

# *Partitioning of environmental and taxonomic controls on Brazilian foliar content of carbon and nitrogen and stable isotopes*

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# Partitioning of Environmental and Taxonomic Controls on Brazilian Foliar Content of Carbon and Nitrogen and Stable Isotopes

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The Neotropics harbor some of the most diversified woody species in the world, and to understand the nutrient dynamics in these ecosystems, it is crucial to understand the role of plant taxonomy. In addition, biological nitrogen (N) fixation (BNF) in the tropics is one of the key processes affecting the global N cycle. Our objective was to (i) investigate the role of taxonomy and sampling site as predictors of foliar carbon (C) and N concentration and its stable isotopes (i.e.,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ); (ii) assess differences in foliar N, C:N ratio, and  $\delta^{15}\text{N}$  among three functional groups: species of  $\text{N}_2$ -fixers and non-fixers of the Fabaceae family, as well as non-Fabaceae species; and (iii) examine the effect of wood density on tree foliar properties. We hypothesized that Fabaceae specimens in symbiosis with  $\text{N}_2$ -fixers would possess a higher foliar N than non-fixing plants, including those of the Fabaceae family, as well as high-density trees would have higher foliar C and C:N ratio relative to low-density trees, where the latter invest in nutrients instead of structural C. We used a data set composed of 3,668 specimens sampled in three main biomes of Brazil: Amazon, Atlantic Forest, and Cerrado. The partitioning of variance had a higher influence of taxonomy on leaf C, N, and C:N ratio. Conversely, foliar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were environmentally constrained. While family was the most important taxonomy level for C, N, and C:N ratio, species played a major role for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Foliar N followed the pattern fixers > non-fixers > non-Fabaceae, while C:N ratio had an opposite trend. In addition, foliar C was correlated with wood density, where high-density > medium-density and low-density woods. The large variability of  $\delta^{15}\text{N}$  was observed among Fabaceae species, demonstrates the complexity of using  $\delta^{15}\text{N}$  as an indicator of BNF. The higher foliar N of Fabaceae non-fixers than non-Fabaceae specimens support the hypothesis that an N-demanding lifestyle is an inherent pattern in this family. Lastly, although observed in some studies, the prediction of foliar properties using wood density is challenging, and future research on this topic is needed.

**Keywords:** Neotropics, variance, site, taxonomy, Fabaceae, wood density

## INTRODUCTION

The Neotropics harbor one of the most diversified plant and animal species in the world (Hughes et al., 2013; Ulloa et al., 2017; Meseguer et al., 2020). Almost 40% of the global seed plant populations, approximately 100,000 species, are found in this region (Antonelli and Sanmartín, 2011). Although the reasons for such richness of life are still debatable, it seems that at regional to continental level, past geological events affecting the elevation of the Earth surface (topography) and climate changes (or lack of them), together with biotic processes, like phylogenetic niche conservatism, shape the distribution of species in the Neotropics (Rull, 2011; Cheng et al., 2013; Hughes et al., 2013; Ledo and Colli, 2017; Rangel et al., 2018).

Plant species diversity is also highly controlled by soil nutrients, from the local to the regional level (Hulshof and Spasojevic, 2020). In recent years, it has been elucidated that biodiversity has a pivotal role in ecosystem functioning (Cardinale et al., 2012; Tilman et al., 2014), which is strongly regulated by how plants process nutrients, mainly through biomass accumulation and litterfall (Vitousek and Sanford, 1986). Therefore, plant biodiversity can increase nutrient cycling and its acquisition due to the complement effect among species with varied functional traits, where different spatial and temporal niches are accessed, decreasing nutrient loss from the system (Hooper et al., 2005; Oelmann et al., 2011). The rate at which nutrients will be available to plants also depends on how nitrogen (N)-rich the vegetal detritus is, since the more N-rich it is, the faster it decomposes, returning this limiting nutrient quicker to the soil for subsequent plant uptake (Melillo et al., 1982). That is the case of Fabaceae family, which usually has higher foliar N concentration than other families, being a particularly important botanical family in forests of the tropics (Vitousek et al., 2002). Some species of this family are also able to establish symbiosis relations with the bacteria of the genus *Rhizobium*, enabling atmospheric N<sub>2</sub> fixation, known as biological N fixation (BNF), one of the key processes in the global N cycle and of high importance in the tropics (Hedin et al., 2009; Barron et al., 2011; Cleveland et al., 2011). There are many other plant families that support symbiotic N fixation. However, Fabaceae are considered the most important species regarding N acquisition due to their symbiotic relationship with rhizobia (Valentine et al., 2018). Potentially, the foliar N isotope ratio (i.e.,  $\delta^{15}\text{N}$ ) could be used to estimate BNF in plants, although there are several pitfalls in such use, especially in woody perennial plants (Boddey et al., 2000).

The uniqueness of the Fabaceae family highlights the importance of understanding the taxonomy of plant species and their role on nutrient flow and ecosystem functioning. Taxonomy-driven variances have been reported as a strong source of the variation of photosynthetic and structural traits (Oliveras et al., 2020). Additionally, taxonomy seems to be more limiting to leaf traits than the environment, showing the dominance of taxonomic turnover over the environmental variations (Fyllas et al., 2009; Anderegg et al., 2018; Oliveras et al., 2020). Many plant traits and associations between traits have been hypothesized to be under the control of a common genetic mechanism, reflecting a combination of evolutionary and

community assembly process responding to biotic and abiotic constraints (Chapin et al., 1993; Vasseur et al., 2012; Oliveras et al., 2020).

In tropical regions, investigation of foliar carbon (C) and N concentrations as well as isotopic ratios seems to be appealing because there is limited evidence suggesting that N site availability is an important driver of foliar N isotopic ratio (Högberg, 1997; Martinelli et al., 1999; Schuur and Matson, 2001; Craine et al., 2009). Current scientific understanding state that N-site availability controls foliar N isotope composition. In addition, there is an understanding that the canopy C isotope ratio (i.e.,  $\delta^{13}\text{C}$ ) is mainly controlled by water availability, in which stomata tend to stay open longer in sites with higher water abundance than in the arid ones (Diefendorf et al., 2010; Basu et al., 2019). Consequently, the internal stomatal CO<sub>2</sub> concentration ( $p_i$ ) tends to equilibrate with the atmospheric CO<sub>2</sub> ( $p_a$ ), leading to more negative  $\delta^{13}\text{C}$  values related to the plants in arid sites (Farquhar et al., 1989). Additionally, it is already established that slow-growing trees are shade-tolerant species, where water is more available, but the light is more limiting, leading to leaves with more negative  $\delta^{13}\text{C}$  than fast-growing and light-demanding species (Farquhar and Sharkey, 1982).

In order to understand the life history of trees and their mechanical and physiological strategies, wood density can be an important trait to be considered, since it relates to the whole-plant performance (Westoby et al., 2002; Swenson and Enquist, 2007). Low-density species have higher N and lower C concentrations in comparison with high-density species, following the well-known “leaf economics spectrum” (LES), where low-density trees, being short-lived, invest more on nutrients and less on structural C, while high-density trees, being long-lived, invest less on nutrients and more on structural C (Wright et al., 2004). Therefore, wood density also appears to be a relevant tree trait to unravel the ecosystem functioning, since this trait is key to determine forest biomass and C (Baker et al., 2004).

As part of the Neotropics, Brazil is the country with the largest number of vascular plant species (Ulloa et al., 2017), comprising six major biomes that can be broadly divided into two major rainforests: Amazon and the Atlantic Forest. These two rainforests are separated along a northeast-southwest axis by the Caatinga and Cerrado, representing two major dry areas (Rangel et al., 2018). Besides the above biomes, there is the Pantanal, one of the main wetland areas of the Neotropics, and the Pampas, in the south tip of Brazil, representing the only truly sub-temperate biome of the country. According to a recent cross-taxonomic biogeographic analysis, Amazon stands out as the primary source of diversity in the Neotropics (Antonelli et al., 2018). However, as recognized by several authors, drier regions such as the Caatinga and Cerrado also play a vital role in the biodiversity of the country (Pennington et al., 2009; Antonelli et al., 2018; Rangel et al., 2018).

In a recent study of Martinelli et al. (2020), the foliar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were determined in ~6,500 tree leaves sampled from 57 sites distributed across all major Brazilian biomes. This analysis showed that there is a large variation in foliar chemical properties among vegetation types, demonstrating that parallel to life diversity, there is also a “biogeochemical diversity,” as postulated by Townsend et al. (2008). It was also speculated that

precipitation was the main environmental factor responsible for such variability. However, the weight of taxonomy in these foliar properties was not determined and remains poorly understood. Therefore, our main objective was to determine how much of the variance in canopy foliar traits is explained by taxonomy and site characteristics, considering foliar N and C isotopic ratios, but also on foliar N and C concentrations in a subset of the database provided by Martinelli et al. (2020), containing 3,668 foliar samples with complete taxonomy (family, genus, and species) from three major Brazilian biomes, i.e., Amazon, Atlantic Forest, and Cerrado, including 741 species, distributed in 298 genera and 81 botanical families. Our special interest in stable isotopes lies in the fact that there is limited information regarding taxonomic control on canopy  $\delta^{13}\text{C}$  (Fyllas et al., 2009), and to the best of our knowledge, this aspect has not yet been evaluated for canopy  $\delta^{15}\text{N}$ . If our study proves that taxonomy exerts a major role in determining foliar  $\delta^{15}\text{N}$ , the importance of N-site availability as a key factor will be challenged. The second objective of this study was to investigate foliar N concentration, C:N ratio, and  $\delta^{15}\text{N}$  in specimens of the Fabaceae family and also compare these parameters among Fabaceae N-fixing, non-fixing, and non-Fabaceae species of the Brazilian biomes mentioned above. Finally, we also assessed whether wood density correlates or not with foliar C and N concentration, as well as the C:N ratio.

## MATERIALS AND METHODS

### Data Acquisition and Processing

We used a data set compiled by Martinelli et al. (2020) and available in a public repository at Mendeley<sup>1</sup>, where C and N concentration, C:N ratio, and isotopic ratios are available for each species. Due to the large number of publications present in this data set (including original data provided by the authors), the sampling procedure of leaves slightly differed among studies. First, while several studies used the abundance criteria, sampling the most common species, others prioritized plant species based on logistic facilities (Martinelli et al., 2020). Second, most of the studies followed the protocol of Cornelissen et al. (2003), where mature, fully expanded, and sunlit leaves found in the middle of the canopy were sampled. However, in some studies, sampling of sunlit leaves was constrained by tree height in tropical forests. In such cases, understory leaves were not included in our subset to reduce bias regarding  $^{13}\text{C}$  composition (Ometto et al., 2006).

Overall, sampled leaves were oven-dried at 60–65°C to a constant weight and finely ground in a Wiley mill, and a subsample was transferred to a tin capsule. Samples were analyzed for total C and N concentration as well as their isotopic composition ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) in an elemental analyzer interfaced to an isotope ratio mass spectrometer. C and N concentrations are expressed in percent, while the stable isotopic ratio in the classical “ $\delta$ ” notation is expressed according to the following equation (Craig, 1953):

$$\delta^n X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000 \quad (1)$$

<sup>1</sup><https://doi.org/10.17632/38npddpnts.1>

where X is C or N;  $n$  is the mass number of the heavier isotope ( $^{13}\text{C}$  or  $^{15}\text{N}$ ); and  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the isotopic ratio of the samples and the standard, respectively. The primary standards were Pee Dee Belemnite for C and atmospheric air for N.

Of a total of ~6,500 foliar samples, 3,668 had botanical identification. We used a similar procedure described by Chave et al. (2006) and by Oliveira et al. (2019). We first compared species names with the currently accepted names using the R package “*flora*” (Carvalho, 2020), with the purpose of correcting spelling errors and checking species names that have changed. We found a high number of spelling errors, which seems to be a recurrent problem in large data sets (Chave et al., 2006), and a smaller number of invalid species names that were discarded. This process resulted in a subset composed of tree foliar samples with accurate botanical classification at the species level from three major Brazilian biomes (Figure 1 and Supplementary Data Sheet 1). Vegetation types found in the data set were those previously described in Martinelli et al. (2020) for Amazon, Atlantic Forest, and Cerrado, namely, Dense Ombrophilous Forest (DOF), Open Ombrophilous Forest (OOF), Mixed Ombrophilous Forest (MOF), Campinarana Forest (CAM), Restinga Forest (MV), and Savanna (SAV). DOF had sampling sites located in the Amazon as well as in the Atlantic biome.

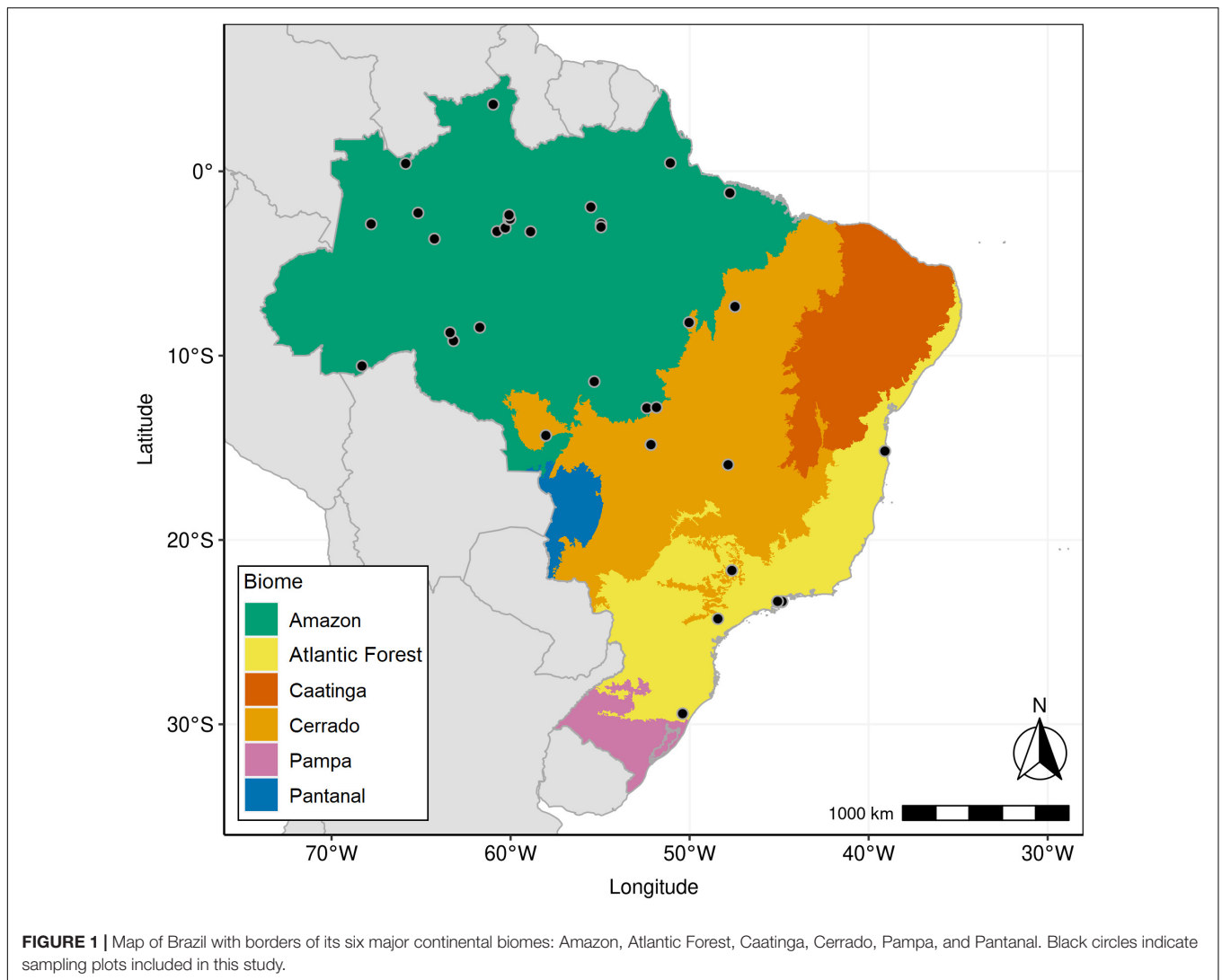
### Variance Partitioning of Foliar Properties Into Taxonomy and Site

To partition the variance between taxonomy and site according to each foliar property, we used a similar statistical procedure employed by Fyllas et al. (2009) and Asner and Martin (2016), among others. A multilevel generalized linear mixed model (GLMM) was used, and model parameters were estimated by a residual maximum likelihood method (REML). To perform this analysis, we first checked normality of residuals using the R package “*fitdistrplus*” (Delignette-Muller and Dutang, 2015). Only foliar N concentration was log-transformed to ensure normality. The “*lmerTest*” R package was used to run the GLMM (Kuznetsova et al., 2017). To estimate the pseudo- $R^2$  for GLMM ( $R^2_{\text{GLMM}}$ ), the R package “*MuMIn*” was used (Bartón, 2020). This package estimated the variance explained by the fixed effect (marginal  $R^2_{\text{GLMM}}$ ), the variance explained by the whole model, and the sum of fixed and random variables (conditional  $R^2_{\text{GLMM}}$ ; Nakagawa et al., 2017).

The response variables (foliar properties) were modeled assuming the following random variables within the GLMM: (i) species, nested within genus and family; and (ii) site, to account for environmental filters. Adopting the same nomenclature as that found in previous studies (Fyllas et al., 2009; Asner and Martin, 2016; Oliveras et al., 2020), the total variance of the model for each response variable was then partitioned as follows:

$$T = \mu + ss + f/g/s + \varepsilon(2)$$

where  $\mu$  is the overall mean value of each foliar trait ( $T$ ), presenting C and N elemental concentration and stable isotopic ratios;  $ss$  is the name of each sampling site;  $f/g/s$  represents the nested taxonomic structure of the data, species ( $s$ ), nested in a



genus ( $g$ ) and nested in a family ( $f$ ); and  $\epsilon$  is the residual term that includes any variability not captured by the model.

### Influence of the Fabaceae Family on Foliar Properties

To test the differences in foliar properties of species of Fabaceae, we first compared this family with each of the other families through multiple box-plots without conducting any statistical test since the main purpose here was to check, in descending order, how these traits varied among families. Second, we grouped Fabaceae according to their capability or not of establishing associations with  $N_2$ -fixing bacteria (“fixers” and “non-fixers,” respectively), following Tedersoo et al. (2018). We compared these two groups with the third group consisting of species belonging to other botanical families (“non-Fabaceae”) with regard to foliar N, C:N, and  $\delta^{15}N$ . With the exception of foliar N, which was log-transformed before analysis, all variables were normally distributed. Then, we developed a GLMM considering these three groups as fixed effect and sampling site as a random

effect. In case of significance ( $p \leq 0.05$ ), means were separated by Sidak’s *post hoc* test through the “emmeans” R package (Lenth et al., 2021). Given the often high foliar N concentration of legumes, a GLMM was used to assess the variance partitioning for foliar N of Fabaceae fixers and non-fixers into each sampling site, taxonomy (species nested in a genus), and residual, based on the statistical procedure described above (Equation 2).

### Influence of Wood Density on Leaf Foliar Properties

Finally, we tested the effects of wood density on foliar properties by assigning a wood density ( $\rho$ ) value to each species found in the data set published by Chave et al. (2006). Unfortunately, not all species included in our data set were found in the database provided by Chave et al. (2006). Accordingly, we ended up with 262 species belonging to 235 genera with values of  $\rho$ , with  $\sim 35\%$  of the total number of species included in our data set (**Supplementary Data Sheet 2**). As Chave et al. (2006) pointed out, most of the variance of  $\rho$  was explained at the



genus level; accordingly, we grouped plants by their respective genera. We also grouped  $\rho$  in three classes considering the 33rd and 66th percentiles in the frequency distribution of  $\rho$  from Chave's data set, as follows: low density ( $\rho < 0.54 \text{ g cm}^{-3}$ ); medium density ( $0.54 < \rho < 0.71 \text{ g cm}^{-3}$ ), and high density ( $\rho > 0.71 \text{ g cm}^{-3}$ ). These values are similar to the ones used for classifying mechanical wood properties. However, we acknowledge that these classes of wood density were solely based on the frequency distribution of  $\rho$  found in Chave et al. (2006). We tested the effects of  $\rho$  on foliar composition through a simple linear model using the normal distribution, since residuals were normally distributed for all variables (foliar C, N, and C:N ratio). A similar procedure used to assess the influence of the Fabaceae family in foliar properties compared with other botanical families was adopted here as related to significances and *post hoc* tests. The R code used in this study can be found in **Supplementary Data Sheet 3**.

## RESULTS

Site had the lowest effect (~21%) on the variance partitioning of foliar C, while ~40% of the variance was explained by the sum of family, genus, and species, and ~39% of the variance was attributed to the residual, not accounted by the above factors (**Figure 2** and **Table 1**). With regard to foliar N, taxonomy explained ~44% of the variance, while sampling site and residual represented 28% each (**Figure 2** and **Table 1**). Approximately 26% of the variance of foliar C:N ratio was explained by site, but taxonomy still represented ~44% of the variance, while the remaining percentage (~30%) was not explained by the model (**Figure 2** and **Table 1**). The effect of sampling site was the highest (~40%) in explaining the variance of foliar  $\delta^{13}\text{C}$ , followed by residual (~35%) and taxonomy (~25%), with the lowest importance (**Figure 2** and **Table 1**). Similar to foliar  $\delta^{13}\text{C}$ , most of the variance of  $\delta^{15}\text{N}$  was explained by site (~63%), while taxonomy and residual accounted for ~16 and ~22%, respectively (**Figure 2** and **Table 1**). Overall, the partitioning of leaf trait variance within the taxonomy levels indicated that botanical family explained mostly the total variance for C, N, and C:N ratio (~23%, on average), while species was the most important group for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (~13%, on average; **Figure 2** and **Table 1**). The pseudo- $R^2$  for GLMM of foliar properties ranged from 0.61 to 0.78 (**Table 1**).

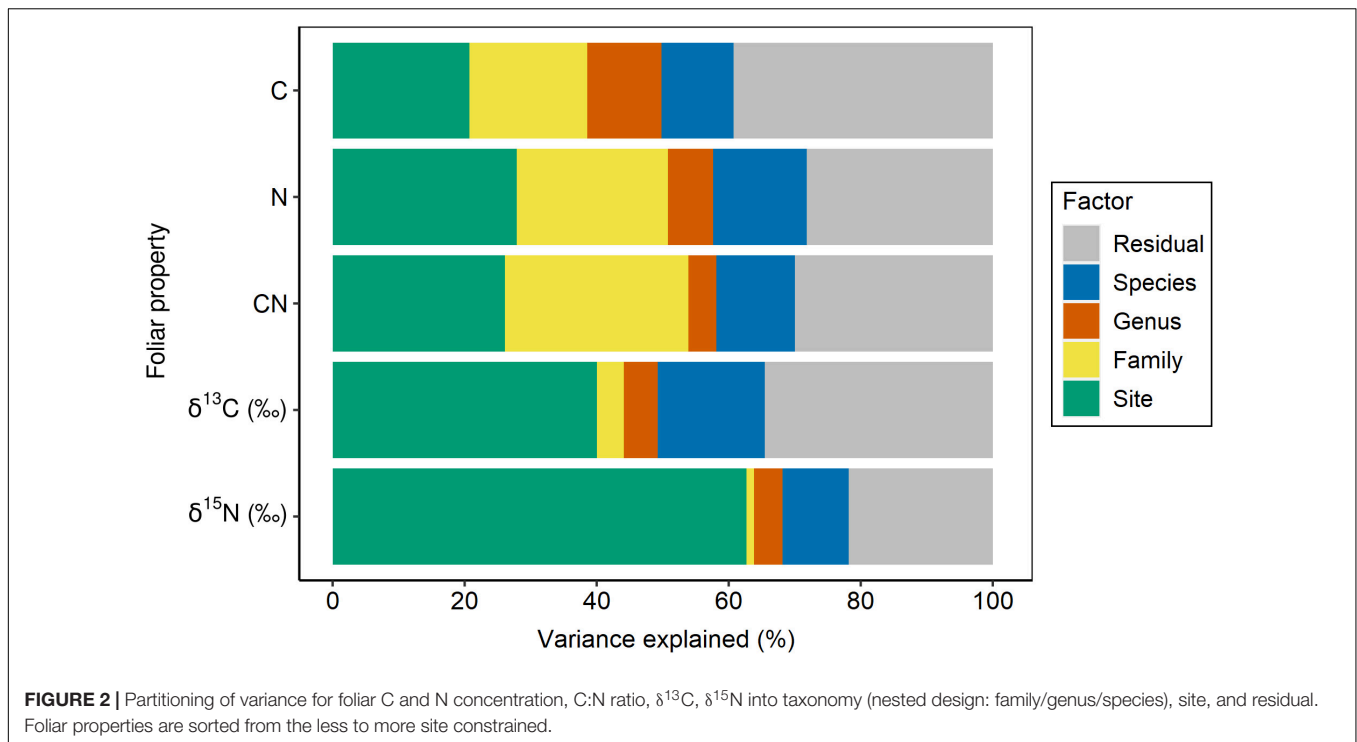
Although Fabaceae ranked among the N-rich families, it was not the family with the highest foliar N concentration or even the lowest C:N ratio (**Figures 3, 4**). However, the family-median values for Fabaceae were higher and lower than the overall median for foliar N and C:N ratio, respectively. Finally, the family-median  $\delta^{15}\text{N}$  of Fabaceae was essentially similar to the overall median (**Figure 5**). By grouping Fabaceae family according to their N-fixing capacity at genera level, a clearer picture emerges. The foliar N concentration of fixers was significantly higher than the other two groups (**Figure 6** and **Supplementary Table 1**). The foliar C:N ratio, as expected, followed an inverse trend, with fixers showing the lowest median values, followed by non-fixers and non-Fabaceae (**Figure 6** and

**TABLE 1** | Variance partitioning according to taxonomy (nested design: family/genus/species), site, and residual for foliar C, N, C:N ratio,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$ .

Factor	Variance	SD
<b>C</b>		
Species:(genus:family)	1.7	1.3
Genus:Family	1.7	1.3
Family	2.7	1.7
Site	3.2	1.8
Residual	6.0	2.5
Total variance	15.4	
$R^2_{GLMM} = 0.61$		
<b>N</b>		
Species:(genus:family)	0.019	0.137
Genus:family	0.009	0.095
Family	0.031	0.175
Site	0.037	0.192
Residual	0.038	0.194
Total variance	0.133	
$R^2_{GLMM} = 0.72$		
<b>C:N ratio</b>		
Species:(genus:family)	12.9	3.6
Genus:family	4.6	2.1
Family	30.1	5.5
Site	28.3	5.3
Residual	32.5	5.7
Total variance	108.4	
$R^2_{GLMM} = 0.70$		
<b><math>\delta^{13}\text{C}</math></b>		
Species:(genus:family)	0.71	0.84
Genus:family	0.22	0.47
Family	0.18	0.42
Site	1.75	1.32
Residual	1.51	1.23
Total variance	4.38	
$R^2_{GLMM} = 0.65$		
<b><math>\delta^{15}\text{N}</math></b>		
Species:(genus:family)	0.88	0.94
Genus:family	0.38	0.62
Family	0.10	0.32
Site	5.53	2.35
Residual	1.92	1.39
Total variance	8.83	
$R^2_{GLMM} = 0.78$		

SD, standard deviation of the variance;  $R^2_{GLMM}$ , conditional coefficient of determination for the generalized linear mixed model according to Nakagawa et al. (2017).

**Supplementary Table 1**). On the other hand, foliar  $\delta^{15}\text{N}$  was higher for non-fixers compared with non-Fabaceae and fixers (**Figure 6** and **Supplementary Table 1**). The variance partitioning across the two groups of Fabaceae indicated that ~50, ~14, and ~36% of the variance of foliar N was explained by taxonomy, site, and residual, respectively, for fixers, while for non-fixers, taxonomy and site represented ~35 and ~27% of the variance, respectively, with the remaining percentage (~38%) attributed to residual (**Figure 7** and **Supplementary Table 2**). For both



groups, genus played a major role relative to species across the taxonomic levels. In addition, foliar composition varied among different classes of wood density (Figure 8 and Supplementary Table 3). However, differences were only significant for foliar C concentration. Thus, C concentration was the highest in the high-density class, exhibiting the following trend: high density > medium density  $\approx$  low density (Figure 8 and Supplementary Table 3).

## DISCUSSION

### Taxonomy and Site Affecting the Canopy Chemistry

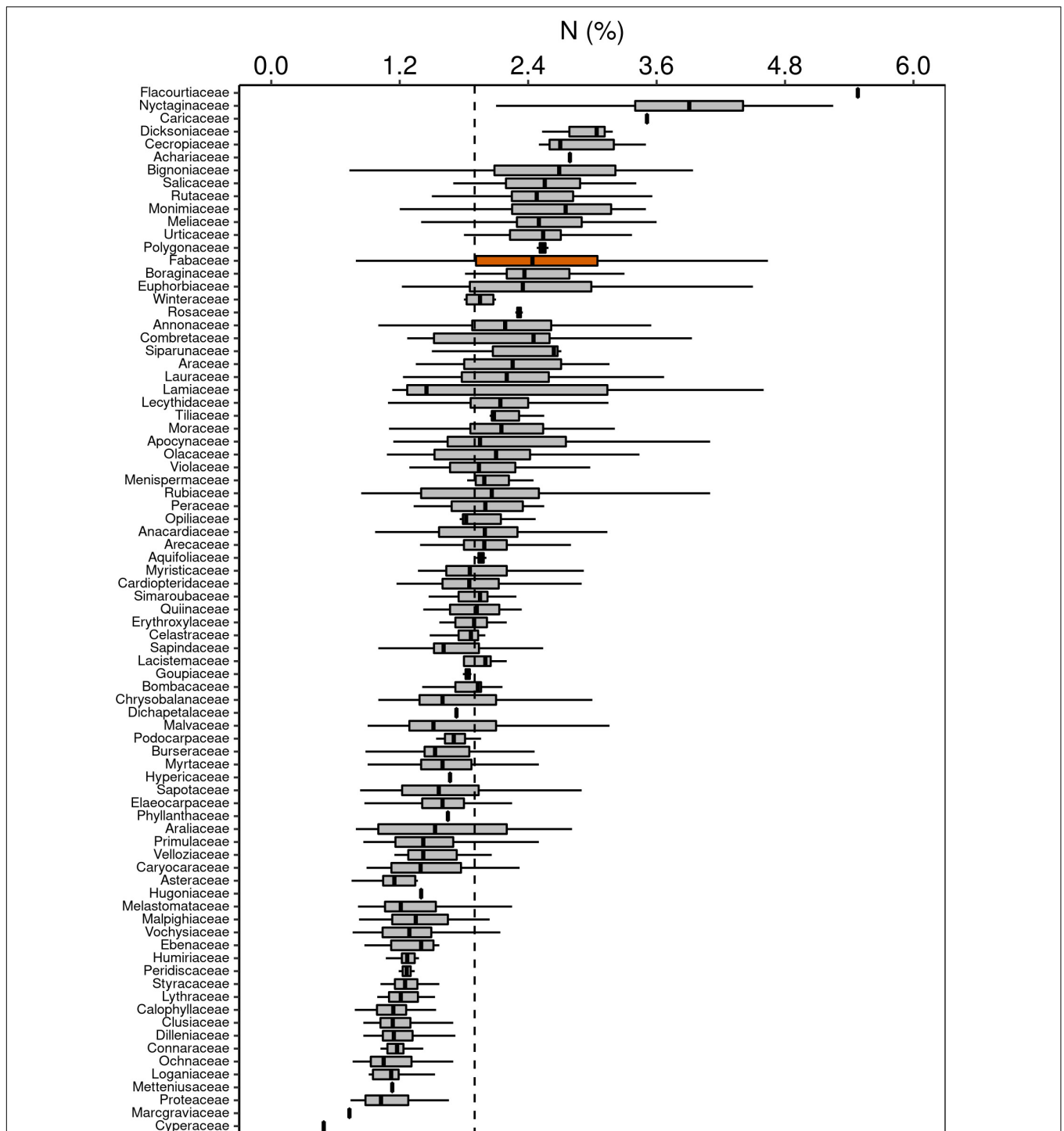
We observed that foliar  $\delta^{15}\text{N}$  was strongly environmentally constrained, therefore presenting a high level of plasticity, with taxonomy playing a minor role in the respective variation (Figure 2). This finding indicates that local abiotic conditions, represented here by the site factor within the variance partitioning model, are tightly associated with  $\delta^{15}\text{N}$  of leaf trees in comparison with the genetic (i.e., taxonomic levels) component. Despite being unable to identify the specific abiotic factors contributing to this strong relationship, soil N availability at the local scale and precipitation at a larger scale have been proven to be the key in controlling the  $\delta^{15}\text{N}$  of plants (Craine et al., 2009, 2015). Many studies postulate that foliar  $\delta^{15}\text{N}$  tends to be higher in sites where the N availability (i.e., mineral N forms) is also high (Högberg, 1997; Martinelli et al., 1999; Craine et al., 2009), in addition to the lower soil available N in wet than dryer areas (Amundson et al., 2003; Pardo et al., 2006). Moreover, a recent study developed an isoscape of soil  $\delta^{15}\text{N}$  for

South America, suggesting a high variation of values across the Brazilian territory (Sena-Souza et al., 2020), which also helps to explain the dominance of the environmental filter on predicting foliar  $\delta^{15}\text{N}$ .

It is important to notice that still  $\sim 16\%$  of the variance of foliar  $\delta^{15}\text{N}$  was explained by taxonomy (Figure 2). Plant species can be important in driving N isotopic ratios in several ways (Högberg, 1997), such as “preferential” uptake of a particular mineral; atmospheric or organic N form (Chalk and Smith, 2020); and metabolic-induced changes of N isotopic ratios (Robinson, 2001; Yoneyama et al., 2001). For instance, *Roupala montana* (Proteaceae), a common species found in the Cerrado, is more effective in acquiring  $\text{NH}_4^+$  related to  $\text{NO}_3^-$ . This species tends to have higher foliar  $\delta^{15}\text{N}$  than other species of the same biome (Bustamante et al., 2004), as  $\text{NH}_4^+$  tends to be more  $^{15}\text{N}$ -enriched than  $\text{NO}_3^-$  (Högberg, 1997; Houlton et al., 2007). In our data set, there are 44 specimens of *R. montana*, most of them from savannas of Central Brazil, and a minor part from savannas encroached on the Amazon region. The median  $\delta^{15}\text{N}$  of *R. montana* was  $5.6\text{‰}$  (25th percentile =  $4.3\text{‰}$ ; 75th percentile =  $6.5\text{‰}$ ), while the overall foliar  $\delta^{15}\text{N}$  median of plants found in savannas was  $-0.6\text{‰}$  (25th percentile =  $-1.6\text{‰}$ ; 75th percentile =  $0.6\text{‰}$ ; Supplementary Data Sheet 1).

Another example of taxonomy influencing foliar  $\delta^{15}\text{N}$  is in the fixers grouping. Taxonomy determines which species of Fabaceae family can establish symbiotic relations with bacteria of the genus *Rhizobium*, which fix atmospheric N in exchange for carbohydrates (Sprent, 1995). As the atmospheric N has a  $\delta^{15}\text{N}$  of  $0\text{‰}$ , and the fractionation between the *Rhizobium* and the plant is almost negligible (Högberg, 1997), species that rely mainly on BNF potentially have a lower  $\delta^{15}\text{N}$  in

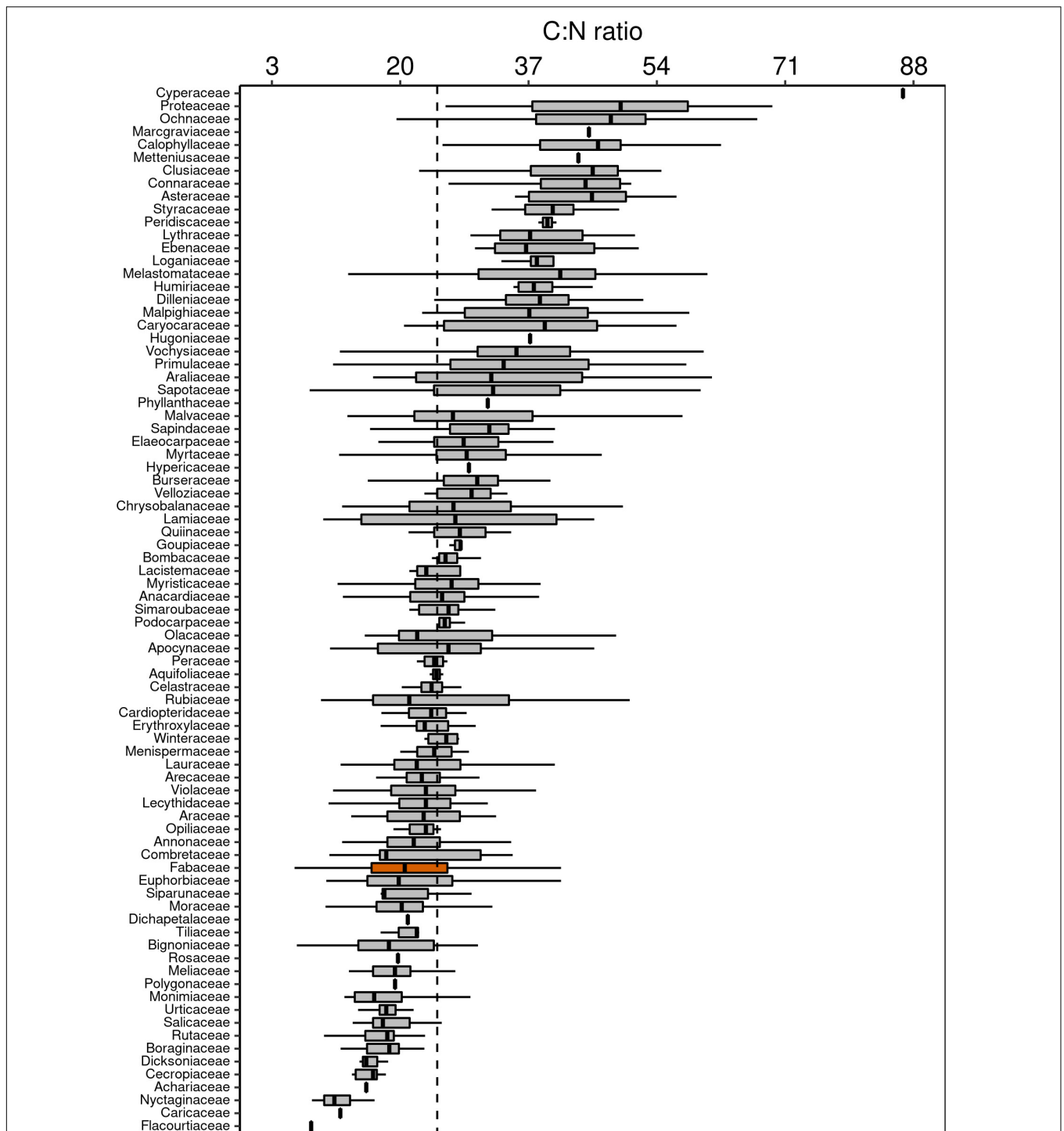




**FIGURE 3 |** Box-plots of foliar N concentration of botanical families in decreasing order. The vertical lines inside each box represent the median; the box represents the first and third quartiles; and the bars represent the interquartile range. The vertical dashed line represents the mean of the entire population. The orange box is evidencing the Fabaceae family, where a notable part of the species is capable of fixing atmospheric N through symbiotic associations.

comparison with plants relying on the soil as the unique N source (Virginia and Delwiche, 1982). Although Fabaceae is an important family in tropical biomes, often specimens do not rely on BNF as a source of N (Vitousek et al., 2002).

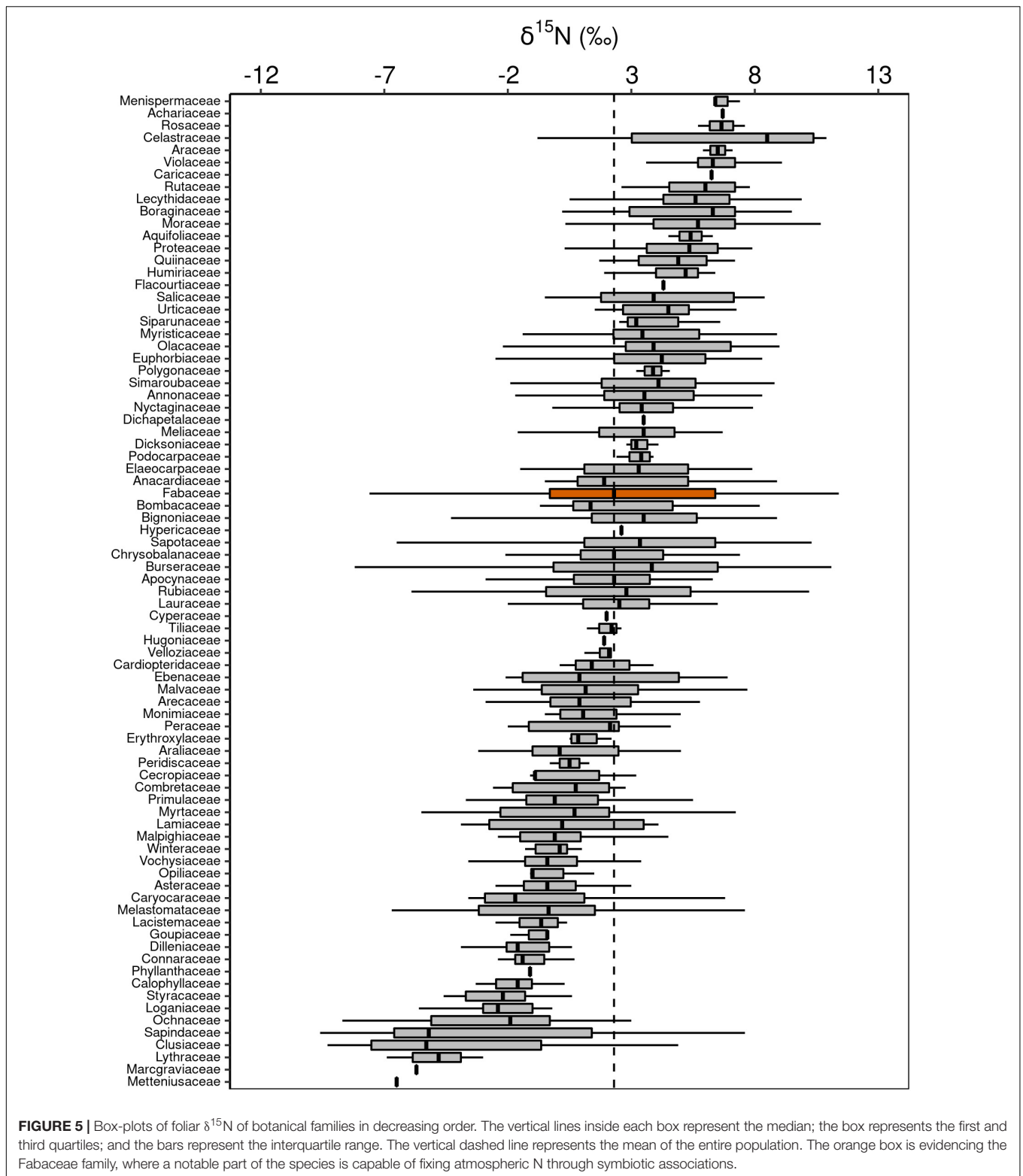
There was a large range of  $\delta^{15}\text{N}$  values in our data set. However, the median  $\delta^{15}\text{N}$  in sampling sites with DOF (including forests of the Amazon and Atlantic Forest biome) was equal to 4.6‰ (25th percentile = 1.8‰; 75th percentile = 7.1‰;



**FIGURE 4 |** Box-plots of foliar C:N ratio of botanical families in decreasing order. The vertical lines inside each box represent the median; the box represents the first and third quartiles; and the bars represent the interquartile range. The vertical dashed line represents the mean of the entire population. The orange box is evidencing the Fabaceae family, where a notable part of the species is capable of fixing atmospheric N through symbiotic associations.

Supplementary Data Sheet 1), which is well above the overall median for foliar  $\delta^{15}\text{N}$ , supporting the hypothesis of low and facultative BNF in tropical forests (Hedin et al., 2009; Barron et al., 2011; Nardoto et al., 2014). Most of

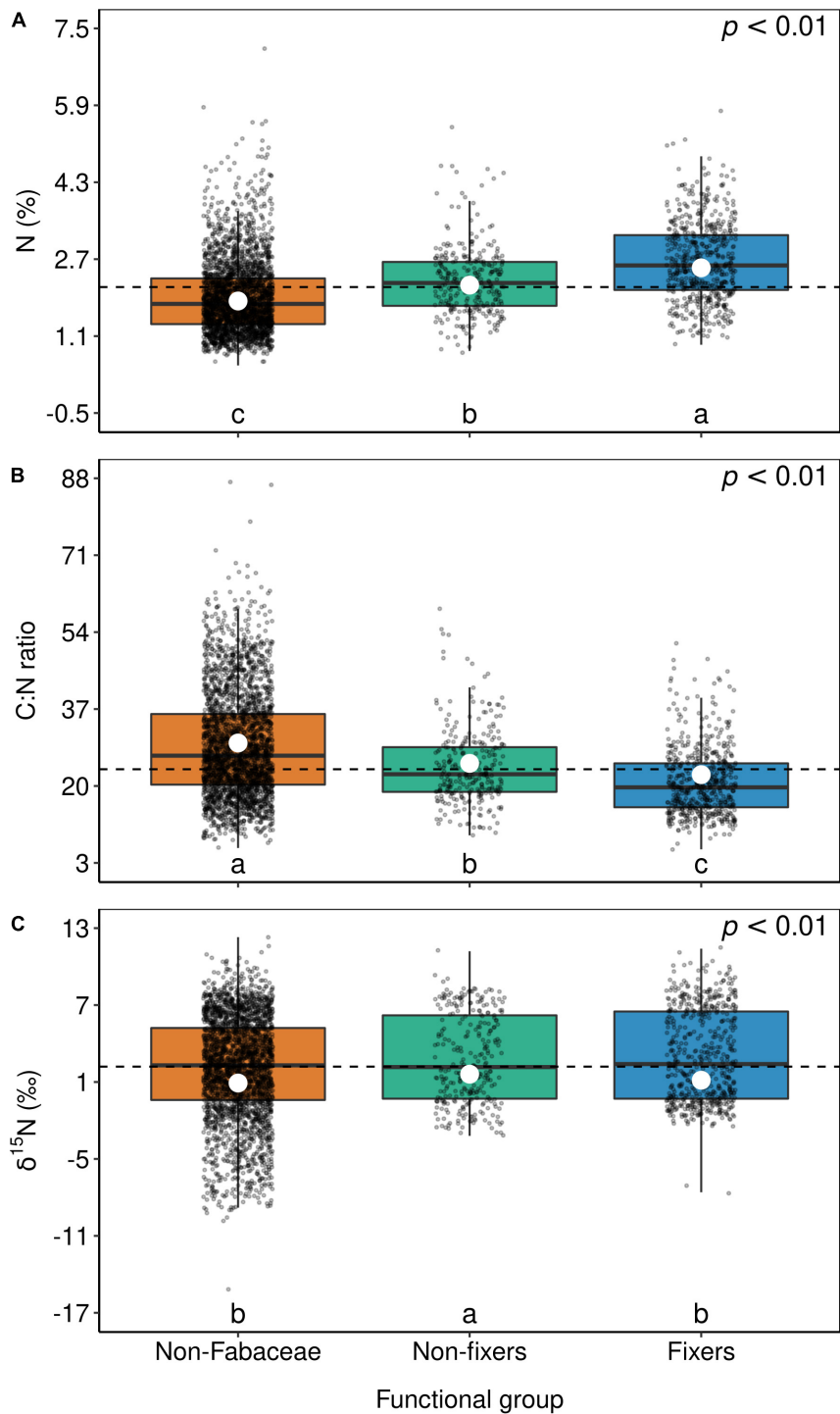
the negative or near-zero  $\delta^{15}\text{N}$  values were observed in the savanna's sites, with a median equal to  $-0.6\text{‰}$  (25th percentile =  $-1.2\text{‰}$ ; 75th percentile =  $0.1\text{‰}$ ; Supplementary Data Sheet 1). Despite many specimens with negative foliar



$\delta^{15}\text{N}$  in sites with savannas vegetation, it is not possible to assess the role of BNF since near-zero  $\delta^{15}\text{N}$  values in the savannas can be derived from BNF as well as the association of legumes with mycorrhizal fungi in N-limited systems,

where depleted  $\delta^{15}\text{N}$  compounds are delivered to the plant (Bustamante et al., 2004).

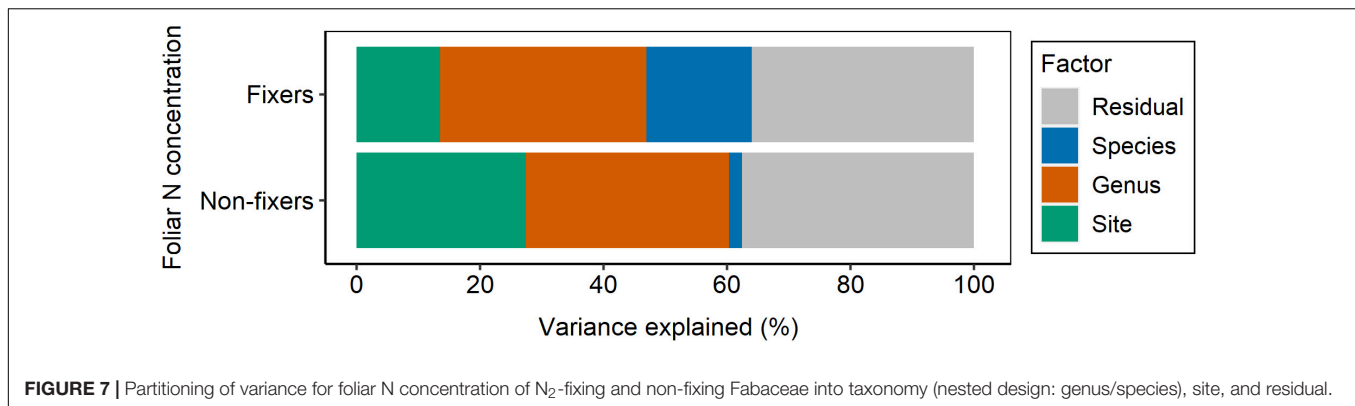
Due to the importance of water availability in driving foliar  $\delta^{13}\text{C}$  values by the photosynthetic discrimination against  $^{13}\text{CO}_2$



**FIGURE 6 |** Box-plots of foliar N (A), C:N ratio (B), and  $\delta^{15}\text{N}$  (C) of non-Fabaceae species as well as non-fixer and  $\text{N}_2$ -fixer species of the Fabaceae family. The horizontal lines inside each box represent the median; the box represents the first and third quartiles; and the bars represent the interquartile range. The horizontal dashed line represents the mean of the entire population. White circles represent estimate means obtained from the generalized linear mixed model. The summary of the statistical model can be seen in **Supplementary Table 1**. Levels followed by a common letter are not different according to the Sidak-test at  $p \leq 0.05$ .

(Lloyd and Farquhar, 1994; Diefendorf et al., 2010; Cornwell et al., 2018), we hypothesized that climatic and soil conditions would be more important than taxonomy (Fyllas et al., 2009;

Cornwell et al., 2018). This hypothesis was confirmed since sampling site explained a major share of the variance, with taxonomy representing a lower but representative percentage



(Figure 2). Taxonomy also exerts influence on foliar  $\delta^{13}\text{C}$  by modulating environmental seasonal changes, such as water availability, mainly through leaf adjustments like stomatal conductance and photosynthetic rates (Read and Farquhar, 1991; Comstock and Ehleringer, 1992). As explained by Cernusak et al. (2013), water availability seems to be a key control of foliar  $\delta^{13}\text{C}$ ; however, the degree of response to this variable depends on how each species will counteract the environmental forcing with leaf adjustments.

Strictly speaking about the taxonomic levels and their role on functional leaf traits, our study is in line with Fyllas et al. (2009); Asner and Martin (2016), and Oliveras et al. (2020), who found a higher proportion of the C and N variance explained by the botanical family compared with genus and species. For  $\delta^{13}\text{C}$ , while we observed that species was the more constrained factor, Fyllas et al. (2009) reported that genus was the dominant genetic level at a local scale for Amazonian sites. To the best of our knowledge, this is the first study reporting the partitioning of variance for foliar  $\delta^{15}\text{N}$ , therefore restricting any comparison with previously published studies, especially those under similar geographic conditions to ours.

## The Role of Fabaceae in the Nitrogen Cycle

Taxonomy was the most important factor in explaining foliar N variance ( $\sim 50\%$ ) for N<sub>2</sub>-fixers, while the variance explained by taxonomy and residual was similar for Fabaceae non-fixers. This trend is in line with previous studies conducted in tropical regions (Fyllas et al., 2009; Asner and Martin, 2016). An iconic example of such control is the higher foliar N concentration found in the Fabaceae family, especially those able to establish an association with N-fixing organisms (Figures 3, 6), a trend already observed by many authors (McKey, 1994; Vitousek et al., 2002; Townsend et al., 2007; Fyllas et al., 2009; Nasto et al., 2014; Adams et al., 2016; Taylor and Ostrowsky, 2019). However, the interpretation of this result is still unknown.

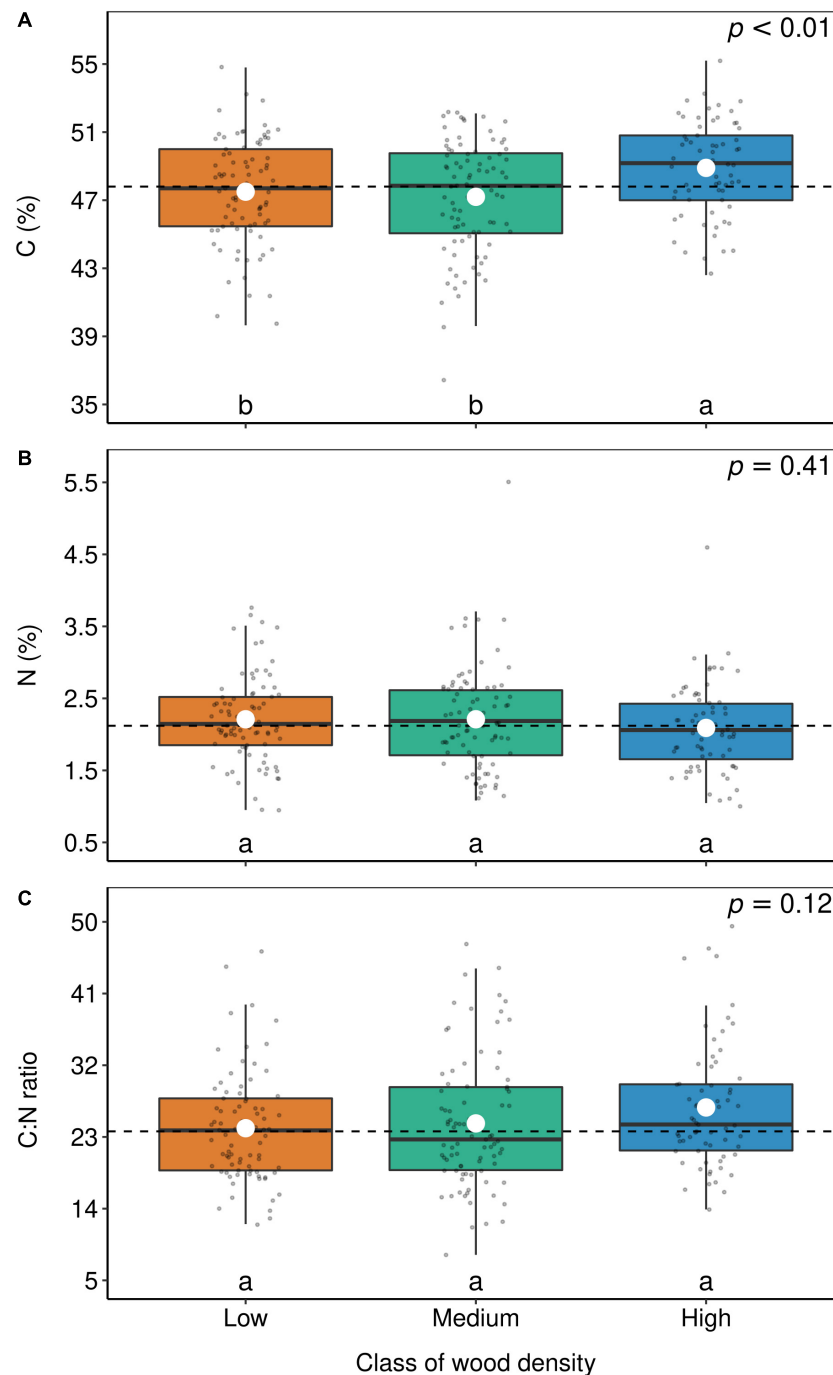
McKey (1994) proposed that the high foliar N concentration in Fabaceae evolved before the association with N-fixing organisms, the so-called “N-demanding lifestyle” of Fabaceae. In this sense, Wolf et al. (2017) defined the term “hardwired”

(i.e., having an inherent pattern) for this N-rich lifestyle and found that in fact Fabaceae are not hardwired, but instead, this family appears to have a high N plasticity, responding to site N availability and rhizobia colonization. Our results indeed show a large variability in foliar N concentration of Fabaceae specimens (Figure 3), suggesting high plasticity, as proposed by Wolf et al. (2017). The ability of specimens in this botanical family in assimilating nitrate efficiently *via* the nitrate reductase enzyme was also postulated (Smirnov and Stewart, 1985). On the other hand, foliar N concentration of non-fixers was also higher than that of non-Fabaceae, favoring the hypothesis that the Fabaceae family is indeed hardwired to have an N-demanding lifestyle.

If a higher N concentration is hardwired in Fabaceae, most of the variance of foliar N in this family would be explained by taxonomy. Therefore, the variance partitioning for foliar N had a comparable weight for taxonomy and site control, regardless of the Fabaceae group (fixers and non-fixers), with no dominant contribution from either of these factors on the variance (Figure 7 and Supplementary Table 2). However, it seems that site characteristics are driving potential associations with root symbionts, which likely increase even more the foliar N contents of fixers in these species (Figure 6). Further research on this topic is needed to mechanistically partition the effects of root symbionts.

Another topic less discussed is the fact that the foliar C:N ratio in fixers was also lower than in non-fixers, which in turn was lower than in non-Fabaceae (Figure 6). This is an ecologically key functional trait of Fabaceae in their role to regulate C accumulation mainly in tropical systems (Davidson et al., 2007; Siddique et al., 2008; Russell and Raich, 2012; Batterman et al., 2013), besides the fact that the LES links N and C acquisition by photosynthesis (Wright et al., 2004). Lower C:N ratios are also important in accelerating litter decomposition, therefore enhancing N cycling (Melillo et al., 1982). As expected, foliar  $\delta^{15}\text{N}$  in fixers was lower than in non-fixers (Figure 6), which can be attributed to the relatively low isotopic fractionation of BNF (Högberg, 1997). In addition, a large number of non-Fabaceae families presented lower values of foliar  $\delta^{15}\text{N}$  relative to Fabaceae (overall median; Figure 5), which could explain the similar results between non-Fabaceae and Fabaceae fixers (Figure 6).





**FIGURE 8 |** Box-plots of foliar C (**A**), N (**B**), and C:N ratio (**C**) according to wood density groups. Each dot represents the mean of a botanical genus. The horizontal lines inside each box represent the median; the box represents the first and third quartiles; and the bars represent the interquartile range. The horizontal dashed line represents the mean of the entire population. White circles represent estimate means obtained from the linear model. The summary of the statistical model can be seen in **Supplementary Table 3**. Levels followed by a common letter are not different according to the Sidak-test at  $p \leq 0.05$ .

## Could Wood Density Predict Foliar Properties?

Based on the LES, we expected that trees with low density would have a higher foliar N content than high-density trees (Wright et al., 2004; Chave et al., 2009). Therefore, in the successional

continuum of tropical forests, fast-growing, low-density trees would invest more on nutrients and less on structural C, while slow-growing, high-density trees would invest less on nutrients and more on structural C (Poorter et al., 2004; Wright et al., 2007). However, only foliar C concentration fully followed the

expected trend, where higher C was found in high-density trees (Figure 8). The fact that Wright et al. (2007) could not have found a correlation between wood density and specific leaf area, one of the pillars of the LES, and neither could we find a correlation between wood density and foliar N, reinforces the idea that a convergence between leaf and wood traits, if it exists, is more complex than we initially thought.

On the other hand, the increase of foliar C with wood density was expected since wood density is defined as the dry mass of wood per volume of fresh wood. The wood C concentration in tropical forests is larger in relation to other elements, but it is not very variable. For example, Elias and Potvin (2003) found a wood C variability of 45–50% among 32 species of tropical trees in Panama. Vieira et al. (2011) found the same range of variability in the coastal Atlantic Forest in southeast Brazil. As the variability in wood density is much higher than the wood C content, high-density trees tend to have higher C concentration than low-density trees simply because the latter has more mass packed per unit of volume than the former. This connection between wood C and density also explains why most of the variance of foliar C is explained by taxonomy (Figure 2), since most of the variance of wood density is highly connected with taxonomy (Chave et al., 2006, 2009).

Finally, we also expected that a correlation between wood density and foliar  $\delta^{13}\text{C}$  would arise, first because foliar  $\delta^{13}\text{C}$  is correlated with wood  $\delta^{13}\text{C}$  (Lins et al., 2016), and second because low-density, light-demanding trees have higher growth rates compared with high-density trees, shade-tolerant trees (Chave et al., 2009; Wright et al., 2010). Therefore, it is fair to suppose that shade-tolerant trees have lower water limitation related to light-demanding trees, which tend to be taller and more exposed to the sunlight (Bucci et al., 2004; Wright et al., 2010). Moreover, lower water limitation leads to more negative foliar  $\delta^{13}\text{C}$  values (Farquhar and Sharkey, 1982). Such light effect was clearly shown in a subtropical forest of China (Ehleringer et al., 1986) and forests along an Andes-to-Amazon elevation gradient (Martin et al., 2020). Although differences were not statistically significant, we observed an opposite trend, with foliar  $\delta^{13}\text{C}$  values of high-density trees, on average, less negative than low-density trees (data not shown). Therefore, our initial hypothesis was rejected. Perhaps because as concluded by Wright et al. (2003), there are few species at both ends of the light-demanding/shade-tolerant spectrum, but instead, most of the trees in a tropical forest stand have intermediate light requirements.

## CONCLUSION

In this paper, we tested how taxonomy and local effects modulate the variance of canopy foliar traits, including the two rainforests

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(Amazon and Atlantic) and the most iconic savanna of Brazil (Cerrado). Therefore, we extended variance partitioning of canopy foliar traits beyond the wet vegetation types of the tropics included across our sampling sites. Overall, we found that the variance of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was explained by different proportions of random variables rather than the elemental composition of C and N. The weight of sampling site in explaining the variance of C and N stable isotopes was more accentuated compared with the elemental composition. On the other hand, taxonomy seems to be more important for foliar C, N, and C:N ratio. This is especially true for the Fabaceae family, which independently of fixing  $\text{N}_2$  or not had higher N foliar concentration in comparison with other families. Even among Fabaceae, those genera capable of establishing symbiotic associations with fixing bacteria had higher N foliar concentration than non-fixing genera. However, there are still several open questions. For instance, there were a dozen families that had higher N foliar concentrations compared with Fabaceae. Is there also a type of taxonomic control in these families? The answer to this type of question would be important to simulate how families with low trait plasticity would respond to increases in temperature and changes in precipitation patterns in comparison with families with high trait plasticity. These findings would contribute to model future tropical vegetation and its biodiversity under a scenario of global changes, and the feedbacks of those changes on the climate and nutrient cycling.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

LAM, GBN, and PBC idealized the manuscript. SAV and NH worked on trees taxonomy. ALA-F, TFG, SRML, EM, and AS built the dataset and worked on statistical tests. LAM did the primary writing with the contribution of all co-authors. All authors contributed to the article and approved the submitted version.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2021.662801/full#supplementary-material>

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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