

# Isotopic composition of leaf carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) of deciduous and evergreen understory trees in two tropical Brazilian Atlantic forests

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**Abstract:** Isotopic composition of leaf carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) is determined by biotic and abiotic factors. In order to determine the influence of leaf habit and site on leaf  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the understory of two Atlantic forests in Brazil that differ in annual precipitation (1200 and 1900 mm), we measured these isotopes in the shaded understory of 38 tropical tree species (20 in the 1200-mm site and 18 in the 1900-mm site). Mean site values for  $\delta^{15}\text{N}$  were significantly lower at the 1200-mm site ( $-1.4\text{‰}$ ) compared with the 1900-mm site ( $+3.0\text{‰}$ ), and  $\delta^{13}\text{C}$  was significantly greater in the 1200-mm site ( $-30.4\text{‰}$ ) than in the 1900-mm site ( $-31.6\text{‰}$ ). Leaf C concentration was greater and leaf N concentration was lower at 1200-mm than at 1900-mm. Leaf  $\delta^{15}\text{N}$  was negatively correlated with  $\delta^{13}\text{C}$  across the two sites. Leaf  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of evergreen and deciduous species were not significantly different within a site. No significant phylogenetic signal for any traits among the study species was found. Overall, site differences were the main factor distinguishing traits among species, suggesting strong functional convergence to local climate and soils within each site for individuals in the shaded understory.

**Key Words:** leaf habit, leaf traits, N and C concentration, tropical rain forest, water use efficiency

## INTRODUCTION

Tropical forests are extremely diverse environments (Mollato & Haddad 2000, Putz *et al.* 2001, ter Steege *et al.* 2013) and the identification of functional groups facilitates understanding of more complex ecological processes (Powers & Tiffin 2010). Leaf  $\delta^{13}\text{C}$  can be used to organize plant functional groups because it reflects conditions during photosynthesis, with increasing discrimination against  $^{13}\text{C}$  as intercellular  $\text{CO}_2$  availability increases (Farquhar *et al.* 1982), leading to lower values of  $\delta^{13}\text{C}$ .

Leaf  $\delta^{13}\text{C}$  can also vary with leaf habit, morphology, genetics and irradiance (Dawson *et al.* 2002, Franco *et al.* 2005, Rossatto *et al.* 2013, Sobrado & Ehleringer 1997, Vitoria *et al.* 2016), which may reflect differences in photosynthetic water use efficiency (WUE).

In sites with high mean annual precipitation (MAP), lower  $\delta^{13}\text{C}$  values are observed (Cornwell *et al.* 2018, Leffler & Enquist 2002, Ma *et al.* 2012). Cornwell *et al.* (2018) showed that woody evergreen species have lower leaf  $\delta^{13}\text{C}$  than woody deciduous species when MAP is higher than 1000 mm, and an inverse pattern when MAP is lower than 1000 mm. In temperate zones, deciduous species and species with short leaf lifespans generally exhibit lower  $\delta^{13}\text{C}$  and presumably lower WUE than

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evergreen species and species with long leaf lifespans (Ehleringer & Cooper 1988, Marshall & Zhang 1994). However, the relationship between leaf  $\delta^{13}\text{C}$  and deciduousness has been somewhat controversial in the tropics, with reports of lower discrimination against  $^{13}\text{C}$  in deciduous compared with evergreen species in Venezuela (Sobrado & Ehleringer 1997), greater discrimination against  $^{13}\text{C}$  in deciduous compared with evergreen species in Brazil, and no difference in other studies (Franco *et al.* 2005, Leffler & Enquist 2002, Powers & Tiffin 2010). Variation of  $\delta^{15}\text{N}$  in plants and soil can reflect temporal and spatial variation in N sources, soil N availability and N acquisition from alternative sources such as biological  $\text{N}_2$ -fixation, mycorrhizal associations and atmospheric deposition (Bai *et al.* 2009, Bustamante *et al.* 2004, Dawson *et al.* 2002, Ometto *et al.* 2006, Powers & Tiffin 2010). Values of  $\delta^{15}\text{N}$  in soil and plants systematically decrease with the increasing of MAP due to variation in the openness of the N cycle until the point of soil waterlogging (Amundson *et al.* 2003, Austin & Vitousek 1998, Handley *et al.* 1999, Nardoto *et al.* 2008, Santiago *et al.* 2004, Schuur & Matson 2001). Differences in the frequency of leaf N re-translocation with leaf lifespan have been suggested to also influence  $\delta^{15}\text{N}$  values of tropical trees, where shorter leaf lifespans re-translocate leaf N more frequently than evergreen species (Santiago *et al.* 2004) and  $\delta^{15}\text{N}$  becomes enriched during re-assimilation of nitrate and leaf N re-metabolism (Evans 2001).

We determined leaf  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in evergreen and deciduous species in the understorey from two tropical Atlantic forests in Brazil that differ in MAP: 1200 mm and 1900 mm in order to determine the influence of leaf habit and site on leaf  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . We hypothesized that in understorey leaves: (1) deciduous species have traits associated with maximizing C gain with higher leaf N and lower  $\delta^{13}\text{C}$  than evergreen species; (2) evergreen species show lower  $\delta^{15}\text{N}$  than deciduous species; and (3) values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are greater at the drier site.

## METHODS

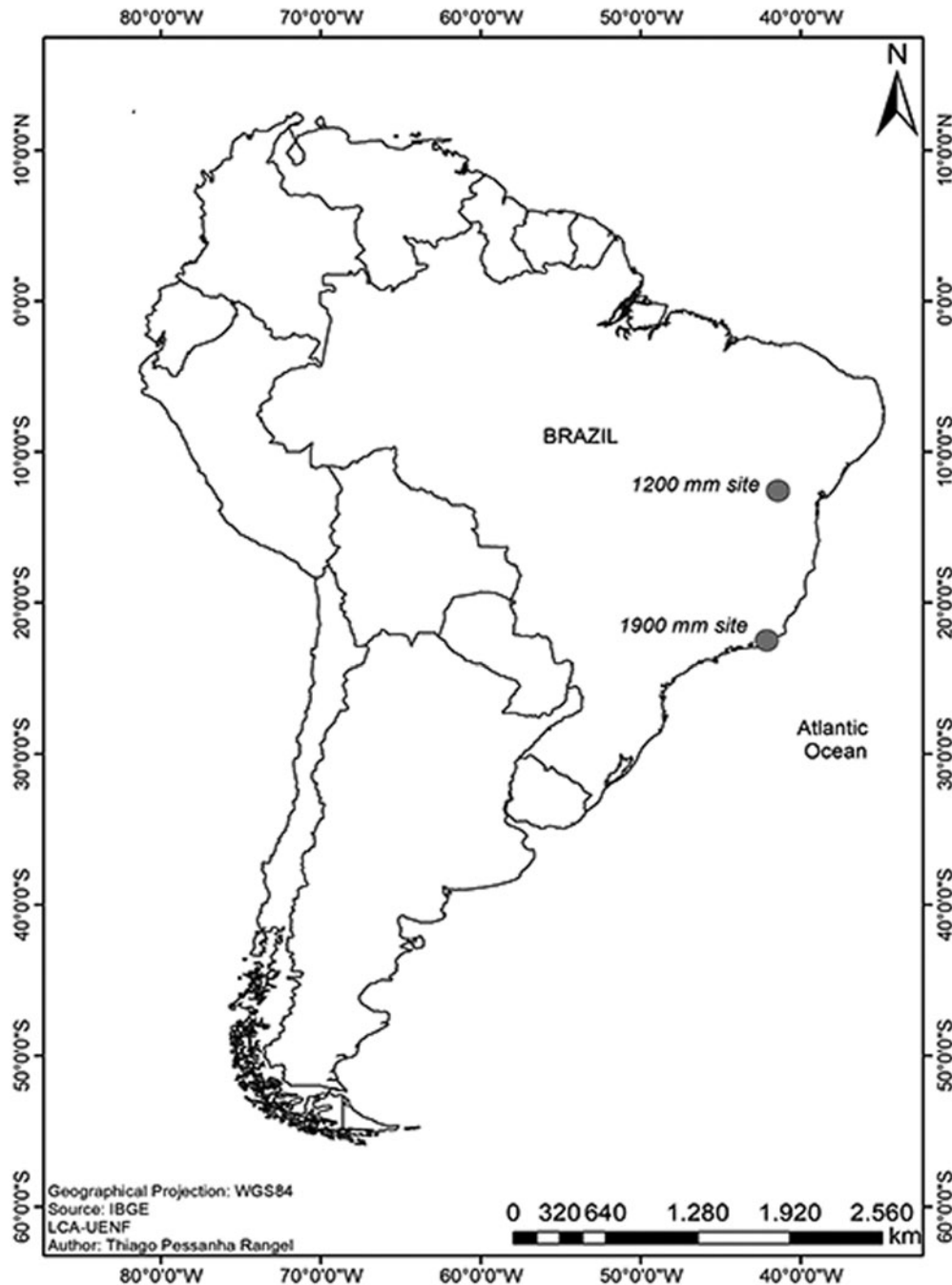
### Study sites and species

This study was carried out in two evergreen tropical Atlantic forests in Brazil (IBGE 2012): one in Bahia state that receives 1200 mm of annual precipitation and one in Rio de Janeiro state that receives 1900 mm  $\text{y}^{-1}$  (Figure 1). The 1200-mm site is located within a semi-arid region of Brazil in Chapada Diamantina National Park (CDNP), north-eastern Brazil ( $12^\circ 28'\text{S}$ ,  $41^\circ 23'\text{W}$ ). However, due to moist air masses, altitude, orography and climatic conditions, it shows floristic similarities to the humid forest along the Brazilian coast (Funch *et al.*

2008). The vegetation of the 1200-mm site is classified as evergreen seasonal forest (Richards 1998) and the soil is yellowish-red latosol with sandy clay loam texture (Funch *et al.*, 2008). The topography is undulating and the altitude ranges between 400 and 600 m asl, which confers a mesothermal climate to this site (*Cwb*) (Kottek *et al.* 2006). Mean temperature oscillates around  $18^\circ\text{C}$  in April–September and exceeds  $22^\circ\text{C}$  in the hotter months of October–February (Funch *et al.* 2002). The 1200-mm site has clouds for much of the year, and the rainy season occurs between December–April, with a peak between March–April, whereas the dry season varies between 5–6 mo occurring between May–October.

The 1900-mm site is located in south-eastern Brazil, in the União Biological Reserve ( $22^\circ 27'\text{S}$ ,  $42^\circ 02'\text{W}$ ). This site has a gently undulating topography ranging from sea level to 370 m asl. The vegetation of the 1900-mm site is classified as lowland wet forest (Braga *et al.* 2016). The soil is dystrophic red-yellow podzolic with sandy clay texture (Lima *et al.* 2011). Climate is tropical humid (*Aw*) (Kottek *et al.* 2006), with an average annual temperature of  $25^\circ\text{C}$ , and about 85% of precipitation occurring between October–April. Although precipitation does occur throughout the year, there are 3–4 mo of dry season.

Thirty-eight species of 18 families were sampled: 20 at the 1200-mm site and 18 at the 1900-mm site. Species were chosen according to previous information about leaf habit. The classification scheme for leaf habit of the 38 species is based on Frankie *et al.* (1974) and included evergreen, evergreen with discontinuous production, deciduous and semi-deciduous species. For data presentation and analyses, evergreen species and species evergreen with discontinuous production were grouped under the category evergreen, whereas deciduous and semi-deciduous species were considered deciduous. For each species, five adult individuals 10–15 m tall with diameter at breast height of 10–30 cm had leaves at heights of 2.5–5.0 m sampled, except for the following species at the 1900-mm site: *Ficus gomelleira* ( $n = 4$ ), *Cupania racemosa* ( $n = 4$ ), *Brosimum glazioui* ( $n = 4$ ), *Virola gardneri* ( $n = 2$ ), *Micropholis guianensis* ( $n = 2$ ), *Ocotea diospyrifolia* ( $n = 2$ ). Photosynthetically active leaves of the second or third pair, under shade conditions ( $< 40 \mu\text{mol m}^{-2} \text{s}^{-1}$  in both sites) were collected. We opted to collect samples in the shade conditions due to the differences in the cloud cover between forests, and consequently irradiance differences in the canopy leaves, since irradiance has an influence on the  $\delta^{13}\text{C}$  (Vitoria *et al.* 2016). Our leaf isotopic composition data can therefore be considered as conservative in terms of emphasizing differences in climate between the two sites. Leaves were collected between 2011–2013 at the 1200-mm site and in 2015 at the 1900-mm site. No substantial variation in MAP in these areas was observed in these years.



**Figure 1.** Map of the studied areas. Geographic map indicating the two tropical forests in Brazil that differ in mean annual precipitation: the 1200-mm site is in Bahia state, located in Chapada Diamantina National Park, and the 1900-mm site is in Rio de Janeiro state, located in the União Biological Reserve.

### C and N analyses

Leaves were dried at 60°C for at least 2 d and ground to a fine powder before analysis. Samples of around 1 mg were combusted on a continuous flow elemental analyser (Flash 2000 Organic Elemental Analyser), which measured elemental concentrations of C and N, coupled to a stable isotope ratio mass spectrometer (IRMS Delta

V Advantage, Thermo Scientific, Germany), which measured isotopic composition of C and N. Pee Dee Belemnite (PDB) and atmospheric N were used as standard values for C and N analyses, respectively. The analytical precision was  $\pm 0.1\%$  for  $\delta^{13}\text{C}$  and  $\pm 0.2\%$  for  $\delta^{15}\text{N}$  and the accuracy for elemental and isotopic compositions were determined by certified standard (Protein OAS/Isotope Cert 114859; Elemental Microanalysis).

**Table 1.** Mean ( $\pm$  SD) of C and N concentrations (%), C/N ratio,  $\delta^{13}\text{C}$  (‰), and  $\delta^{15}\text{N}$  (‰) of evergreen and deciduous species from two tropical forests in Brazil that differ in mean annual precipitation (MAP), 1200-mm in Bahia state and 1900-mm in Rio de Janeiro state. Capital letters compare between sites (same leaf habit), and lowercase letters compare between leaf habit (same site). Factorial ANOVA.  $P \leq 0.05$ . Mean = mean of all species (evergreen + deciduous) in the same site.

MAP (mm)	Leaf habit	C (%)	$\delta^{13}\text{C}$ (‰)	N (%)	$\delta^{15}\text{N}$ (‰)	C/N ratio
1200	Evergreen	44.4 $\pm$ 2.5 Aa	-30.5 $\pm$ 1.5 Aa	1.6 $\pm$ 0.3 Ba	-1.4 $\pm$ 1.1 Ba	28.3 $\pm$ 5.9 Aa
1200	Deciduous	44.0 $\pm$ 2.0 Aa	-30.0 $\pm$ 1.8 Aa	1.8 $\pm$ 0.6 Ba	-1.6 $\pm$ 1.4 Ba	26.9 $\pm$ 7.9 Aa
1200	Mean	44.3 $\pm$ 2.4 A	-30.4 $\pm$ 1.6 A	1.7 $\pm$ 0.4 B	-1.4 $\pm$ 1.2 B	27.9 $\pm$ 6.5 A
1900	Evergreen	42.5 $\pm$ 2.2 Ba	-31.6 $\pm$ 1.5 Ba	2.1 $\pm$ 0.5 Aa	3.0 $\pm$ 2.2 Aa	21.0 $\pm$ 4.2 Ba
1900	Deciduous	40.4 $\pm$ 4.0 Bb	-31.5 $\pm$ 1.0 Ba	2.3 $\pm$ 0.4 Aa	2.9 $\pm$ 2.0 Aa	17.8 $\pm$ 2.6 Ba
1900	Mean	42.0 $\pm$ 2.9 B	-31.6 $\pm$ 1.3 B	2.2 $\pm$ 0.4 A	3.0 $\pm$ 4.0 A	20.2 $\pm$ 4.0 B

## Data analyses

Comparisons of parameters between evergreen and deciduous leaf habit and the 1200-mm and 1900-mm sites were performed using factorial ANOVA (Statistica 6.0) followed by Tukey's test ( $P \leq 0.05$ ). Regression and correlation coefficients were calculated with the software package Sigma Plot 11.0 (SPSS; Chicago, IL, USA).

Non-metric multidimensional scaling (nMDS) was used to explore the spatial distribution of species characterized by leaf habit and site and potential grouping with leaf C, leaf N, leaf  $\delta^{13}\text{C}$ , leaf  $\delta^{15}\text{N}$  and leaf C/N ratio for the 38 study species. Traits were first normalized and then used to compute pairwise Euclidean distances among plant species. Significant differences ( $P \leq 0.05$ ) between leaf habit or site were assessed with analysis of similarity (ANOSIM, Clarke & Gorley 2006). Analysis of similar percentages (SIMPER) was used to identify which leaf traits contributed most to the differentiation among defined groups. All multivariate analyses were performed using Primer v6.0 (Clarke & Gorley 2006).

In order to detect phylogenetic effects, phylogenetic independent contrasts (PIC) were performed using the 'Ape' package in R. Analysis for phylogenetic signal of each leaf trait was performed with the 'Picante' package in R (Kembel *et al.* 2010). PICs were calculated for each leaf trait and correlations on these contrasts were performed and compared to Pearson's correlation coefficients on the raw data. To build a trait-based phenogram, a distance matrix based on the values of leaf C concentration,  $\delta^{13}\text{C}$ , N concentration,  $\delta^{15}\text{N}$  and C/N ratio was computed among species. The leaf traits were first normalized and then used to compute pairwise Euclidean distances among plant species with Primer v6.0 (Clarke & Gorley 2006). The matrix file was edited and imported into PAUP\*4.0a146 (Swofford 2002) to build a trait phenogram using the Neighbour joining method. The trait-based phenogram was refined using Adobe Illustrator v. 16.0.4. Finally, the trait-based phenogram was contrasted against the molecular-based phylogeny of the same plant species. The phylogenetic tree was obtained using Phylomatic

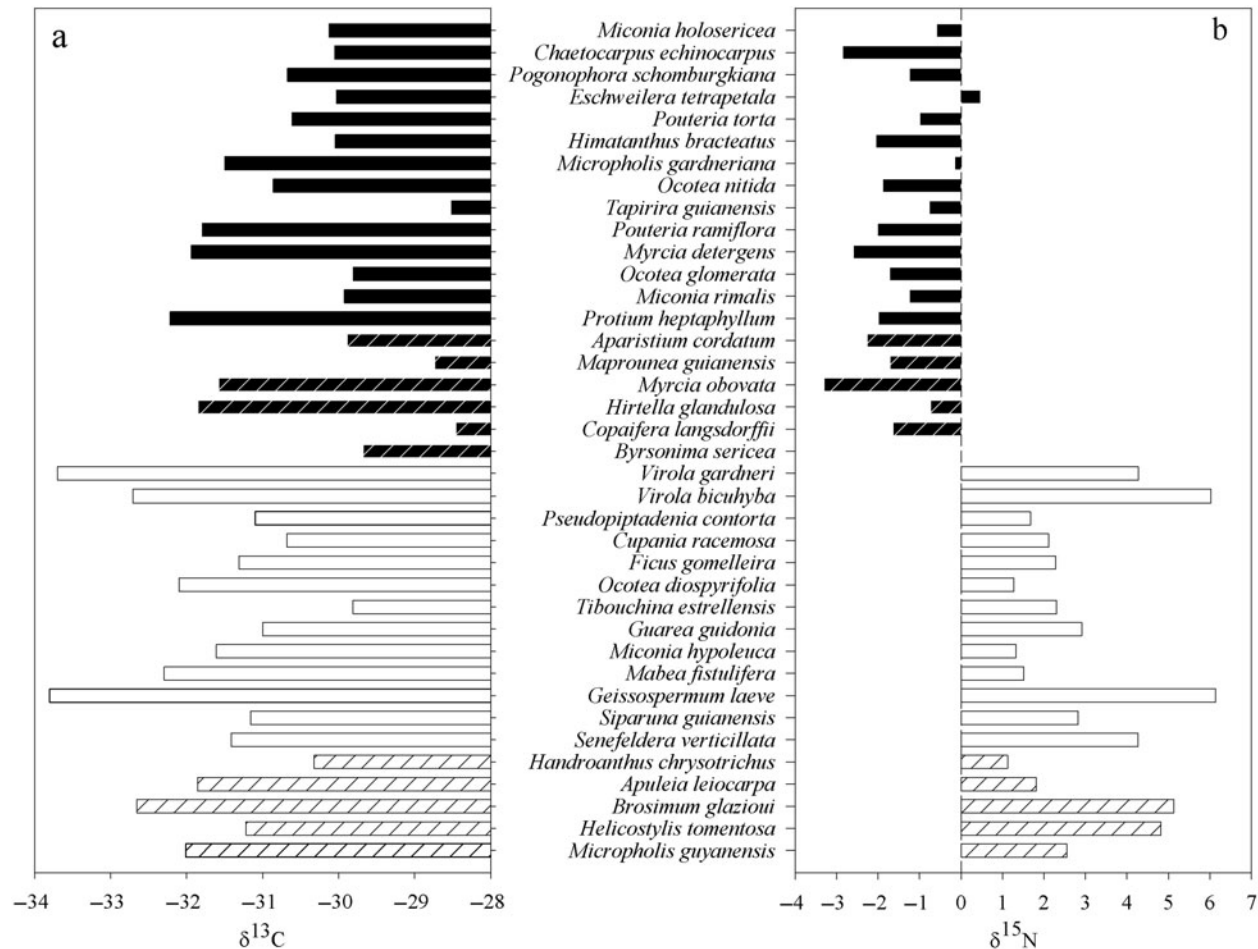
v3 (<http://www.phylodiversity.net/phyloomatic>) and the stored megatree by Zanne *et al.* (2014).

## RESULTS

No significant differences for mean values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between deciduous and evergreen species were observed (Table 1). Leaf  $\delta^{13}\text{C}$  ranged from -28.4‰ in *Copaifera langsdorffii*, a deciduous species from the 1200-mm site, to -33.8‰ in *Geissospermum laeve*, an evergreen species from the 1900-mm site (Figure 2). *Geissospermum laeve* also presented the highest leaf  $\delta^{15}\text{N}$  (+6.1‰), whereas the lowest leaf  $\delta^{15}\text{N}$  was -3.3‰ in *Myrcia obovata*, a deciduous species from the 1200-mm site. Significant differences for leaf C concentration between deciduous and evergreen species were observed only at the 1900-mm site, with higher values in evergreen than in deciduous species (Table 1 and 2). Higher values of leaf C, leaf C/N ratio and leaf  $\delta^{13}\text{C}$  were found at the 1200-mm site, whereas higher leaf  $\delta^{15}\text{N}$  and N were found at the 1900-mm site, regardless of the leaf habit (Tables 1 and 2). There were no significant differences in  $\delta^{15}\text{N}$  between potentially  $\text{N}_2$ -fixing leguminous species (*Apuleia leiocarpa* +1.8‰, *Copaifera langsdorffii* -1.6‰ and *Pseudopiptadenia contorta* +1.6‰) and species from other families. Rather, leaf  $\delta^{15}\text{N}$  values were strongly influenced by site (Figure 2). No statistical differences in N concentration between leguminous species and species from other families were found.

The nMDS analysis identified two major clusters structured by site with the 1200-mm site differing significantly from the 1900-mm site ( $P = 0.001$ ). No grouping based on leaf habit was observed (Figure 3, Table 3). The two sites were mostly differentiated by leaf  $\delta^{15}\text{N}$  (25.3%), although the contribution of leaf C/N ratio (19.9%), leaf N concentration (18.5%), leaf  $\delta^{13}\text{C}$  (18.3%) and leaf C concentration (18.0%) were also important.

A bi-plot between leaf  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  showed negative correlation ( $P = 0.0003$ , Figure 4) with 1900-mm samples distributed in a broader range of  $\delta^{15}\text{N}$  values. However, no correlation was observed between leaf  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from the 1200-mm site, whereas leaf  $\delta^{13}\text{C}$  and



**Figure 2.** Leaf C and N isotopic composition of 38 tree species from two tropical forests in Brazil that differ in mean annual precipitation: 1200-mm site (black bars) and 1900-mm site (white bars). Leaf C ( $\delta^{13}\text{C}$ ) (a) and leaf N ( $\delta^{15}\text{N}$ ) (b) isotopic composition. Evergreen (unhatched) and deciduous (hatched) species are differentiated within each site.

$\delta^{15}\text{N}$  from 1900-mm site showed significant correlation ( $r = -0.59$ ,  $P = 0.0099$ ).

Correlations between leaf traits revealed that in the 1200-mm site, species with lower C/N ratio also had higher  $\delta^{13}\text{C}$  and N concentration, and species with lower C concentration also had lower  $\delta^{15}\text{N}$  (Table 4). Correlations between leaf traits in the 1900-mm site species showed: (1) weak, but significant differences between deciduous and evergreen for C/N ratio; (2) species with higher  $\delta^{13}\text{C}$  also had lower  $\delta^{15}\text{N}$ ; and (3) species with lower C/N ratio had higher N concentration as also shown by the species in the 1200-mm site (Table 4). In general, these correlations were robust compared with PIC analysis, except for the relationship between  $\delta^{13}\text{C}$  and C/N ratio in the species from the 1200-mm site, which became non-significant in PIC analysis, and the relationships between  $\delta^{15}\text{N}$  and N concentration and between  $\delta^{15}\text{N}$  and C/N ratio, which became significant in species from the 1900-mm site (Table 4). However, when the more conservative method Bonferroni was applied,

only the negative correlation between C/N ratio and N in the 1900-mm site remained (for Pearson's correlation ( $r$ ) and PIC). The trait-based phenogram, suggested no phylogenetic effect on the grouping pattern according to the data, and clearly reinforced the influence of site on leaf traits (Appendix 1).

## DISCUSSION

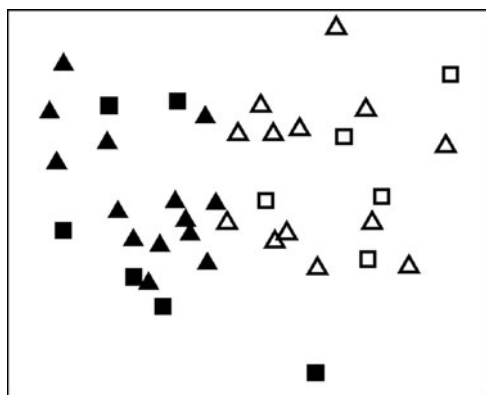
Our data show that in shaded leaves, there were no consistent differences between species based on leaf habit. Within sites, only leaf C varied with leaf habit at the 1900-mm site. Across all traits, nMDS showed two major clusters, highly structured by site. We also anticipated large differences between sites based on differences in mean annual precipitation, with the wetter site expected to show relatively low  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Our data supported the pattern that leaf  $\delta^{13}\text{C}$  values were significantly lower at the wetter site, but did not support the pattern of lower

**Table 2.** Species, plant family, leaf habit (EG: evergreen, EG-DP: evergreen with discontinuous production, DC: deciduous, SM-DC: semi-deciduous), mean annual precipitation (MAP) and concentration of leaf C (C; %), N (N; %) and C/N ratio from two tropical forests in Brazil that differ in MAP: 1200-mm in Bahia state, and 1900-mm in Rio de Janeiro state.

Family/Species	Leaf habit	MAP	C (%)	N (%)	C/N
<b>Anacardiaceae</b>					
<i>Tapirira guianensis</i> Aubl.	EG -DP	1200	43.2 ± 0.4	1.6 ± 0.0	26.2 ± 0.9
<b>Apocynaceae</b>					
<i>Geissospermum laeve</i> (Vell.) Miers.	EG	1900	43.9 ± 2.0	2.4 ± 0.1	18.4 ± 0.5
<i>Himatanthus bracteatus</i> (A. DC.) Woodson	EG	1200	44.2 ± 3.9	2.1 ± 0.1	21.4 ± 2.4
<b>Bignoniaceae</b>					
<i>Handroanthus chrysotrichus</i> (Mart. ex DC.) Mattos.	DC	1900	41.4 ± 5.8	2.0 ± 0.4	20.5 ± 2.0
<b>Burseraceae</b>					
<i>Protium heptaphyllum</i> (Aubl.) Marchand	EG	1200	41.7 ± 0.6	1.7 ± 0.1	25.0 ± 1.9
<b>Chrysobalanaceae</b>					
<i>Hirtella glandulosa</i> Spreng.	SM-DC	1200	41.4 ± 0.3	1.4 ± 0.2	29.9 ± 5.1
<b>Euphorbiaceae</b>					
<i>Aparisthium cordatum</i> (A. Juss.) Baill.	SM-DC	1200	43.5 ± 1.7	3.0 ± 0.1	14.4 ± 0.4
<i>Chaetocarpus echinocarpus</i> (Baill.) Ducke	EG -DP	1200	41.0 ± 3.7	1.1 ± 0.1	37.7 ± 2.4
<i>Mabea fistulifera</i> Mart.	EG -DP	1900	41.7 ± 0.4	1.8 ± 0.2	23.2 ± 3.0
<i>Maprounea guianensis</i> Aubl.	SM-DC	1200	44.7 ± 0.6	1.7 ± 0.4	26.7 ± 4.9
<i>Pogonophora schomburgkiana</i> Miers ex Benth.	EG -DP	1200	45.4 ± 0.8	1.9 ± 0.1	23.9 ± 1.6
<i>Senefeldera verticillata</i> (Vell.) Croizat	EG -DP	1900	41.3 ± 1.4	1.8 ± 0.2	23.4 ± 2.6
<b>Fabaceae</b>					
<i>Apuleia leiocarpa</i> (Vogel) J.F. Macbr.	DC	1900	39.1 ± 3.0	2.1 ± 0.4	18.5 ± 3.0
<i>Copaifera langsdorffii</i> Desf.	SM-DC	1200	44.4 ± 0.9	1.9 ± 0.2	23.2 ± 2.6
<i>Pseudopiptadenia contorta</i> (DC.) G.P. Lewis & M.P.	EG -DP	1900	44.3 ± 1.8	2.2 ± 0.1	19.6 ± 1.1
<b>Lima</b>					
<b>Lauraceae</b>					
<i>Ocotea diospyrifolia</i> (Meisn.) Mez	EG	1900	45.7 ± 0.4	2.5 ± 0.4	18.8 ± 3.4
<i>Ocotea glomerata</i> (Nees) Mez	EG	1200	45.3 ± 0.9	1.8 ± 0.2	25.4 ± 2.5
<i>Ocotea nitida</i> (Meisn.) Rohwer	EG -DP	1200	44.8 ± 0.4	1.8 ± 0.1	24.2 ± 1.6
<b>Lecythidaceae</b>					
<i>Eschweilera tetrapetala</i> Mori	EG -DP	1200	47.0 ± 0.6	1.7 ± 0.2	27.6 ± 4.0
<b>Malpighiaceae</b>					
<i>Byrsonima sericea</i> DC	DC	1200	47.1 ± 1.7	1.4 ± 0.3	34.3 ± 6.9
<b>Melastomataceae</b>					
<i>Miconia holosericea</i> (L.) DC.	EG -DP	1200	42.4 ± 3.7	1.8 ± 0.2	23.0 ± 0.7
<i>Miconia hypoleuca</i> (Benth.) Triana	EG -DP	1900	41.5 ± 1.7	1.6 ± 0.2	25.8 ± 3.5
<i>Miconia rimalis</i> Naudin.	EG -DP	1200	42.9 ± 0.6	1.7 ± 0.2	25.9 ± 3.0
<i>Tibouchina estrellensis</i> (Raddi) Cogn.	EG -DP	1900	43.8 ± 1.2	1.8 ± 0.1	24.3 ± 1.8
<b>Meliaceae</b>					
<i>Guarea guidonia</i> (L.) Sleumer	EG -DP	1900	42.0 ± 1.6	3.0 ± 0.4	14.2 ± 2.4
<b>Moraceae</b>					
<i>Brosimum glazioui</i> Taub.	SM-DC	1900	37.2 ± 15.7	2.4 ± 0.9	15.7 ± 5.8
<i>Ficus gomelleira</i> Kunth & C.D. Bouché	EG	1900	40.0 ± 3.5	1.6 ± 0.2	25.1 ± 3.1
<i>Helicostylis tomentosa</i> (Poepp. & Endl.) Rusby	SM-DC	1900	41.5 ± 1.0	2.5 ± 0.2	16.8 ± 1.3
<b>Myristicaceae</b>					
<i>Virola bicuhyba</i> (Schott ex Spreng.) Warb.	EG	1900	43.1 ± 1.1	2.0 ± 0.5	21.7 ± 1.3
<i>Virola gardneri</i> (A. DC.) Warb.	EG	1900	39.6 ± 4.0	1.6 ± 0.5	24.7 ± 4.5
<b>Myrtaceae</b>					
<i>Myrcia detergens</i> Miq.	EG	1200	44.9 ± 0.8	1.2 ± 0.1	37.7 ± 2.8
<i>Myrcia obovata</i> (Berg) Niedenzu	SM-DC	1200	42.7 ± 0.7	1.3 ± 0.1	33.0 ± 3.5
<b>Sapindaceae</b>					
<i>Cupania racemosa</i> (Vell.) Radlk.	EG	1900	44.7 ± 1.8	2.2 ± 0.2	20.3 ± 1.1
<b>Sapotaceae</b>					
<i>Micropholis gardneriana</i> (DC.) Pierre	EG -DP	1200	45.1 ± 1.2	1.3 ± 0.2	34.1 ± 5.3
<i>Micropholis guyanensis</i> (A. DC.) Pierre	SM-DC	1900	44.5 ± 1.1	2.7 ± 0.3	16.3 ± 1.7
<i>Pouteria ramiflora</i> (DC.) Pierre	EG	1200	47.1 ± 0.8	1.4 ± 0.1	34.1 ± 2.8
<i>Pouteria torta</i> (DC.) Pierre	EG -DP	1200	46.8 ± 1.3	1.6 ± 0.2	29.4 ± 3.6
<b>Siparunaceae</b>					
<i>Siparuna guianensis</i> Aubl.	EG -DP	1900	41.4 ± 0.8	2.6 ± 0.3	15.9 ± 2.0

**Table 3.** R values and significance levels (P) from the analysis of similarity (ANOSIM) for the different comparison tests assessing differences in leaf traits, C and N concentrations, C and N isotopic composition, and C/N ratio between two tropical forests in Brazil that differ in mean annual precipitation (1200 mm and 1900 mm). \* Significance:  $P \leq 0.05$ .

Comparisons	R Statistic	Significance level
Site		
1200 mm vs. 1900 mm	0.576	0.001*
Leaf habit		
Evergreen vs. deciduous	0.098	0.105
Site and Leaf habit		
1200 mm evergreen vs. 1200 mm deciduous	0.117	0.156
1200 mm evergreen vs. 1900 mm evergreen	0.543	0.001*
1200 mm deciduous vs. 1900 mm evergreen	0.593	0.001*
1900 mm evergreen vs. 1900 mm deciduous	0.033	0.379



**Figure 3.** Non-metric multidimensional scaling (nMDS) analysis considering mean of all leaf traits, C and N concentrations, C and N isotopic composition, and C/N ratio (2D Stress: 0.13), from two tropical forests in Brazil that differ in mean annual precipitation. The 1200-mm site and the 1900-mm site are represented by closed and open symbols, respectively. Leaf habits (evergreen: triangles, deciduous: squares) are also given for each species.

$\delta^{15}\text{N}$  at the wetter site, suggesting that factors besides openness of the N cycle were important in differentiating N cycling processes between these two forests (Evans 2001). Overall, site differences were the main factor distinguishing traits among species, suggesting strong functional convergence to local climate and soils among shade leaves within each site. In addition, we suggest that higher leaf N concentration, and associated higher leaf  $\delta^{15}\text{N}$ , in the 1900-mm site than in the 1200-mm site allows trees to increase photosynthesis, leading to lower  $\delta^{13}\text{C}$  in the 1900-mm site (Figure 4).

One of the most notable findings of this study was the muted difference in leaf isotopic composition and elemental concentrations between evergreen and de-

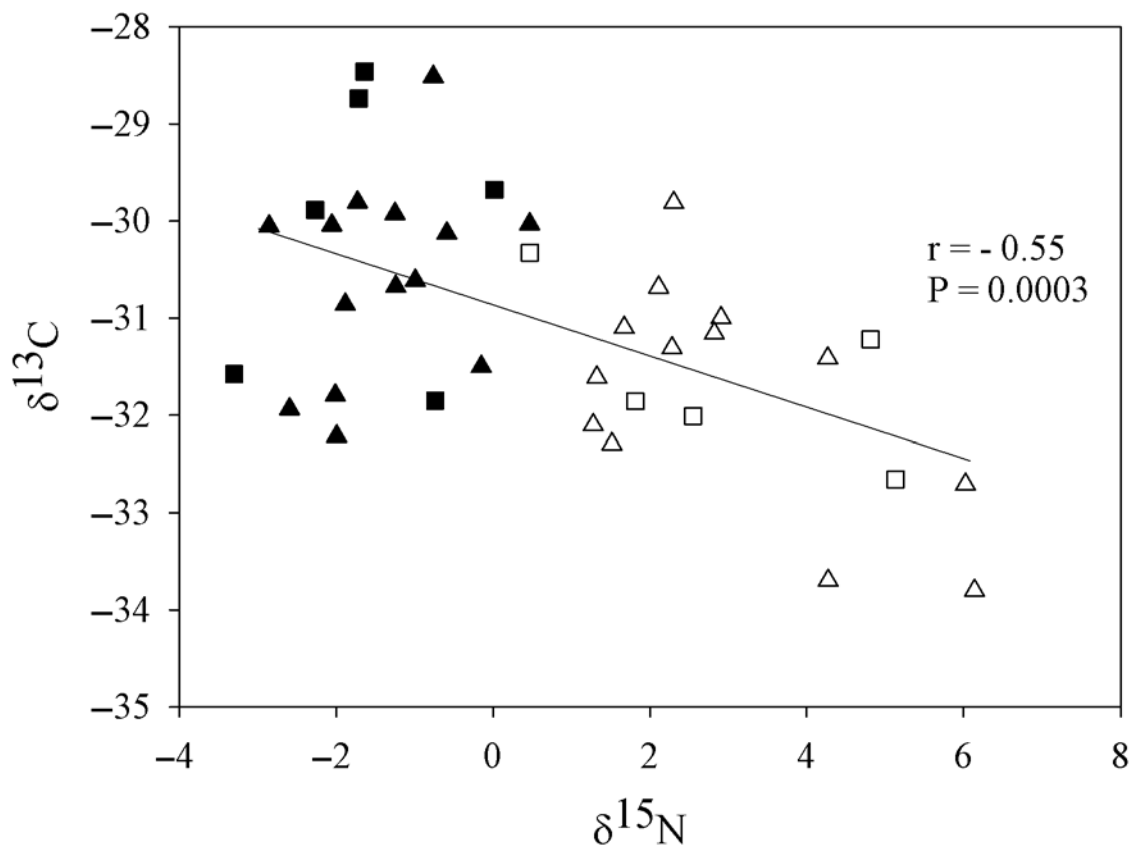
ciduous species. The lack of differences in  $\delta^{13}\text{C}$  values between coexisting deciduous and evergreen species in both forests indicates that over the long term, these species maintained similar ranges of internal  $\text{CO}_2$  concentration during photosynthesis (Farquhar & Richards 1984, Farquhar *et al.* 1982, 1989). This finding runs counter to a number of studies showing that although dry-season-deciduous tropical tree species do not gain C through leaf photosynthesis during the unfavourable dry season, they often have greater maximum rates of photosynthesis than evergreen species during the growing season (Chabot & Hicks 1982, Eamus & Prior 2001, Santiago *et al.* 2004), potentially leading to differences in leaf  $\delta^{13}\text{C}$ . Deciduous species are generally considered less conservative in the use of water than evergreen species (Lloyd & Farquhar 1994), as they appear to maximize photosynthetic C gain during a shorter season than evergreen species. Our study on shaded leaves represents a conservative assay and does not exclude the possibility that differences in evergreen and deciduous leaf function are manifested under high light conditions. For example, in dry tropical ecosystems, greater  $\delta^{13}\text{C}$  in deciduous than evergreen species has been shown and related to maximizing C gain in deciduous species through higher concentrations of leaf N and P and higher photosynthetic rates than in evergreen species (Franco *et al.* 2005, Hasselquist *et al.* 2010a, Sobrado & Ehleringer 1997). For a given stomatal aperture, greater rates of photosynthesis results in lower leaf internal  $\text{CO}_2$  concentration, leading to relatively more incorporation of  $^{13}\text{CO}_2$  (Farquhar & Richards 1984). Yet, Leffler & Enquist (2002) showed no significant differences in  $\delta^{13}\text{C}$  between evergreen and deciduous tropical tree species in Guanacaste, Costa Rica, similar to our findings. Consistent with our lack of difference in  $\delta^{13}\text{C}$  between deciduous and evergreen species, we also found no differences in leaf N concentration between evergreen and deciduous species, suggesting similar photosynthetic capacity across these groups.

However, it is important to highlight that our results were obtained from shaded leaves in the understorey due to constant cloudiness in the 1200-mm site, whereas most of the literature data is from sun leaves. It is widely known that  $\delta^{13}\text{C}$  varies between sun and shade leaves, with shade leaves being  $^{13}\text{C}$ -depleted (Leffler & Enquist 2002, Medina *et al.* 1991, Xiao *et al.* 2013). Thus, it is expected that canopy leaves are  $^{13}\text{C}$ -enriched in comparison with understorey leaves and the possibility that differences between deciduous and evergreen species could still be evident in canopy leaves can not be ruled out, although no differences due to leaf habit were described in  $\delta^{13}\text{C}$  for canopy leaves in wet forest (Leffler & Enquist 2002).

In general, deciduous species compensate for the shorter leaf payback period with an increase in the

**Table 4.** Correlation coefficients ( $r$ ) for leaf traits of tropical tree species from two tropical forests in Brazil that differ in mean annual precipitation (MAP), 1200-mm in Bahia state and 1900-mm in Rio de Janeiro state. Cross-species  $r$  in the upper right section of the matrix ( $n = 20$  for 1200-mm site;  $n = 18$  for 1900-mm site). Phylogenetic independent contrast in lower left section of the matrix ( $n = 19$  for 1200 mm site;  $n = 17$  for 1900-mm site). Significance: \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ .

	Leaf habit	C	$\delta^{13}\text{C}$	N	$\delta^{15}\text{N}$	C/N ratio
1200 mm						
Leaf habit	1	0.051	0.093	0.067	-0.093	-0.276
C	-0.192	1	0.093	0.019	0.388*	0.122
$\delta^{13}\text{C}$	0.120	0.193	1	0.375	0.216	-0.402*
N	-0.067	-0.068	0.212	1	-0.008	-0.933***
$\delta^{15}\text{N}$	-0.161	0.389*	0.278	-0.006	1	-0.050
C/N ratio	-0.204	0.199	-0.247	-0.928***	-0.053	1
1900 mm						
Leaf habit	1	0.374	-0.055	-0.291	0.010	0.456*
C	0.325	1	0.193	0.269	-0.181	-0.044
$\delta^{13}\text{C}$	-0.031	0.295	1	0.040	-0.590***	0.025
N	-0.383	0.231	-0.050	1	0.112	-0.958***
$\delta^{15}\text{N}$	-0.053	0.064	-0.428*	0.419*	1	-0.194
C/N ratio	0.532**	-0.037	0.103	-0.964***	-0.457*	1



**Figure 4.** Correlation between leaf  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for two tropical forests in Brazil that differ in mean annual precipitation. The 1200-mm site and the 1900-mm site are represented by closed and open symbols, respectively. Leaf habits (evergreen: triangles, deciduous: squares) are also given for mean of each species.



potential payback capacity or maximum photosynthetic rate (Kikuzawa 1991, Pringle *et al.* 2011). However, no significant correlation between  $\delta^{13}\text{C}$  and N were found for deciduous species in either site. It is likely that direct measurements of photosynthetic gas exchange or RUBISCO activity are needed to fully evaluate these relationships. It is also necessary to consider that understorey leaves are not at maximum photosynthetic capacity due to irradiance limitations and that RUBISCO activity may not be sufficient to assimilate all the C available in the substomatal cavity, as occurs in the sun leaves of deciduous species, which leads to lower  $^{13}\text{C}$  discrimination.

When analysing all leaf traits together, no significant differences between deciduous and evergreen were found, even in the 1200-mm site, suggesting that differences in water scarcity between the two sites is not felt in understorey leaves to the same degree as canopy leaves in other studies (Franco *et al.* 2005, Hasselquist *et al.* 2010a, Sobrado & Ehleringer 1997). Despite the lower MAP, the presence of clouds could also modulate water loss from the 1200-mm site, leading to more similar water deficits than expected based on MAP alone.

Deciduousness is more evident in dry than wet tropical forests (Borchert 1998). In addition, more variability in growth and photosynthetic gas exchange has been described in trees in dry sites than in wet sites (Ruzicka *et al.* 2017). Thus, both sites showed a greater degree of functional convergence than expected, which has been described under low-disturbance conditions (ter Steege & Hammond 2001), as well as a function of limited resources including light (Valladares *et al.* 2002) and phosphorus (Fonseca *et al.* 2000). However, when water is the limited resource, physiological trade-offs become extreme, resulting in greater divergence between strategies that on one hand favour withstanding the long dry season as evergreen trees, or on the other hand, reducing costs due to transpiration and respiratory C losses as deciduous trees (Santiago *et al.* 2004). The lack of difference between evergreen and deciduous species in the understorey of these sites reinforces the idea that water is not the only limiting factor for these forests. Although the question of variable functional convergence along precipitation gradients has not been addressed much in the literature, there is some evidence based on the fact that differences in leaf traits of lianas and trees are greatest in seasonally-dry tropical forests and converge above around 2500 mm (Asner & Martin 2012), suggesting that reduced seasonal variability of water availability leads to a loss of an advantage for some drought resistance strategies and promotes functional convergence as annual precipitation increases.

A difference in leaf  $\delta^{15}\text{N}$  of approximately 4.4‰ was found between the 1200-mm and 1900-mm sites, showing a strong influence of the environment on this leaf trait. Plants are integrators of  $\delta^{15}\text{N}$  from different

sources, and their  $\delta^{15}\text{N}$  values reflect source  $\delta^{15}\text{N}$  and internal N cycling (Dawson *et al.* 2002). At ecosystem and landscape scales  $\delta^{15}\text{N}$  is thought to be controlled by pathways of N loss, in which losses favour the light isotope and lead to enrichment of  $\delta^{15}\text{N}$ , proportional to the amount of loss (Amundson *et al.* 2003). Ecosystems rich in N can become progressively enriched in  $^{15}\text{N}$  relative to ecosystems with relatively little N (Amundson *et al.* 2003, Hasselquist *et al.* 2010b, Martinelli *et al.* 1999). At the global scale, decreases of leaf  $\delta^{15}\text{N}$  occur with increasing water availability (Amundson *et al.* 2003). However, at local scales, where differences in climate may be more subtle, other factors may play a larger role (Nardoto *et al.* 2008). Lower leaf  $\delta^{15}\text{N}$  values have also been interpreted as an indication of relatively low N availability in the ecosystem (Bai *et al.* 2009, Högberg 1997, Martinelli *et al.* 1999). The drier conditions of the 1200-mm site compared to the 1900-mm site could limit aqueous N dissolution and plant uptake, consequently limiting N availability, consistent with lower leaf N values at the 1200-mm site. Other factors including temperature, N re-translocation in plants, N fractionation after plant uptake, atmospheric deposition and plant leaf habit are known to influence leaf  $\delta^{15}\text{N}$  (Amundson *et al.* 2003, Dawson *et al.* 2002, Högberg 1997, Martinelli *et al.* 2009, Ometto *et al.* 2006, Santiago *et al.* 2005, 2017). Alternative N sources, such as biological  $\text{N}_2$ -fixation and mycorrhizal associations also affect  $\delta^{15}\text{N}$  (Evans 2001). Fabaceae, the legume plant family with the potential for biological  $\text{N}_2$ -fixation, is the richest family in both of our sites with similar contribution to the community composition: 11.9% at the 1200-mm site (Couto *et al.* 2011), and 14.3% at the 1900-mm site (Carvalho *et al.* 2008). However, no clear pattern was observed between potentially  $\text{N}_2$ -fixing and non-legume plant species. Thus it is likely that differences in water availability, soil type, or sources of atmospheric deposition are the main factors influencing mean site  $\delta^{15}\text{N}$  (Nardoto *et al.* 2008, Schulze *et al.* 1998).

Our results indicate that deciduous and evergreen species in the understorey from two sites in Brazilian Atlantic forest that differ in annual precipitation show similar leaf C and N concentration and isotopic composition. This indicates that leaf habit is not a strong factor in distinguishing C and N resource acquisition in shaded leaves, as in sun leaves of other tropical forest regions. Overall, our data showed that leaf isotopic composition and elemental concentration data tended to group species according to site and not according to broad categorical functional groups, such as evergreen and deciduous species. Such functional information as provided by leaf isotopic and elemental analysis can aid in interpreting responses of vegetation to environmental changes. If trends in decreasing precipitation in the tropics continue (Joetzier *et al.* 2014, Pan *et al.* 2011), patterns of resource

acquisition revealed by isotopic analysis may serve as indicators of ecological change.

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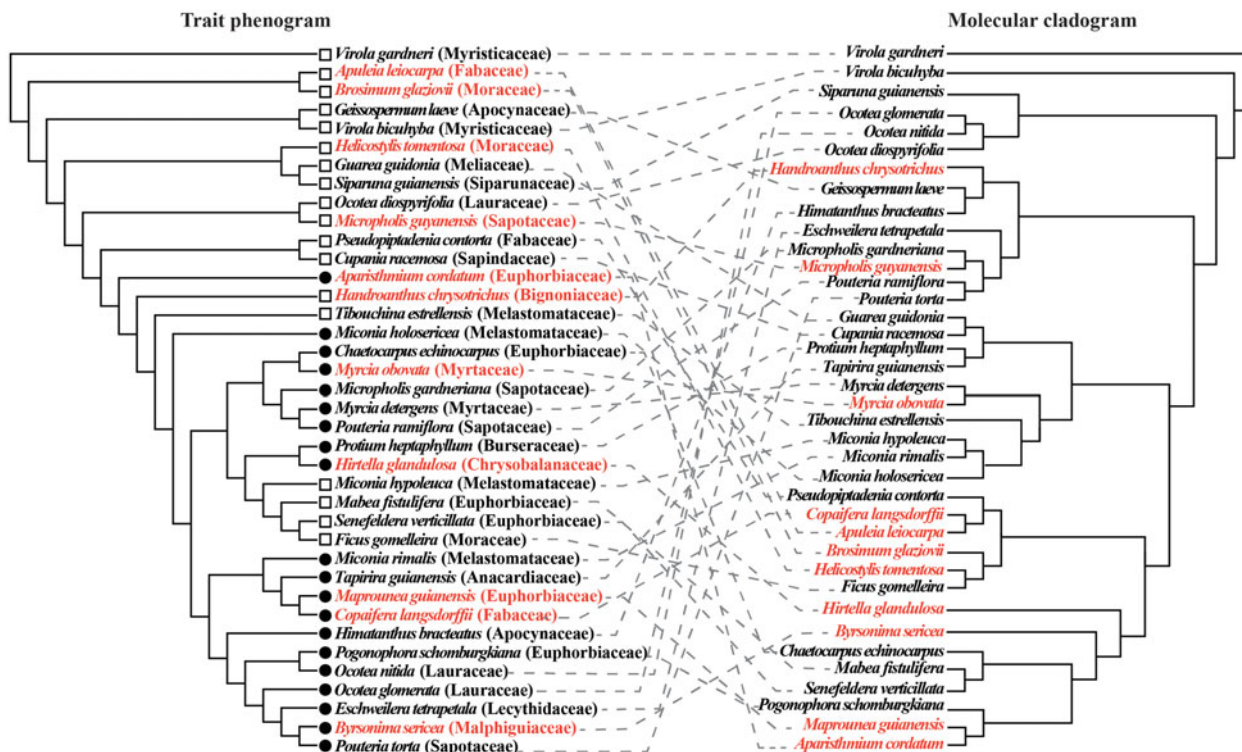
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### Appendix 1.



A comparison between a trait-phenogram and a molecular phylogeny including the 38 plant species (18 families) studied in two tropical forests in Brazil (1200 mm and 1900 mm) using five leaf traits = leaf C concentration,  $\delta^{13}\text{C}$ , N concentration,  $\delta^{15}\text{N}$  and C/N ratio. Crossing lines indicate inconsistencies between evolutionary relationships and trait relationships among species. 1200-mm site and 1900-mm sites are represented by closed circles and open squares, respectively. Species names in black represent evergreen species, whereas deciduous species are represented in red.