{15}N$ values indicated no nitrogen fixing during the survey (Table 1, Table S3). Comparing foliar characteristics of the Fabaceae (nitrogen, phosphorus and their $\delta^{15}N_F$), the only difference observed among F+, F- and $\delta^{15}N_{nF}$ was a significantly lower $\delta^{15}N_F$ ($F_{(2,71)} = 1.72$, $P = 0.19$) in F+ compared with both F- and $\delta^{15}N_{nF;}$ the latter two not differing from each other (Table 1). We also did not find any statistically significant differences in $[N]_{DW}$ and $[P]_{DW}$ as well as in nitrogen:phosphorus ratios among them (Table 1).

Discussion

Overall trends in foliar $\delta^{15}N$

Much of the variation in $\delta^{15}N_F$ (a range of 21‰) could be attributed to local conditions, especially precipitation and soil characteristics, with little variation attributable to a species' taxonomic identity/affiliation. The mean $\delta^{15}N_F$ (± standard deviation) of +3.1±2.3‰ (n = 1255) found in our study for Amazon forest is significantly greater than $\delta^{15}N_F$ typically reported for temperate forests. For example, in a survey involving different temperate tree species of North America and Europe, $\delta^{15}N_F$ varied from –8‰ to +4‰ but usually less than 0‰ (Pardo et al. 2006). The mean difference in $\delta^{15}N_F$ between tropical and temperate forests was anticipated as it has already been shown that N-rich/leaky forests have higher $\delta^{15}N_F$ than N-poor/tightly cycling forests due to losses of ^{14}N in relation to ^{15}N in processes such as denitrification (Martinelli et al. 1999; Houlton et al. 2006).

Is the coupling of phosphorus and nitrogen cycles modulated by precipitation?

Lowland tropical forest productivity is often considered to be more limited by phosphorus than nitrogen (Vitousek 1984; Crews et al. 1995; Chadwick et al. 1999; Hedin et al. 2003; McGroddy et al. 2004; Reich and Oleksyn 2004), especially in the case of old and highly leached soils (Porder et al. 2007; Vitousek et al. 2010; Quesada et al. 2012). Nevertheless, there are indications that productivity can also be limited by nitrogen in some cases, especially in response to transient nitrogen limitation (Davidson et al. 2007) and/or on younger soils where the phosphorus availability is relatively high (Quesada et al. 2010; Mercado et al. 2011).

As pointed out earlier, Quesada et al. (2010) found that to ca. 30 mg kg^{-1} of $[\mathrm{P}]_{\mathrm{ex}}$ there was a direct relation between this soil property and $\delta^{15}\mathrm{N}_{\mathrm{S}}$ (see Figure S1) suggesting that at the lowest $[\mathrm{P}]_{\mathrm{ex}}$ there were potentially limitations on plant productivity due to low levels of nitrogen availability which may have even exceeded those expected as a consequence of low plant available phosphorus concentrations in the soil. For these reasons, and considering that ca. 70% of $\delta^{15}\mathrm{N}_{\mathrm{F}}$ variance was attributed to local conditions (soil and climate), and more than 60% of the foliar $\delta^{15}\mathrm{N}_{\mathrm{F}}$ variance was explained by $\delta^{15}\mathrm{N}_{\mathrm{S}}$, and with a clear pattern between $[\mathrm{P}]_{\mathrm{ex}}$ and $\delta^{15}\mathrm{N}_{\mathrm{S}}$ previously found by Quesada et al. (2010), it seemed useful to investigate the role of $[\mathrm{P}]_{\mathrm{ex}}$ in modulating $\delta^{15}\mathrm{N}_{\mathrm{F}}$. For plots with $P_{\mathrm{D}} < 100$ mm a similar trend exists between $\delta^{15}\mathrm{N}_{\mathrm{P}}$ and $[\mathrm{P}]_{\mathrm{ex}}$ as is the case for $\delta^{15}\mathrm{N}_{\mathrm{s}}$ and $[\mathrm{P}]_{\mathrm{ex}}$. More specifically, in the interval from very low concentrations up to 30-50 mg kg^{-1} of $[\mathrm{P}]_{\mathrm{ex}}$, an increase in the $\delta^{15}\mathrm{N}_{\mathrm{P}}$ value was observed, with the highest $\delta^{15}\mathrm{N}_{\mathrm{P}}$ values observed mainly in Ferralsols and Acrisols of eastern Amazonia. Beyond this $[P]_{ex}$ threshold, similar to the $\delta^{15}N_S$, $\delta^{15}N_P$ then slightly decreases, with this associated with progressively younger soils.

Plants on lowest $[P]_{ex}$ white-sandy soils in the Amazon region (sites with relatively large annual precipitation) have already been noted to have low $\delta^{15}N_F$ (Pons et al. 2007; Nardoto et al. 2008; Mardegan et al. 2009), perhaps due to a low availability of nitrogen in these soils, as is also the case for other forests on Podzols, such as our SCR-04 plot in Venezuela. Nitrogen limitations in such soils appears to be caused by low rates of mineralisation and nitrification (Vitousek and Matson 1988; Luizão et al. 2004; Nardoto et al. 2008; Mardegan et al. 2009), in the presence of relatively high levels of phosphorus availability in litter mats and humic horizons despite the low overall $[P]_{ex}$ (Tiessen et al. 1994). In response to such conditions, both $[N]_{DW}$ and $[P]_{DW}$ were low (Figure 3c,d,e,f). Consistent with this are the results from a recent modelling analysis using the RAINFOR data (Mercado et al. 2011). They found the only Podzol site in their dataset (SCR-04) to be one of the few plots across the Amazon for which photosynthetic productivity was limited by low foliar nitrogen rather than by phosphorus, despite the vegetation at this site having low foliar phosphorus concentrations (Table S1). This result was consistent with the suggestion of Quesada et al. (2010) that, due to feedback effects of litter quality on nitrogen mineralisation rates, the oldest and/or most chemically weathered forest soils of the Amazon may end up supporting ecosystems with self-imposed limitations to productivity through low levels of nitrogen availability.

As the $[P]_{ex}$ increased beyond its lowest observed values, $\delta^{15}N_P$ also increased, reaching a maximum in the Ferralsols of Tapajós National Forest (TNF) and in Caixuanã (TAP-123, TAP-04, CAX-06, Table S1). These three sites have an intermediate $[N]_{DW}$ content, and a rather low $[P]_{DW}$ resulting in high foliar N:P ratios

(Table S1). This suggests that these forests are more limited by phosphorus than by nitrogen (Silver et al. 2000), a result also consistent with the analysis of the dual nitrogen-phosphorus photosynthesis limitation model of Mercado et al. (2011). Clay soils in the TNF, such as Ferralsols, showed higher mineralisation, nitrification, and denitrification than sandier soils of the same region (Silver et al. 2000) and Keller et al. (2005) found the highest N_2O soil flux among tropical forests on these soils. Soil N_2O fluxes have recently been linked to forest productivity and nitrogen availability (Wolf et al. 2011). Taken together, this information suggests that nitrogen availability is relatively high in these forests (Davidson et al. 2007). Given that high nitrogen availability (Vitousek et al. 1989; Martinelli et al. 1999; Houlton et al. 2006; Pardo et al. 2006; Craine et al. 2009; Posada and Schuur 2011), and moderate soil water availability constraints (Heaton 1987; Austin and Vitousek 1998; Handley et al. 1999; Amundson et al. 2003; Nardoto et al. 2008; Craine et al. 2009; Posada and Schuur, 2011) are both associated with higher $\delta^{15}N_F$, it therefore seems reasonable to suggest that the high $\delta^{15}N_F$ and $\delta^{15}N_S$ values found on Ferralsols of the TNF are a consequence of relatively clay-rich old soils combined with moderate soil water deficits and with phosphorus limitation on plant productivity (Ometto et al. 2006; Nardoto et al. 2008; Quesada et al. 2012) leading to relatively high levels of both nitrogen availability and loss (Ometto et al. 2006; Nardoto et al. 2008).

It is interesting to note that, as was observed for soils in Quesada et al. (2010), beyond approximately 50 mg kg^{-1} of $[P]_{ex}$ there was a decline in the $\delta^{15}N_P$ in plots with $P_D > 100$ mm. In this end part of the curve, trees were growing in plots where less strongly weathered soils dominated (Umbrisols, Cambisols, Gleysols and Alisols, Figure 3a). This is the same relationship as for nitrogen isotopes in the soil and, evoking the same explanation presented by Quesada et al. (2010): that younger soils have not yet experienced sufficient nitrogen losses from the system (Houlton et al. 2006) to enrich such soils in ^{15}N atoms with a consequent increase in their $\delta^{15}N_F$ and $\delta^{15}N_S$.

Through analysing foliage of trees of the same plot we can, however, add another layer of interpretation to that possible from soil analyses alone. Although $\delta^{15}N_F$ values are clearly influenced by the $\delta^{15}N_S$, additional factors, such as soil nitrogen absorption fractionation, internal plant processes (nitrogen retranslocation), and plant functional differences might also influence patterns of nitrogen cycling (Robinson 2001). For example, even though $[N]_{DW}$ were not greatly different when older soils were compared with younger soils where $P_D < 100$ mm (Figure 3c), there was a general tendency for $[P]_{DW}$ content to not positively correlate with $[P]_{ex}$, with $[P]_{DW}$ actually tending to decrease only beyond a threshold of $[P]_{ex}$ ca. 200 mg kg^{-1}. Soils with highest $[P]_{ex}$ do not necessarily support tropical forests with higher foliar phosphorus concentration than those with lower levels of phosphorus availability - with dry-season precipitation being a likely modulating factor. The very low foliar nitrogen:phosphorus ratios of the vegetation on younger soils (Figure 3g) can, however, be taken as an indication of a vegetation limited by nitrogen (Koerselmann and Meuleman 1996; Gusewell 2004). The stand-level photosynthesis modelling analysis of Mercado et al. (2011) also inferred that some of the younger sites (Figure 3g) tended to be limited by nitrogen rather than phosphorus, even though foliar nitrogen values were relatively high (Figure 3e,f). This is consistent with the Walker and Syers's (1976) hypothesis, suggesting a stronger nitrogen- than phosphorus-limitation in less developed soils, with their relatively low $\delta^{15}N_F$ suggestive of a less leaky nitrogen cycle, but with phosphorus availability also modulated by dry-season precipitation regime.

For plots with $P_D > 100$ mm there was a strong correlation between foliar $\delta^{15}N_P$ and $[P]_{ex}$ with nearly 0.7 of the variance in $\delta^{15}N_P$ explained by $[P]_{ex}$ (Figure 3b). In this case, as was also observed for $P_D < 100$ mm, $\delta^{15}N_P$ were sometimes less than 1‰ at very low $[P]_{ex}$. But contrary to $P_D < 100$ mm, the highest $\delta^{15}N_P$ values were found at the highest $[P]_{ex}$ (Figure 3b) and with the highest $\delta^{15}N_P$ for the higher dry-season precipitation plots ca. 2‰ less than the highest $\delta^{15}N_P$ observed for plots with $P_D < 100$ mm (Figures 3a,b). Thus, it seems that once differences in $[P]_{ex}$ are accounted for that stand-level $\delta^{15}N_F$ tend to be reduced at higher P_D.

There have already been several studies similarly indicating that $\delta^{15}N_F$ values may decrease with precipitation (Heaton 1987; Schulze et al. 1998; Austin and Vitousek 1998; Handley et al. 1999; Amundson et al. 2003; Santiago et al. 2004; Nardoto et al. 2008; Craine et al. 2009; Posada and Schuur 2011). Explanations for this effect are still unclear, with explanations encompassing a range of factors including nutrient availability, and leaf physiological traits (Santiago et al. 2004) and with Austin and Vitousek (1998) hypothesising that in drier areas nitrogen cycling may be more open compared to wetter areas; this leading to a $\delta^{15}N_S$ enrichment. Analysing seven lowland neotropical forests plots along a gradient of P_A from ca. 2700 mm year^{-1} to more than 9500 m year^{-1} Posada and Schuur (2011) suggested that at wetter sites there was limitation by both nitrogen and phosphorus, which in turn caused a slow decomposition rate due to poor litter quality, this then leading to carbon accumulation in the soil (Vieira et al. 2011). As a result, nitrogen availability was argued to be lower at wetter sites, leading to nitrogen-retentive forests, and lower $\delta^{15}N_S$ values. One possibility is that this extra nitrogen is lost through complete denitrification of the nitrate pool (Houlton et al. 2006).

Contrasting these results, Santiago et al. (2005) working along a precipitation gradient from 1800 mm year^{-1} to 3500 mn year^{-1} in Panamá did not find any systematic effects of rainfall on stand-level foliar $[N]_{DW}$, but with lower $\delta^{15}N_F$ occurring at the higher precipitation sites (Santiago et al. 2004, 2005). This contradiction suggests that there is a decoupling of the soil nitrogen cycle and leaf traits, suggesting, in turn, that foliar nitrogen concentration and isotopic composition may depend more on leaf structure and physiology than nitrogen availability (Santiago et al. 2005). Therefore, the findings of Santiago et al. (2004, 2005) point to the fact that our interpretation should be viewed with caution, although several other studies have shown a strong relation between soil nitrogen availability and $\delta^{15}N_F$ (Vitousek et al. 1989; Martinelli et al. 1999; Houlton et al. 2006; Pardo et al. 2006; Craine et al. 2009; Posada and Schuur 2011; Wolf et al. 2011). Cernusak et al. (2009) confirmed by observation that for tropical trees large differences in $\delta^{15}N_F - \delta^{15}N_S$ may occur as a consequence of considerable inter-specific variation in the transpiration efficiency of nitrogen acquisition. This, therefore, might explain, at some extent, the differences of rainfall effects in the relationship between soil nitrogen and $\delta^{15}N_F$ in tropical forests.

The role of Fabaceae in the tropical forests

Because of their abundance, diversity, and N_2-fixing ability the Fabaceae have often been considered to play a prominent role in tropical forests (Vitousek et al. 2002; Hedin et al. 2009; Barron et al. 2011). The monophyletic Fabaceae constitute one of the most important botanical families in the Amazon region (Gentry 1988; ter Steege et al. 2006) and the ability of some legumes to establish a symbiotic relationship with rhizobia make this family even more important because of their ability to fix N_2 from the atmosphere (Hoering and Ford 1960). Whether fixing or not, there are numerous reports of members of this family typically having greater $[N]_{DW}$ than other trees (McKey 1994; Roggy, Prevost, Garbaye, et al. 1999; Vitousek et al. 2002; Ometto et al. 2006; Nardoto et al. 2008; Fyllas et al. 2009) and it is thought that, because decomposition depends on the litter quality (Santiago 2007; Cornwell et al. 2008), legume-rich forests should also produce a nitrogen-rich litter, stimulating decomposition and liberating nitrogen for all species present (Although we do note there may be exceptions to this general pattern; e.g., Palm and Sanchez 1991).

Estimates of N_2-fixation in the Guiana lowland forests ranged from ca. 4 to 7 kg ha^{-1} $year^{-1}$ for some Amazonian Oxisols (Roggy, Prevost, Garbaye, et al. 1999; Pons et al. 2007), which is equivalent to ca. 6% of the total annual nitrogen input to the forest. On the other hand, several other studies have shown that putatively Fabaceae N_2-fixing species often do not fix nitrogen from the atmosphere in mature, undisturbed tropical forests (Sylvester-Bradley et al. 1980; Salati et al. 1982; Shearer and Kohl 1986; Yoneyama et al. 1993; Högberg and Alexander 1995; Vitousek et al. 2002; Gehring et al. 2005; Ometto et al. 2006; Nardoto et al. 2008; Barron et al. 2011). It has thus been argued that it is only advantageous to Fabaceae to fix in these environments where the cost of nitrogen fixation becomes affordable and/or the natural nitrogen cycle has become disturbed (Sylvester-Bradley et al. 1982; Vitousek et al. 2002; Hedin et al. 2008; Barron et al. 2011).

Foliar $\delta^{15}N$ composition has been used for four decades as an indicator of nitrogen fixation since the $\delta^{15}N$ of the air is approximately 0‰ and is generally lower than $\delta^{15}N_S$ (Hoering and Ford 1960; Amarger et al. 1977; Delwiche et al. 1979; Högberg 1986; Shearer and Kohl 1986; Peoples and Craswell 1992; Sprent et al. 1996; Roggy, Prevost, Goubiere, et al. 1999; Gehring et al. 2005; Pons et al. 2007; Unkovich et al. 2008). The $\delta^{15}N_F$ of the fixing species is compared with the $\delta^{15}N_F$ of non-fixing species. If the $\delta^{15}N_F$ of fixing species is significantly closer to 0‰ than the $\delta^{15}N_F$ of non-fixing species this means that the fixing species is receiving some nitrogen from its symbiont (Delwiche et al. 1979). This method is not, however, without its problems. First, it has to be assumed that there is no fractionation from air-bacteria-plant. In most cases this assumption appears to be true, or if not, the fractionation is rather small (Hoering and Ford 1960; Högberg 1997). Second, as pointed out by Shearer and Kohl (1986) it has to be assumed that the non-fixer species are taking up the same nitrogen form as the fixing species (NH_4, NO_3, organic nitrogen forms), and that the $\delta^{15}N$ of the bulk soil is a good indicator of the $\delta^{15}N$ of the inorganic or organic form that the plants are taking up. Both assumptions are difficult to prove unequivocally, and most of the time, are probably, to some degree, violated (Högberg 1997). Therefore estimates presented here are only a rough indication of whether some nitrogen biological fixation occurred or not and need to be interpreted with caution due to these methodological limitations (Table S3).

Nevertheless, our results based on $\delta^{15}N_F$ of potentially fixing Fabaceae and non-fixing species confirm the low extent of N_2-fixation in mature lowland tropical forests across the Amazon Basin (Table S3), with the $\delta^{15}N_F$ analyses suggesting that only a small portion of potentially N_2-fixing Fabaceae fix N_2. Where observed, this N_2-fixation was usually for trees growing on less weathered soils, which are characterised by having a high $[P]_{ex}$, high total base reserves (Σ_{RB}) and often with some type of physical constraint, which is expressed by the high value of the Π_1 index (see Quesada et al. 2010 for details). These constraints include depth, structure, topography and anoxia that are thought to translate into the field as characteristics that prevent plant development such as a shallow soil and rooting depth, steep topography, and structural and drainage deficiencies and it may be relevant that, in addition to there being a tendency for a higher relative frequency of nodulation in the species in tropical secondary forests (Gehring et al. 2005; Davidson et al. 2007; Barron et al. 2011) it also seems that nodulation may be more common for undisturbed stands exposed to regular flooding (Moreira et al. 1992; De Faria et al. 2010). Consistent with this observation, Martinelli et al. (1992) and Krebich et al. (2006) also found N_2-fixation to be important in the Amazon várzea (flooded forests) and Koponen et al. (2003) found fixing Fabaceae in a freshwater swamp forest in French Guiana. Likewise, Sprent (2001) has emphasised the importance of flooding for N_2-fixation in legumes in the Orinoco Basin and with James et al. (2001) describing flooding tolerant legumes from the Brazilian Pantanal wetland, including one species that only nodulates under flooding conditions. Taking a parallel with our younger soils mostly in the plots with $P_D > 100$ mm, we thus suggest that soils with greater physical constraints that are – other characteristics being similar – exposed to more frequent and longer periods of waterlogging associated with anoxia, might explain the more frequent occurrence of actively fixing Fabaceae on such soils. Here we note that pressurised or diffusive gas transport strategies are sometimes capable of maintaining an adequate O_2 supply to the root system under anaerobic conditions, and may also serve to maintain symbiotic N_2 fixation during these times (Martinelli et al. 1992; James et al. 2001; Kreibich et al. 2006). Moreover, high rates of denitrification under anaerobic conditions, occasional spatial N limitation and the significant high seasonal N losses due to the exchange with rapid water flows all support the notion that a seasonally waterlogged environment is one where a capability for symbiotic N_2 fixation might be most beneficial (Roggy, Prevost, Garbaye et al. 1999; Vitousek et al. 2002).

We also speculate that the same may occur in the *baixio* of Manaus, an N-poor system on sandy soils that is frequently inundated (Nardoto et al. 2008), even though the relative abundance of putative N-fixers is not exceptionally high (Table S1). However the negative $\delta^{15}N_F$ values of several plants in this area prevented the use of $\delta^{15}N_F$ values as an indicator of N fixation by putatively N_2-fixing Fabaceae.

As discussed above, tropical forests on mature soils, such as the Ferralsols of Brazil are generally considered phosphorus-poor and with nitrogen being relatively abundant (Martinelli et al. 1999; Ometto et al. 2006; Nardoto et al. 2008; Quesada et al. 2010; Quesada et al. 2012) and it might therefore be expected that N_2-fixation rates would be minimal (Vitousek et al. 2002; Houlton et al. 2008; Hedin et al. 2009; Baron et al. 2011). Our data tend to confirm this view with N_2-fixing Fabaceae being more common on less developed soils, but nevertheless, it is clear that some fixation may occur in old mature tropical soils in some circumstances. The Ferralsols for which we found some indications of N_2-fixation occurring also tended to have slightly higher relative abundances of putative N_2-fixing Fabaceae than the Ferralsols on which we failed to detect any N_2-fixation. One possible explanation for this apparent anomaly is that on the oldest most strongly weathered Ferralsols, a gradual transition from phosphorus to nitrogen limitation has already occurred, this being due to an immobilisation of nitrogen associated with high lignin/tannin contents of decomposing foliage growing on the oldest soils (Quesada et al. 2010).

As we measured the relative abundance of potentially N_2-fixing Fabaceae in most of our plots (Table S1) we can also use our data here to provide a ‘back of the envelope’ estimate of the extent of N_2–fixation by Amazon forest. Noting that, on average, only 8% of the tree population consists of putative N_2- fixers (Table S1) and with only 36% of such trees fixing nitrogen and with the differences in $\delta^{15}N_F$ in Table S3 suggesting that at most those trees fixing nitrogen obtain 30% of their total N from the atmosphere, then the total amount of N_2 fixed should be ca. 0.08 x 0.35 x 0.03, or 1% of the average nitrogen utilised by the Amazon forest each year. From recent work of Aragão et al. (2009) estimated that, although somewhat variable, leaf and fine-root production account for about 70% of total net plant primary production, a reasonable overall value, being about 10 Mg C ha^{-1} $year^{-1}$ which for a plant carbon concentration of 50% with $[N]_{DW}$ = 20 mg g^{-1} (Fyllas et al. 2009) and assuming a similar nitrogen concentration for fine roots as foliage (Cuevas and Medina 1988) yields an estimate for a (leaf +fine root) nitrogen requirement of 0.01 x 2 x 10 x 20 x 0.7, or 2.8 kg N ha^{-1} $year^{-1}$. To it must be added the nitrogen requirements for new wood growth. Measurements of nitrogen of tropical tree stem are very scarce, but taking a value of 1 mg g^{-1} based on what little data are available - mostly from Australian tropical/subtropical forests (Meerts 2002) - then calculating using the nitrogen requirement for new wood production of stems, branches and coarse roots as for leaves and fine roots above, we obtain a value of around 0.1 kg N ha^{-1} $year^{-1}$. Taking every assumption made above to account for the potential input of nitrogen via symbiotic biological fixation, we therefore estimate the potential rate of N_2-fixation by the Fabaceae Amazon trees of being ca. 3 kg N $ha^{-1}year^{-1}$. This is likely an overestimate as retranslocation of nitrogen from senescing tissues has not been considered, but is still less than the 4 to 7 kg N $ha^{-1}year^{-1}$ for lowland tropical forests of Guiana and Rondônia (Roggy, Prevost, Garbaye, et al. 1999; Cleveland et al. 2010), and much less than the estimate of Cleveland et al. (1999) for tropical forest symbiotic nitrogen fixation of 16 kg N $ha^{-1}year^{-1}$. It is, however, important to realise that the global average value for tropical forest symbiotic N_2-fixation presented in Cleveland et al. (1999) was an indirect estimate; having being extrapolated from measurements of free-living N_2-fixation for a solitary Amazon forest site (Jordan et al. 1980).

Our estimate here also differs vastly from those predicted from a self-proclaimed ‘unifying framework’ for N_2 fixation where values for lowland tropical forest of 20 to 25 kg N $ha^{-1}year^{-1}$ are suggested (Houlton et al. 2008). That model is based on the simple assumption that N_2-fixing legumes maintain their advantage over other tropical tree species by virtue of being able to have higher rates of release of phosphatase enzymes enabling access to phosphorus pools that non N_2-fixers do not. There are, however, numerous other means by which plants can improve their access to phosphorus, for example through organic acid exudation and/or mycorrhizal associations (Lloyd et al. 2001; Turner 2008). There may also be other characteristics of members of the Fabaceae contributing to their success in tropical forest systems as is also suggested by the relatively high abundance of non-nodulating Fabaceae in the Amazon (Table S1). For example, two of the fastest-growing of all tree genera in Amazonia – *Inga* and *Tachigali* – are often characterised by the presence of a symbiotic relationship with ants who aggressively defend their leaves against insect and other herbivores (Gentry 1993), and the most abundant genus, *Inga*, also being characterised by a relatively rare but highly efficient photosynthetic xanthophyll cycle (García-Plazaola et al. 2007). Moreover, many members of the Fabaceae have compound leaves of potential benefit under nutrient poor or water-stressed conditions (Malhado et al. 2010), also often being with large seeds conferring advantages on nutrient poor and/or sandy soils (ter Steege et al. 2006). In this respect it is interesting to note that Corby et al. (2011) reported that seed weight was higher and the concentration of nitrogen in the seed was lower in non-nodulating Fabaceae compared to those that cannot nodulate but that the seeds of non-nodulating species had a higher absolute nitrogen content than those of nodulating species.

Finally, we note that our results here regarding the importance of N_2-fixing Fabaceae in influencing the nitrogen cycle of the Amazon forest probably do not apply to the tropical forests of other regions of the world. For example, Raven and Pothill (1981) have argued that as the rainforest flora of parts of Asia was well established before legumes were able to reach the area, then there was no great pressure for nitrogen fixation in this region. Thus, relatively few N_2-fixing legumes have penetrated these habitats. This also seems to be the case for many parts of Africa where non-nodulating legumes are often more abundant in tropical forests than putative N_2-fixing Fabaceae (Sprent 2009); and with ectomychorrizae substituting as the principle mode of symbiotic nitrogen acquisition (Sprent 2007). By contrast, around the time that symbiotic N_2-fixation is thought to have evolved in the Fabaceae ca. 55 million years ago (Ma), not only would the global climate have been extremely favourable for plant-microbe carbon/nitrogen trading with high temperatures and high [CO_2] also prevailing (Sprent 2007) but, especially when considered in conjunction with the wetter climate (Bowen et al. 2004) the transformation in Amazonia from an ancient 'cratonic' to a much more dynamic 'Andean' dominated landscape (Hoorn et al. 2010) around the same time also providing uniquely favourable conditions for the evolution of legume symbiosis in the neotropics for at least 30 Ma afterwards: These conditions being the continual emergence of nitrogen-depauperate, disturbed and often waterlogged landscapes, especially in western Amazonia as a consequence of proto-Andean uplift and the associated geomorphological instability. As would be suggested by the hypothesis of Walker and Syers (1976), under such circumstances the development of an ability to acquire atmospheric N_2 and hence overcome potential ecosystem level nitrogen limitations in the young forests emerging in Western Amazonia may have provided an unprecedented opportunity for N_2-fixing Fabaceae to evolve and proliferate in an otherwise resource-rich environment. With many of these legume lineages then having survived to the present through a range of adaptations to the continually changing Amazon tropical forest environment as outlined above, the clade has been able to radiate and persist as a dominant taxonomic group. We suggest that, because of this presence of N_2-fixing Fabaceae in the Amazon Basin over the last 50 Ma, that nitrogen has now accumulated in many of the soils of the Amazon Basin to the extent that N_2 fixation is now rarely actually required. This idea is consistent with the current distribution of Fabaceae that is considered to relate more to factors, such as rainfall and temperature, than to rafting of land masses; it is also in accordance with the tendency of Fabaceae establishing in seasonally dry areas prior to their colonisation in the moist tropical forests (Schrire, Lavin, Lewis 2005; Schrire, Lewis, Lavin 2005).

Conclusions

The high number of plots encompassing different soil types and precipitation regimes coupled with the high number of plants analysed in this study, has allowed us to untangle the complex factors influencing $\delta^{15}N_F$ values in one of the most important tropical forest regions of the world.

It had been known from several previous studies that mean annual precipitation had an important influence on the $\delta^{15}N_F$ values. However, our study advanced the understanding of the role of climate factors by showing that for tropical forests precipitation during the dry season has a strong influence on the relationship between $\delta^{15}N_F$, $[N]_{DW}$, $[P]_{DW}$ and $[P]_{ex}$. We have also shown for the first time that, through effects on $[P]_{ex}$, soil age has a fundamental effect in influencing not only foliar nitrogen and phosphorus concentrations (Fyllas et al. 2009), but also foliar ^{15}N:^{14}N ratios. Finally, our study has confirmed the low incidence of N_2 fixation by Fabaceae members, irrespective of whether they have this ability or not. We also show that although young less-weathered soils have a high foliar phosphorus and nitrogen content, they have a low nitrogen:phosphorus ratio, potentially making them more limited by nitrogen than by phosphorus, with, generally speaking, more nitrogen fixation in these younger soils than in old weathered soils that dominate the tropics. Overall, we estimate an average maximum symbiotic N_2-fixation rate occurring within Amazon forest at maximum 3 kg N ha^{-1} $year^{-1}$. This is necessarily a rough estimate is much less than that suggested by several other scaling and/or modelling analyses which may have been based on unrealistic assumptions. Due to the unique tectonic history of the Amazon Basin our results cannot be simply extrapolated to the tropical forest of other continents with rates of symbiotic nitrogen fixation by tropical forests on other continents probably even less than those calculated here.

Acknowledgements

We thank our many South American collaborators, also involved in the work described in Fyllas et al. (2009) and Patiño et al. (2009), for help with logistics and practical help in the field. Much of the data collection phase of this work was supported through the EU FP5 “LBACarbonsink” project and a UK Natural Environment Research Council (NERC) TROBIT Consortium grant. Support for Oliver Phillips, Carlos Quesada, and Ted Feldpausch came from the Gordon and Betty Moore Foundation with additional support for the RAINFOR project from the NERC-AMAZONICA Consortium. Shiela Lloyd provided invaluable help with the nodulation database and manuscript preparation.

References

Amager N, Mariotti A, Mariotti F. 1977. Essai d’estimation du taux d’azote fixé symbiotiquement chez le lupin par le tracage isotopique naturel 15N. Comptes Rendus Académie des Sciences Paris 284: 2179–2182.

Amaral DD, Viera ICC, Almeeida SS, Salamão, R , da Silva ASL, Jardim MAG. 2009. Checklist da flora arbórea de remanescentes florestais da região metropolitana de Belém e valor histórico dos fragmentos, Pará, Brasil. Boletim do Museu Paraense Emílio Goeldi: Ciências Naturais 4: 231-289.

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: No.1031.

Aragão LEOC, Malhi Y, Metcalfe DB, Espejo JS, Jimenez EM, Navarrete D, Almeida S, da Costa ACL, Salinas N, Phillips OL, et al. 2009. Above- and below-ground net primary productivity across ten Amazonian forests on contrasting soils. Biogeosciences 6: 2759-2778.

Austin AT, Vitousek PM. 1998. Nutrient dynamics on a precipitation gradient in Hawai'i. Oecologia 113: 519-529.

Barron AR, Purves DW, Hedin LO. 2011. Facultative nitrogen fixation by canopy legumes in a lowland tropical forest. Oecologia 165: 511-520.

Bowen GJ, Beerling DJ, Koch PL, Zachos JC, Quattlebaum T. 2004. A humid climate state during the Palaeocene/Eocene thermal maximum. Nature 432: 495–499.

Cernusak LA, Winter K, Turner, BL. 2009. Plant δ^{15}N correlates with the transpiration efficiency of nitrogen acquisition in tropical trees. Plant Physiology 151: 1667-1676.

Chadwick OA, Derry LA, Vitousek PM, Huebert BJ, Hedin LO. 1999. Changing sources of nutrients during four million years of ecosystem development. Nature 397: 491-497.

Cleveland CC, Houlton BZ, Neill C, Reed SC, Townsend AR, Wang YP. 2010. Using indirect methods to constrain symbiotic nitrogen fixation rates: A case study from an Amazonian rain forest. Biogeochemistry 99: 1-13.

Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedin LO, Perakis SS, Latty EF, Von Fischer JC, Elseroad A, et al. 1999. Global patterns of terrestrial biological nitrogen (N_2)fixation in natural ecosystems. Global Biogeochemical Cycles 13: 623-645.

Corby HDL, Smith DL, Sprent JI. 2011. Size, structure and nitrogen content of seeds of Fabaceae in relation to nodulation. Botanical Journal of the Linnean Society167: 251–280.

Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Perez-Harguindeguy N, et al. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11: 1065-1071.

Craine JM, Elmore AJ, Aidar MP, Bustamante MMC, Dawson TE, Hobbie EA, Kahmen A, Mack MC, McLauchlan KK, Michelsen A, et al. 2009. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytologist 183: 980-992.

Crews TE, Kitayama K, Fownes JH, Riley RH, Herbert DA, Mueller-Dombois D, Vitousek PM. 1995. Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. Ecology 76: 1407-1424.

Cuevas E, Medina E. 1988. Nutrient dynamics within Amazonian forests II. Fine root growth, nutrient availability and leaf litter decomposition. Oecologia 76: 222-235.

Davidson EA, Carvalho CJR, Figueira AMS, Ishida FY, Ometto JPHB, Nardoto GB, Saba RT, Hayashi SN, Leal EC, Vieira ICG, et al. 2007. Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. Nature 447: 995-998.

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. Global Change Biology 10: 718–30.

Delwiche CC, Zinke PJ, Johnson CM, Virginia RA. 1979. Nitrogen isotope distribution as a presumptive indicator of nitrogen fixation. Botanical Gazette 140 Suppl: S65-S69.

De Faria SM, Diedhiou AG, de Lima HC, Ribeiro RD, Galiana A, Castilho AF, Henriques JC. 2010. Evaluating the nodulation status of leguminous species from the Amazonian forest of Brazil. Journal of Experimental Botany 61: 3119-27.

De Faria SM, Lewis GP, Sprent JI, Sutherland JM. 1989. Occurrence of nodulation in the Leguminosae. New Phytologist 111: 607-619.

De Faria SM, Lima H.C. 1998. Additional studies of the nodulation status of legume species in Brazil. Plant and Soil 200: 185-192.

Dutilleul P. 1993. Spatial heterogeneity and the design of ecological field experiments. Ecology 74: 1646-1658.

Fyllas NM, Patiño S, Baker TR, Nardoto GB, Martinelli LA, Quesada CA, Paiva R, Schwarz M, Horna V, Mercado LM, et al. 2009. Basin-wide variations in foliar properties of Amazonian forest: phylogeny, soils and climate. Biogeosciences 6: 2677-2708.

García-Plazaola JI, Matsubara S, Osmond CB. 2007. The lutein epoxide cycle in higher plants: its relationships to other xanthophyll cycles and possible functions. Functional Plant Biology 34: 759–773.

Gehring C, Vlek PLG, de Souza LAG, Denich M. 2005. Biological nitrogen fixation in secondary regrowth and mature rainforest of central Amazonia. Agriculture, Ecosystems and Environment 111: 237-252.

Gentry AH. 1988. Changes in Plant Community Diversity and Floristic Composition on Environmental and Geographical Gradients. Annals of the Missouri Botanical Garden 75: 1-34.

Gentry AH. 1993. A field guide to the families and genera of wood plants of northwest South America (Colombia, Ecuador, Peru), with suplementary notes on herbaceous taxa. Washington: Conservation Biology Series Contribution no 1.

Güsewell S. 2004. N: P ratios in terrestrial plants: variation and functional significance. New Phytologist 164: 243–266.

Handley LL, Austin AT, Robinson D, Scrimgeour CM, Raven JA, Heaton THE, Schmidt S, Stewart GR. 1999. The^{15}N natural abundance (δ^{15}N) of ecosystem samples reflects measures of water availability. Australian Journal of Plant Physiology 26: 185-199.

Heaton THE. 1987. The ^{15}N/^{14}N ratios of plants in South Africa and Namibia: relationship to climate and coastal/saline environments. Oecologia 74: 236-246.

Hedin LO, Brookshire E, Menge DNL, Barron AR. 2009. The nitrogen paradox in tropical forest ecosystems. Annual Review of Ecology, Evolution and Systematics 40: 613–635.

Hedin LO, Vitousek PM, Matson PA. 2003. Pathways and implications of nutrient losses during four million years of tropical forest ecosystem development. Ecology 84: 2231–2255.

Hedley MJ, Stewart JWB, Chauhan BS. 1982. Changes in inorganic and organic phosphorus fractions induced by cultivation practices and laboratory incubation. Soil Science Society of America Journal 46: 970–976.

Hobbie EA, Jumpponen A, Trappe J. 2005. Foliar and fungal ^{15}N:^{14}N ratios reflect development of mycorrhizae and nitrogen supply during primary succession: testing analytical models. Oecologia 146: 258-268.

Hoering TC, Ford HT. 1960. The isotope effect in the fixation of nitrogen by Azotobacter. Journal of the American Chemical Society 82: 376-378.

Högberg P. 1986. Nitrogen-fixation and nutrient relations in savanna woodland trees (Tanzania). Journal of Applied Ecology 23: 675-688.

Högberg P. 1997. Tansley review No 95 – ^{15}N natural abundance in soil-plant systems. New Phytologist 137(2): 179–203.

Högberg P, Alexander IJ. 1995. Roles of roots symbioses in African woodland and forest: evidence from 15N abundance and foliar analysis. Journal of Ecology 83: 217-224.

Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, et al. 2010. Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. Science 330: 927-931.

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: 87450–8750.

Houlton BZ, Wang Y-P, Vitousek PM, Field CB. 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454: 327-330.

James EK, Loureiro MF, Pott A, Martins CM, Franco AA, Sprent JI. 2001. Flooding tolerant legume symbiosis from the Brazilian Pantanal. New Phytologist 150: 723-738.

Jenny H, Bingham FT, Padilla-Saravia B. 1948. Nitrogen and organic matter contents of equatorial soils of Colombia, South America. Soil Science 66: 173-186.

Jenny H, Gessel SP, Binghan FT. 1949. Comparative study of decomposition rates of organic matter in temperate and tropical regions. Soil Science 68: 419-432.

Jordan C, Golley F, Hall J, Hall J. 1980. Nutrient scavenging of rainfall by the canopy of an Amazonian rain forest. Biotropica 12(1): 61-66.

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 Interactions 9: No.23.

Koponen P, Nygren P, Domenach AM, Le Roux C, Saur E, Roggy JC. 2003. Nodulation and dinitrogen fixation of legume trees in a tropical freshwater swamp forest in French Guiana. Journal of Tropical Ecology 19: 655-666.

Koerselmann W, Meuleman AFM. 1996. The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. Journal of Applied Ecology 33: 1441–1450.

Kreibich H, Kern J, de Camargo PB, Moreira MZ, Victoria RL, Werner D. 2006. Estimation of symbiotic N_2 fixation in an Amazon floodplain forest. Oecologia 147: 359-368.

Lewis WM, Melack JM, McDowell WH, McClain M, Richey JF. 1999. Nitrogen yields from undisturbed watersheds in the Americas. Biogeochemistry 46: 149-162.

Lloyd J, Bird MI, Veenendaal E, Kruijt B. 2001. Should phosphorus availability be constraining moist tropical forest responses to increasing CO2 concentrations? In: Schulze E-D, Harrison SP, Heimann M, Holland EA, Lloyd J, Prentice IC, Schimel D, editors. Global biogeochemical cycles in the climate system. San Diego (CA): Academic Press. p. 96–114.

Lloyd J, Patiño S, Paiva R, Nardoto GB, Quesada CA, Santos AJB, Baker TR, Brand WA, Hilke I, Gielmann H, et al. 2010. Optimisation of photosynthetic carbon gain and within-canopy gradients of associated foliar traits for Amazon forest trees. Biogeosciences 7: 1833-1859.

Lloyd J, Goulden M, Ometto JP, Fyllas NM, Quesada CA, Patiño S. 2009. Ecophysiology of forest and savanna vegetation. In: Keller M, Gash, JHC, Silva Dias P, editors. Amazonia and climate change. Washington DC: American Geophysical Union. p. 463-484.

Lopez-Gonzalez G, Lewis SL, Burkitt M, Phillips OL. 2011. ForestPlots.net: a web application and research tool to manage and analyse tropical forest plot data. Journal of Vegetation Science 22: 610–613.

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 Change Biology 10: 592-600.

Malhado ACM, Whittaker RJ, Malhi Y, Ladle RJ, ter Steege H, Phillips OL, Aragão LEOC, Baker TR, Arroyo L, Almeida S, et al. 2010. Are compound leaves an adaptation to seasonal drought or to rapid growth? Evidence from the Amazon rain forest. Global Ecology and Biogeography 19: 852–862.

Malhi Y, Wright J. 2004. Spatial patterns and recent trends in the climate of tropical forest regions. Philosophical Transactions of the Royal Society of London Series B—Biological Sciences 359: 311–329.

Mardegan SF, Nardoto GB, Higuchi N, Moreira MZ, Martinelli LA. 2009. Nitrogen availability patterns in white-sand vegetations of Central Brazilian Amazon. Trees 23: 479-488.

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: 45-65.

Martinelli LA, Victoria RL, Trivelin PCO, Devol AH, Richey J.E. 1992. ^{15}N natural abundance in plants of the Amazon River floodplain and potential atmospheric N_2 Fixation. Oecologia 90: 591-596.

McGroddy ME, Silver WL, de Oliveira RC. 2004. The effect of phosphorus availability on decomposition dynamics in a seasonal lowland Amazonian forest. Ecosystems 7: 172-179.

McKey D. 1994. Legumes and nitrogen: the evolutionary ecology of a nitrogen-demanding lifestyle. In: Sprent JL, McKey D, editors. Advances in legume systematics: Part 5 - the nitrogen factor. Richmond: Kew Publishing. p 211-228.

Meerts P. 2002. Mineral nutrient concentrations in sapwood and heartwood: a literature review. Annual of Forest Science 59: 713-722.

Mercado LM, Patiño S, Domingues TF, Fyllas NM, Weedon GP, Sitch S, Quesada CA, Phillips OL, Aragao LEOC, Malhi Y, et al. 2011. Variations in Amazon forest productivity correlated with foliar nutrients and modelled rates of photosynthetic carbon supply. Philosophical Transactions of the Royal Society B: Biological Sciences 366: 3316-3329.

Moreira FMS, da Silva MF, de Faria SM. 1992. Occurrence of nodulation in legume species in the Amazon region of Brazil. New Phytologist 121: 563-70.

Nadelhoffer KJ, Shaver G, Fry B, Giblin A, Johnson L, McKane R. 1996. N-15 natural abundances and N use by tundra plants. Oecologia 107: 386-394.

Nardoto GB, Ometto JPHB, Ehleringer JR, Higuchi N, Bustamante MMC, Martinelli LA. 2008. Understanding the influences of spatial patterns on the N availability within the Brazilian Amazon Forest. Ecosystems 11: 1234-1246.

Nye PH. 1960. Organic matter and nutrient cycles under moist-tropical forest. Plant and Soil 13: 333-346.

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: 251-274.

Palm CA, Sanchez PA. 1991. Nitrogen release from leaves of some tropical legumes as affected by their lignin and polyphenolic contents. Soil Biology and Biochemistry 23: 83-88.

Pardo LH, Templer PH, Goodale CL, Duke S, Groffman PM, Adams MB, Boeckx P, Boggs J. et al. 2006. Regional assessment of N saturation using foliar and root δ^{15}N. Biogeochemistry 80: 143-171.

Patiño S, Lloyd J, Paiva R, Quesada CA, Baker TR, Santos AJB, Mercado LM, Malhi Y, Phillips OL, Aguilar A, et al. 2009. Branch xylem density variations across Amazonia. Biogeosciences Discussion 5: 3003-2047.

Patiño S, Lloyd J, Paiva R, Baker TR, Quesada CA, Mercado LM, Schmerler J, Schwartz M, Santos AJB, Aguilar A, et al. 2012. Coordination of physiological and structural traits in Amazon forest trees. Biogeosciences 9: 775-801.

Patiño S, Lloyd J, Lloyd S, Lopez-Gonzalez G. 2012: Nodulation. In: Lopez-Gonzalez G, Baker TR, Lewis SL, Phillips O, editors. Functional traits database, RAINFOR project, University of Leeds 2012. Date of extraction: 29 February 2012.

Peoples RB, Craswell ET. 1992. Biological nitrogen fixation: investiments, expectations and actual contributions to agriculture. Plant and Soil 141: 13-39.

Pires JM, Prance GT. 1977. The Amazon forest: a natural heritage to be preserved.. In: Prance GT, Elias TS, editors. Extinction is forever. Bronx (NY): New York Botanical Gardens. p.158- 194.

Pons TL, Perreijn K, van Kessel C, Werger MJA. 2007. Symbiotic nitrogen fixation in a tropical rainforest: ^{15}N natural abundance measurements supported by experimental isotopic enrichment. New Phytologist 173: 154-167.

Porder S, Vitousek PM, Chadwick OA, Chamberlain, CP, Hilley GE. 2007. Uplift, erosion, and phosphorus limitation in terrestrial ecosystems. Ecosystems 10: 158-170.

Posada JM, Schuur EAG. 2011. Relationships among precipitation regime, nutrient availability, and carbon turnover in tropical rain forests. Oecologia 165: 783-795.

Quesada CA, Lloyd J, Schwarz M, Patiño S, Baker TR, Czimczik C, Fyllas NM, Martinelli LA, Nardoto GB, Schmerler J, et al. 2010. Chemical and physical properties of Amazon forest soils in relation to their genesis. Biogeosciences 7: 1515-1541.

Quesada CA, Phillips OL, Schwarz M, Czimczik CI, Baker TR, Patiño S, Fyllas NM, Hodnett MG, Herrera R, Almeida S, et al. 2012. Basin-wide variations in Amazon forest structure and function are mediated by both soils and climate. Biogeosciences 9:2203-2246.

Rangel TF, Diniz-Filho JA, Bini LM. 2006. Towards an integrated computational tool for spatial analysis in macroecology and biogeography. Global Ecology and Biogeography 15: 321-327.

Raven PH, Pothill RM. 1981. Biogeography of the Leguminose. In Pothill RM, Raven PH. (editors), Advances in legume systematics. Kew (UK): Royal Botanical Gardens. p 27-34.

Reich PB, Oleksyn J. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proceedings of the National Academy of Sciences of the United States of America 101: 11001-11006.

Robinson D. 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. Trends in Ecology and Evolution 16: 153-162.

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 $\delta^{15}N$. Journal of Tropical Ecology 15: 1-22.

Roggy JC, Prevost MF, Gourbiere F, Casabianca H, Garbaye J, Domenach AM. 1999. Leaf natural ^{15}N 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: 171-82.

Salati E, Sylvester-Bradley R, Victoria RL. 1982. Regional gains and losses in the Amazon Basin. Plant and Soil 67: 367-376.

Santiago LS. 2007. Extending the leaf economics spectrum to decomposition: Evidence from a tropical forest. Ecology 88: 1126-1131.

Santiago LS, Kitajima K, Wright SJ, Mulkey S.S. 2004. Coordinated changes in photosynthesis, water relations and leaf nutritional traits of canopy trees along a precipitation gradient in lowland tropical forest. Oecologia 139: 495-502.

Santiago LS, Schuur EAG, Silvera K. 2005. Nutrient cycling and plant–soil feedbacks along a precipitation gradient in lowland Panama. Journal of Tropical Ecology 21: 461-470.

Schrire BD, Lavin M, Lewis GP. 2005. Global distribution patterns of the Leguminosae: insights from recent phylogenies. In Friis I, Balslev H. (editors), Plant diversity and complexity patterns: local, regional and global dimensions. Biologiske Skrifter 55. 375–422.

Schrire BD, Lewis GP, Lavin M. 2005. Biogeography of the Leguminosae. In Lewis G, Schrire B, Mackinder B Lock M, (editors), Legumes of the world. Kew (UK): Royal Botanic Gardens. p. 21–54.

Schulze ED, Williams RJ, Farquhar GD, Schulze W, Langridge J, Miller JM, Walker BH. 1998. Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. Australian Journal of Plant Physiology 25: 413-425.

Shearer G, Kohl DH. 1986. N_2-fixation in field settings: estimations based on natural ^{15}N abundance. Australian Journal of Plant Physiology 13: 699-756.

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: 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. Global Change Biology 11: 290–306.

Sotta ED, Corre MD, Veldkamp E. 2008. Differing N status and N retention processes of soils under old-growth lowland forest in Eastern Amazonia, Caxiuanã, Brazil. Soil Biology and Biochemistry 40: 740-750.

Sprent JI. 1994. Evolution and diversity in the legume-rhizobium symbiosis: chaos theory? Plant and Soil 161: 1-10.

Sprent JI. 1995. Legume trees and shrubs in the tropics: N_2-fixation perspective. Soil Biology and Biochemistry 27: 401-407.

Sprent JI. 2001. Nodulaton in Legumes. Kew (UK): Royal Botanic Gardens.

Sprent JI. 2007. Evolving ideas of legume evolution and diversity: a taxonomic perspective on the occurrence of nodulation. New Phytologist 174: 11-25.

Sprent JI. 2009. Legume nodulation: a global perspective. Chichester (UK): Wiley-Blackwell.

Sprent JI, Geoghegan IE, Whitty PW, James EK. 1996. Natural abundance of ^{15}N and ^{13}C in nodulated legumes and other plantsin the cerrado and neighbouring regions of Brazil. Oecologia 195: 440-446.

Sprent JI, James EK. 2007. Legume Evolution: Where Do Nodules and Mycorrhizas Fit In? Plant Physiology 144: 575–581.

STATSOFT INC. Statistica. Data analysis software system. Version 8 for Windows. 2007.

Sylvester-Bradley R, de Oliveira LA, de Podesta Filho JA, StJohn TV. 1980. Nodulation of legumes, nitrogenase activity of roots and occurence of nitrogen-fixing Azospirillum spp. in representative soils of central Amazonia. Agro-Ecosystems 6: 249-266.

ter Steege H, Pitman NCA, Phillips OL, Chave J, Sabatier D, Duque A, Molino J-F, Prévost M-F, Spichiger R, Castellanos C, et al. 2006. Continental-scale patterns of canopy tree composition and function across Amazonia. Nature 443: 444-447.

Tiessen H, Chacon P, Cuevas E. 1994. Phosphorus and nitrogen status in soils and vegetation along a toposequence of dystrophic rainforests on the upper Rio Negro. Oecologia 99: 145-150.

Torello-Raventos M, Feldpausch TR, Veenendaal EM, Schrodt F, Saiz G, Domingues TF, Djagbletey G, Ford A, Kemp J, Marimon BS, et al. 2013. On the delineation of tropical vegetation types with an emphasis of forest/savanna transitions. Plant Ecology and Diversity. In press.

Turner BL. 2008. Resource partitioning for soil phosphorus: a hypothesis. Journal of Ecology 96: 698-702.

Unkovich M, Herridge D, Peoples M, Cadisch G, Boddey R, Giller K, Alves B, Chalk P. 2008. Measuring plant-associated nitrogen fixation in agricultural systems. Camberra (Australia): ACIAR Monograph No. 136.

Vieira SA, Alves LF, Duarte-Neto PJ, Martins SC, Veiga LG, Scaranello MA, Picolo MC, Camargo PB, do Carmo JB, Neto ES, et al. 2011. Stocks of carbon and nitrogen and partitioning between above- and belowground pools in the Brazilian coastal Atlantic Forest elevation range. Ecology and Evolution 1: 421-434.

Vitousek PM. 1984. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. Ecology 65: 285–98.

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: 1-45.

Vitousek PM, Matson PA. 1988. Nitrogen transformations in a range of tropical forest soils. Soil Biology and Biochemistry 20: 361-367.

Vitousek PM, Porder S, Houlton BZ, Chadwick OA. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. Ecological Applications 20: 5-15.

Vitousek PM, Sanford RL. 1986. Nutrient cycling in moist tropical forest. Annual Review of Ecology and Systematics 17: 137–67.

Vitousek PM, Shearer G, Kohl DH. 1989. Foliar ^{15}N abundance in an Hawaian rainforest: pattern and possible mechanisms. Oecologia 78: 383-388.

Walker TW, Syers JK. 1976. The fate of phosphorus during pedogenesis. Geoderma 15: 1–19.

Wolf K, Veldkamp E, Homeier J, Martinson GO. 2011. Nitrogen availability links forest productivity, soil nitrous oxide and nitric oxide fluxes of a tropical montane forest in southern Ecuador. Global Biogeochemical Cycles 25: No.4009.

Yoneyama T, Muraoka T, Murakami T, Boonkerd N. 1993. Natural abundance of ^{15}N in tropical plants with emphasis on tree legumes. Plant and Soil 153: 295-304.

Table Legends

Table 1. Average foliar traits of Fabaceae trees grouped according the capability of symbiotic nitrogen fixation and current status of fixation. See Table S3 for details. Different superscript letters in the same column indicate significance difference at 5% level by using the Tukey's HSD (Honestly significant difference) test for unequal variance.

Figure Legends

Figure 1. Frequency distribution of foliar nitrogen natural isotopic abundance ($\delta^{15}N_F$) for 1255 trees sampled across Amazonia.

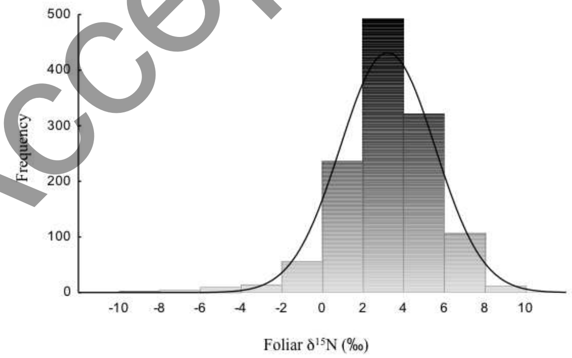

Figure 2. Scatter-plot of average $\delta^{15}N_F$ in the same plot ($\delta^{15}N_P$) versus (A) soil nitrogen natural isotopic abundance ($\delta^{15}N_S$); (B) mean annual precipitation (P_A); and (C) soil sand fraction in the sampled plots in Amazonia. Circles represent old weathered soils (total sum of bases 0 to 100 mmol$_c$ kg^{-1}clay, and total P < 200 mg kg^{-1}); diamonds represent intermediate weathered soils (total sum of bases 100 to 400 mmol$_c$ kg^{-1}clay, and total P < 600 mg kg^{-1}); and squares represent young less weathered soils (total sum of bases > 400 mmol$_c$ kg^{-1}clay, and total P > 600 mg kg^{-1}). Soil data from Quesada et al. (2010).

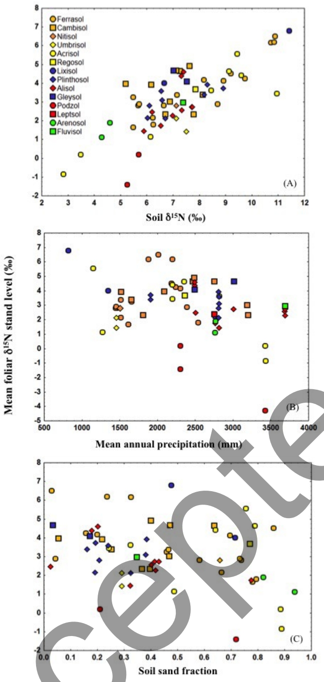

Figure 3. Scatter-plots of soil extractable phosphorus $[P]_{ex}$ versus foliar traits for plots with a mean monthly precipitation during the driest quarter (P_D) of < 100 mm (P_D < 100 mm) and for P_D > 100 mm: (A) and (B) soil $[P]_{ex}$ versus soil nitrogen natural isotopic abundance ($\delta^{15}N_S$); (C) and (D) soil $[P]_{ex}$ versus foliar nitrogen concentrations ($[N]_{DW}$); (E) and (F) soil $[P]_{ex}$ versus foliar phosphorus concentrations ($[P]_{DW}$); and (G) and (H) soil $[P]_{ex}$ versus foliar nitrogen:phosphorus ratios (foliar N:P). Data for soil $[P]_{ex}$ from Quesada et al. (2010); foliar nitrogen, phosphorus, and nitrogen:phosphorus from Fyllas et al. (2009). Circles represent older, more weathered soils (total sum of bases 0 to 100 $mmol_c\ kg^{-1}$clay, and total phosphorus < 200 mg kg^{-1}); diamonds represent intermediate weathered soils (total sum of bases 100 to 400 $mmol_c\ kg^{-1}$clay, and total phosphorus < 600 mg kg^{-1}); and squares represent young less weathered soils (total sum of bases > 400 $mmol_c\ kg^{-1}$clay, and total phosphorus > 600 mg kg^{-1}). Soil chemistry data from Quesada et al. (2010).

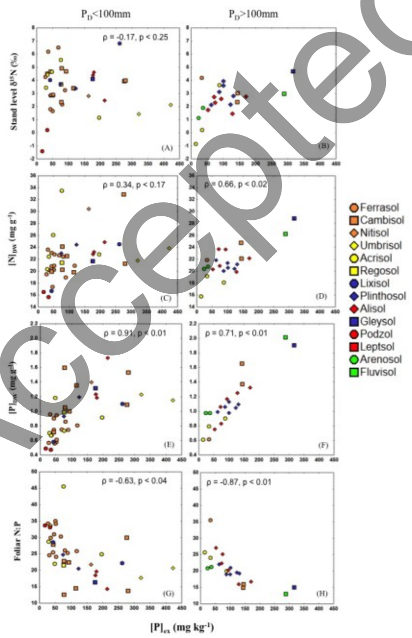

Table 1. Average foliar traits of Fabaceae trees grouped according the capability of symbiotic nitrogen fixation and current status of fixation. See Table S3 for details. Different superscript letters in the same column indicate significance difference at 5% level by using the Tukey's HSD (Honestly significant difference) test for unequal variance.

Potential status	Confirmed status	N^3	$\delta^{15}N_F$ (‰)	$[N]_{DW}$ (mg kg^{-1})	N:P^4	$[P]_{DW}$ (mg g^{-1})
Fixer	F+	14	$+1.4^{b}$	26.4^{a}	26^{a}	1.1^{a}
Fixer	F-	34	$+3.5^{ab}$	25.3^{a}	27^{a}	1.0^{a}
Non-fixer	nF	28	$+2.9^{ab}$	21.9^{a}	23^{a}	1.2^{a}

Fixer, putatively (nodulating) Fabaceae species; non-fixer, non-fixing Fabaceae species; F+, legumes which were assumed to fix nitrogen during the survey (>1‰ criterion); F-, legumes whose leaf $\delta^{15}N$ values indicated no nitrogen fixing during the survey; nF, non-fixer; *N*, number of samples; N:P, foliar nitrogen:phosphorus ratio; different letters in superscript indicate significant differences in a same column.