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Functional differences between woodland savannas and seasonally dry forests from south-eastern Brazil: Evidence from ^{15}N natural abundance studies

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Abstract Nitrogen availability and N-cycling dynamics across ecosystems play a critical role in plant functioning and species distribution. Measurements of ^{15}N natural abundance provides a way to assess ecosystem N dynamics, and the range of nitrogen stable isotope values ($\delta^{15}N$) for plants in an ecosystem can indicate divergent strategies for N uptake. We tested the hypotheses that the N-rich seasonally dry forest would have higher soil and leaf $\delta^{15}N$ and a smaller range of leaf $\delta^{15}N$ values compared to the N-poor cerradão (savanna woodland). We measured N concentration and $\delta^{15}N$ in two soil depths and leaves of 27 woody species in cerradão and 26 in seasonally dry forest. As expected, total soil N concentration decreased while soil $\delta^{15}N$ value increased with soil depth. Regardless of soil depth, seasonally dry forest soils had higher $\delta^{15}N$ and total N concentration compared to cerradão soils. Foliar $\delta^{15}N$ values varied from −6.4‰ to 5.9‰ in cerradão and from −2.3‰ to 8.4‰ in seasonally dry forest plants. Phylogenetically independent contrasts analysis and comparisons of $\delta^{15}N$ mean values of the most abundant species and species co-occurring in both sites confirmed the hypothesis of higher $\delta^{15}N$ for seasonally dry forest in comparison to cerradão. These results corroborate the expectation of higher soil and leaf $\delta^{15}N$ values in sites with higher soil N availability. However, except for the most abundant species, no across-site leaf–soil ($\delta^{15}N$ leaf – $\delta^{15}N$ soil) differences ($\Delta\delta^{15}N$) were found suggesting that differences in leaf $\delta^{15}N$ between cerradão and seasonally dry forest are driven by differences in soil $\delta^{15}N$. Variation of leaf $\delta^{15}N$ was large in both sites and only slightly higher in cerradão, suggesting high diversity of N use strategies for both cerradão and seasonally dry forest communities.

Key words: cerrado, comparative ecology, nitrogen, stable isotope, tropical forest.

INTRODUCTION

Nitrogen availability and N-cycling dynamics across natural ecosystems play a critical role in determining species distributions and biome boundaries (Tanner *et al.* 1998; Schimann *et al.* 2008). Major patches of savanna–forest boundaries in the tropics, for example, can be maintained by differences in soil water and nutrient availability (Ratter 1992; Bowman & Panton 1993; Ruggiero *et al.* 2002; Hoffmann *et al.* 2004). Nutrient-poor soils may favour species that are more conservative in resource use, and the dominance of this functional group will influence the rates of nutrient cycling in the ecosystem (Reich *et al.* 1992; Aerts & Chapin 2000; Nardoto *et al.* 2008). However, nitrogen dynamics in tropical ecosystems is not easy to quantify, mainly because of high spatial and temporal variability in nitrogen pools and losses (Robertson *et al.* 1988) and high functional diversity of plants and decomposers.

The measurement of the natural abundance of $^{15}N/^{14}N$ stable isotopes in soils and plants can provide important insights about the N cycle in natural ecosystems that no other measurements can (Martinelli *et al.* 1999; Amundson *et al.* 2003; Craine *et al.* 2009). At the ecosystem and landscape level the ratio of these isotopes in a sample relative to the standard, or the $\delta^{15}N$, increases significantly with increasing soil N availability (Schmidt & Stewart 2003; Craine *et al.* 2009). Soil N mineralization, denitrification and nitrification discriminate against ^{15}N and lead to soil nitrogen pools enriched in ^{15}N (Mariotti *et al.* 1981; Högberg 1997; Robinson 2001; Houlton & Bai 2009). These processes partly explain lower soil and leaf $\delta^{15}N$ values for N-limited ecosystems when compared to N-rich ecosystems (Schulze *et al.* 1994; Michelsen *et al.* 1996; Nadelhoffer *et al.* 1996; Martinelli *et al.* 1999).

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At the scale of individual plants, leaf δ^{15}N values are influenced by the isotope ratio of the external N source (organic N, NH_4^+ or NO_3^-), by the availability of each N source, and by physiological mechanisms within the plant, such as resorption and reallocation of N (Evans 2001). Moreover, plants associated with mycorrhizal fungi tend to have lower δ^{15}N values than non-mycorrhizal plants, mainly because discrimination against ^{15}N occurs during fungal N assimilation (Högberg *et al.* 1996; Hobbie *et al.* 2000). Legume trees, which might obtain substantial amounts of their N inputs by association with N-fixing bacteria, tend to present higher leaf N concentration relative to nonlegumes (Vitousek *et al.* 2002; Bustamante *et al.* 2004; Bai *et al.* 2009; Coletta *et al.* 2009). In addition, legumes would presumably have δ^{15}N values between 0 and 2‰ (Högberg 1997), because there is no isotopic discrimination during biological fixation of atmospheric N_2, which has a δ^{15}N of near 0‰ (see Dawson *et al.* 2002). However, recent studies have not supported this idea, mainly because in field conditions biological fixation might not be the only or even the main N source for legumes (Medina & Izaguirre 2004; Gehring *et al.* 2005; Nardoto *et al.* 2008).

Assuming that each N source has a distinct δ^{15}N value and that N source will be reflected in plant leaf δ^{15}N (Evans 2001; Kahmen *et al.* 2008), some authors have suggested that a wide range of δ^{15}N for plants in an ecosystem could indicate divergent strategies for N uptake (Schulze *et al.* 1994; Michelsen *et al.* 1996; Nadelhoffer *et al.* 1996; Mardegan *et al.* 2009) and hence resource partitioning among plants for a given environment (Bustamante *et al.* 2004). Community equilibrium models suggest that resource partitioning is essential to allow the coexistence of several species in species-rich communities (Tilman 1982) and it has been proposed that the range of leaf δ^{15}N would be higher in nutrient-limited environments (see Bustamante *et al.* 2004).

Savanna and tropical forests are among the most abundant biomes in the world and frequently form vast contact zones. It is particularly true in south-eastern Brazil, where seasonally dry forest (SDF), a vegetation physiognomy of the Atlantic rainforest biome, and cerradão, the woodland of the Cerrado savannas, are among the dominant vegetation types (Kronka *et al.* 2005; Durigan & Ratter 2006). This savanna–forest boundary may be a good model to test hypotheses related to nutrient dynamics and functional diversity, as both cerradão and SDF occur under similar climates, with the same rainfall levels, but have significant differences in soil nutrient availability.

Previous studies indicated that SDF–savanna boundaries are maintained by differences in soil water and nutrient availability, with SDF occurring over soils with higher nutrient availability (Furley & Ratter 1988, Ratter 1992, Bowman & Panton 1993; Ruggiero *et al.* 2002; Schmidt & Stewart 2003; Viani 2010). Therefore, it is reasonable to hypothesize that SDF would have higher δ^{15}N values and leaf N concentration, as a consequence of higher N availability, and consequently higher N losses and turnover rates, while N-poor cerradão would have a wider range of δ^{15}N values, reflecting a greater diversity of plant strategies for N uptake. To test these hypotheses we measured and compared the ^{15}N natural abundance and N concentration in soil samples and in leaves of several co-occurring, closely related and abundant woody species of a cerradão and a SDF in south-eastern Brazil.

METHODS

Study sites

Soil and leaf samples were collected in a cerradão at the *Estação Ecológica de Assis* (EEA) (22°32–39′S, 50°22–24′W) and in a SDF at the *Estação Ecológica dos Caetetus* (EEC) (22°22–26′S, 49°40–44′W). Both areas are protected lands located in the state of São Paulo, south-eastern Brazil. Cerradão belongs to the Cerrado biome and is a dense woodland with abundant evergreen trees and shrubs. The vegetation has a semi-closed canopy with tree cover varying from 50 to 90% (Ribeiro & Walter 1998). The EEA has a Cwa Koeppen's climate type, with a mean annual temperature of 22.1°C and annual rainfall of approximately 1440 mm. Its mean elevation is 505 m. The main soil type is a deep, well-drained and sandy dystrophic red Latosol with a severe restriction in water availability during the driest months (Juhász *et al.* 2006). Seasonally dry forest is a closed canopy vegetation type with abundance of shrubs and trees, characterized by leaf loss of significant part of the canopy trees (20–50%) during the driest months (Veloso 1992). The EEC has the same climate type (Cwa), but mean annual temperature and rainfall of 21.3°C and 1460 mm, respectively. Its mean elevation is 522 m. The main soil type is a well-drained and sandy clay loam Ultisol. In both vegetation types, less than 25% of total annual precipitation falls during April to September, indicating a strong seasonality of rainfall and the existence of a dry season during this period.

Although distanced only 100 km and under similar climate, EEA and EEC are structurally and floristically distinct. The cerradão site has almost the double of stems per hectare while the SDF site has a mean diameter at breast height almost four times higher. Moreover, they share only 18 of the more than 110 woody species they possess (R.R. Rodrigues, unpubl. data, 2010).

Soil and plant sampling

We collected leaves of 27 (19 families) species in cerradão and 26 (14 families) in the SDF site. Twelve species represented six congeneric pairs (genus with distinct species in each vegetation type), 11 are shared species (species occur-

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ring in both areas) and 12 per site represented the most abundant species in each vegetation type (cerradão and SDF), selected according to previous floristic surveys made in a 10.24-ha permanent plot located within each site. Cerradão and SDF species selected as the most abundant species represent 68.9% and 75.6% of the tree community abundance, respectively (R.R. Rodrigues, unpubl. data, 2010).

Leaves of all species were collected in the end of the dry season (September–October 2008). Within each site, we sampled six plants per species and collected eight leaves per plant. For compound-leaved species, we collected eight leaflets per plant, each one from a different leaf, and we treated them as functional leaves. Within species, the leaflets collected were always in the same leaf position. We collected only fully expanded leaves, from sun-exposed individuals in sunlit portions of the canopy, except for those species only found in the forest understory (*Cupania tenuivalvis,* Sapindaceae and *Siparuna guianensis,* Siparunaceae). Herbivore- and pathogen-damaged leaves were avoided. For *Cecropia pachystachya* (Urticaceae) and *Syagrus romanzoffiana* (Arecaceae) only a portion of each leaf was collected, due to their large individual leaf dimensions.

Plants were classified in nonlegumes, non-fixing legumes and fixing legumes. We treated as fixing legumes all plants with the ability to associate with nitrogen-fixing bacteria, according to Sylvester-Bradley *et al.* (1980) and Faria *et al.* (1984, 1987, 1994). In the case of *Stryphnodendron obovatum*, we assessed this information by checking the presence of nodules in plants grown in pots, at a greenhouse. We did not find any published study documenting nodulation in *Machaerium acutifolium*; however, we considered this species as a nitrogen-fixing legume because of its potential to associate with nitrogen-fixing bacteria (S.M. Faria, pers. comm., 2009).

Soil samples were collected in six points at each vegetation type, in two different depth layers, 0–10 cm and 10–30 cm. In both sites, points were located at least 500 m apart from each other, along a pre-existing transect. After collected in the field, soils were sieved to eliminate plant material such as foliage, branches and other debris.

Isotope analyses

Soil and leaf samples were oven-dried at 60°C for at least 48 h and then ground in a Wiley mill. Determination of $\delta^{15}N$ and total N content (% of dry weight) was done via elemental analyser/continuous flow isotope ratio mass spectrometry (ANCA/SL elemental analyser, Sercon, Cheshire, UK) coupled with a Finnigan MAT Delta^{Plus} XL mass spectrometer (Thermo Scientific, Bremen, Germany) at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley, USA.

The N isotope ratio ($\delta^{15}N$) is expressed in 'delta' notation (‰), where the isotopic composition of a material relative to that of a standard on a per mill deviation basis is given by $(R_{sample}/R_{standard} - 1) \times 1000$, where R is the molecular ratio of heavy to light isotope forms. The standard is the N from the air. The reference material NIST SMR 1547 (peach leaves), was used as calibration standard. Calibration standards were analysed every 10 samples to account for drift during the run and to perform non-linearity correction due to variation in sample weight. Based on the calibration and correction procedures, the long-term precision for N stable isotope analysis is 0.18‰.

Data analyses and statistics

Soil $\delta^{15}N$ and total N concentration were compared between vegetation types (cerradão and SDF) within each soil depth layer, by an unpaired Student's *t*-test, considering each point of soil sampling as an independent sample. Soil $\delta^{15}N$ and total N concentration were also compared between the two depth layers within vegetation type by a paired Student's *t*-test, with each soil sampling point as a pair.

To correct leaf $\delta^{15}N$ values for site-specific differences in background bulk soil $\delta^{15}N$, we also calculated the difference between leaf and soil $\delta^{15}N$, designated here as $\Delta\delta^{15}N$, following the criteria discussed in Amundson *et al.* (2003). Foliar $\Delta\delta^{15}N$ values represent the ^{15}N depletion of a plant leaf compared to the soil $\delta^{15}N$ background (Kahmen *et al.* 2008). Within each vegetation type, soil $\delta^{15}N$ mean value used for $\Delta\delta^{15}N$ calculation was obtained considering the two soil layers (0–10 and 10–30 cm depth).

We performed comparisons between vegetation types with all species together and by congeneric pairs, shared species and the most abundant species. Leaf $\delta^{15}N$, $\Delta\delta^{15}N$ and N concentration values of shared species and congeneric pairs were analysed by a factorial ANOVA, considering vegetation type and species or genus as factors. In the factorial ANOVA, species and genera were treated as random effects, once we are interested in generalization for the vegetation types from the pool of species analysed. Within species, differences between vegetation types for these traits were analysed using an unpaired Student's *t*-test with each plant being a replicate. Overall (all species together) and for the most abundant species comparisons of leaf $\delta^{15}N$, $\Delta\delta^{15}N$ and N concentration values were done between vegetation types by a Student's *t*-test with the mean value of each species as a replicate.

Finally, we used phylogenetically independent contrasts (PICs) for all traits, considering species pooled and site as a binary factor (cerradão and SDF), as described in Webb *et al.* (2008). In this comparison, shared species were treated as sister-taxa contrasts. After tested for normal distribution (Shapiro–Wilk test), the sister-taxa contrasts of each trait were compared by a one-sample *t*-test to test whether they significantly diverged from a null hypothesis where mean is zero (Webb *et al.* 2008). Analyses of PICs were performed using Phylocom (Webb *et al.* 2008). Species were arrayed on a phylogenetic tree by using Phylomatic (Webb & Donoghue 2005). The phylogenies were obtained using the conservative seed plant tree option. All branch lengths were scaled to 1. According to Ackerly (2000), treating all lengths equally may provide a good approximation when branch length is not available for all taxa.

When necessary, data were $\log_{10}$ transformed prior to the analysis to achieve Student's *t*-test and ANOVA assumptions of normality and homogeneity of variances. Because the original $\delta^{15}N$ and $\Delta\delta^{15}N$ values contained both positive and negative values, prior to $\log_{10}$ transformation, we converted them to positive values by adding a value higher than the minimum value of each origin dataset.

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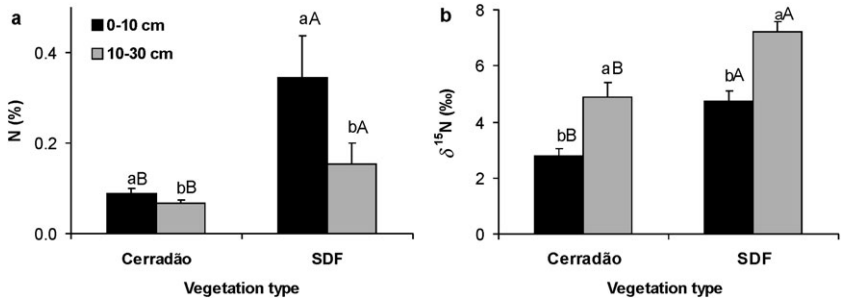

Fig. 1. Cerradão and seasonally dry forest (SDF) mean (+SE) soil N total concentration and $\delta^{15}N$ at two depth layers. Distinct small letters between bars indicate significant differences between depth layers within vegetation types, while distinct capital letters indicate significant differences between vegetation types within depth layer (*t*-test; d.f. = 1,10; $P < 0.05$).

RESULTS

Soil $\delta^{15}N$ and N concentration

Soil $\delta^{15}N$ values varied from 1.9 to 7.0‰ (range of 5.1‰) in cerradão and from 3.0 to 8.5‰ (range of 6.6‰) in SDF. Total soil nitrogen concentration varied from 0.04 to 0.12% in cerradão and from 0.07 to 0.77% in SDF soil. Soil N concentration decreased while soil $\delta^{15}N$ increased from the surface (0–10 cm depth) to the deeper soil layer, for both vegetation types (Fig. 1). Seasonally dry forest soil had greater N concentration and more positive $\delta^{15}N$ compared to cerradão soil, independent of soil depth (Fig. 1).

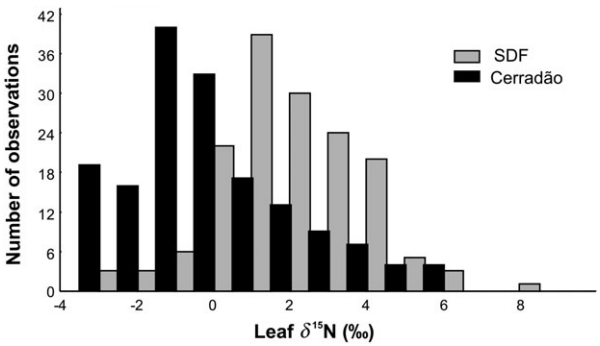

Fig. 2. Frequency histogram of leaf $\delta^{15}N$ values for cerradão ($n = 162$) and seasonally dry forest (SDF, $n = 156$) woody plants.

Leaf $\delta^{15}N$ values

Leaf $\delta^{15}N$ values varied from −6.4‰ (*Ocotea corymbosa*, Lauraceae) to 5.9‰ (*Roupala montana*, Proteaceae) (range of 12.3‰) in cerradão, and from −2.3‰ (*Nectandra oppositifolia*, Lauraceae) to 8.4‰ (*Duguetia lanceolata*, Annonaceae) (range of 10.7‰) in SDF plants. Mean leaf $\delta^{15}N$ values for cerradão and SDF species were −0.5‰ and 2.3‰ (difference of 2.8‰), respectively. Most of the cerradão plants had negative leaf $\delta^{15}N$ values while most of the SDF trees had positive $\delta^{15}N$ values (Fig. 2, Appendix S1). Mean $\Delta\delta^{15}N$ values ($\delta^{15}N$ leaf – $\delta^{15}N$ soil) were −3.9‰ in cerradão and −3.7‰ in SDF site (difference of only 0.2‰) and negative for all species, except for *Duguetia furfuracea* (Annonaceae) and *R. montana* (Proteaceae) from the cerradão site (Appendix S1).

The most abundant species of SDF had higher leaf $\delta^{15}N$ (*t*-value = −6.35, d.f. = 22, $P < 0.001$) and $\Delta\delta^{15}N$ (*t*-value = −2.35, d.f. = 22, $P = 0.028$) values than the most abundant species of cerradão. However, when all species were pooled together, mean foliar $\delta^{15}N$ was different between vegetation type (*t*-value = −6.08, d.f. = 51, $P < 0.001$) but not leaf $\Delta\delta^{15}N$ (*t*-value = −0.50, d.f. = 51, $P = 0.623$). With all species pooled, PICs corroborated these results, as they revealed higher leaf $\delta^{15}N$ in SDF (one-sample *t*-test, d.f. = 19, $P < 0.001$), but no across-site divergence for $\Delta\delta^{15}N$ (one-sample *t*-test, d.f. = 19, $P = 0.965$).

For congeneric pairs, variation in leaf $\delta^{15}N$ and $\Delta\delta^{15}N$ was larger within site (among genera) than across sites. For co-occurring species, variation was greater across sites than among species (Table 1). Populations of species co-occurring in both sites had higher leaf $\delta^{15}N$ in SDF but no difference was found with respect to leaf $\Delta\delta^{15}N$ (Table 1). Individually, seven of the 11 co-occurring species had higher $\delta^{15}N$ in the SDF site, while the other four species did not have across-site divergence in leaf $\delta^{15}N$ (Fig. 3a). Four of the 11 co-occurring species also revealed across-site differences for $\Delta\delta^{15}N$, two with higher $\Delta\delta^{15}N$ values in the cerradão site and the other two with higher values in the SDF (Fig. 3b). Overall, no across-site divergence was found for $\delta^{15}N$ and $\Delta\delta^{15}N$ for congeneric pairs (Table 1). Four of the six species pairs had across-site divergence for $\delta^{15}N$; however, this divergence was not always in the same direction, as three genera had higher $\delta^{15}N$ values in the SDF site while one had higher values in the cerradão site (Fig. 3d). Three genera had higher $\Delta\delta^{15}N$ in the cerradão site (Fig. 3e).

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Table 1. Leaf $\delta^{15}N$, $\Delta\delta^{15}N$ and N concentration (mean ± SE) of cerradão and SDF shared species and congeneric pairs

	Shared species			Congeneric pairs		
	N (%)	$\delta^{15}N$ (‰)	$\Delta\delta^{15}N$ (‰)	N (%)	$\delta^{15}N$ (‰)	$\Delta\delta^{15}N$ (‰)
Cerradão	2.4 ± 0.1	−0.8 ± 0.3	−5.7 ± 0.3	2.0 ± 0.1	1.0 ± 1.5	−4.3 ± 1.5
SDF	2.4 ± 0.1	1.8 ± 0.2	−5.5 ± 0.2	2.5 ± 0.1	2.1 ± 0.6	−5.1 ± 0.6
$P_{\text{vegetation type}}$	0.991	**<0.001**	0.581	0.060	0.175	0.152
P_{species} or P_{genus}	**0.002**	0.706	0.706	**0.008**	**0.021**	**0.003**

Within variables, significance levels in bold indicate significant effect of vegetation type or species (two-factor ANOVA, $P < 0.05$). SDF, seasonally dry forest.

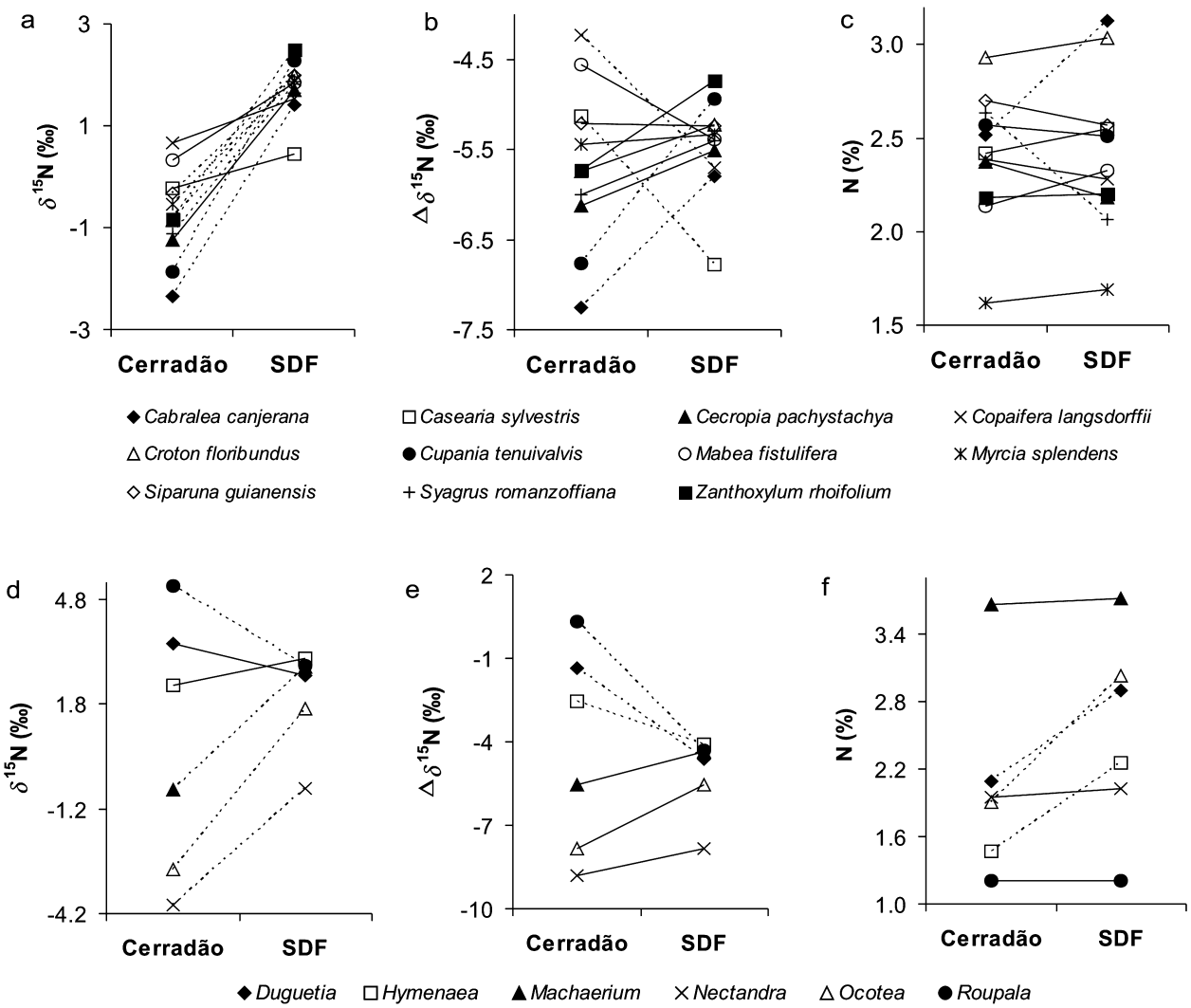

Fig. 3. Leaf N concentration, $\delta^{15}N$ and $\Delta\delta^{15}N$ of cerradão and seasonally dry forest (SDF) congeneric pairs (a–c) and shared species (d–f). Dashed lines indicate significant across-site differences (paired *t*-test; d.f. = 1,10; $P < 0.05$).

Overall, legumes and nonlegumes did not differ in foliar $\delta^{15}N$ (*t*-value = 0.44, d.f. = 51, $P = 0.660$). Mean leaf $\delta^{15}N$ for legumes and nonlegumes were 0.5‰ and −0.7‰ in the cerradão site and 1.8‰ and 2.4‰ in the SDF site, respectively. In both sites, leaf $\delta^{15}N$ values overlapped between nonlegumes and legumes (Appendix S1). Within legumes, mean leaf $\delta^{15}N$ values were −0.4‰ for nitrogen-fixing and 1.5‰ for non-fixing in the cerradão site and 1.4‰ and 2.3‰ in the SDF site. At the cerradão site, the two nitrogen-fixing species

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had lower mean δ^{15}N values compared to the other two non-fixing species, but at the SDF site there was an overlap between δ^{15}N values of nitrogen-fixing and non-fixing species (Appendix S1).

Leaf N concentration

Total leaf N concentration varied from 1.2% (*R. montana*) to 3.7% (*Machaerium acutifolium*) in cerradão and from 1.2% (*Roupala brasiliensis*) to 3.7% (*Machaerium stipitatum*) in SDF plants. Seasonally dry forest species had total leaf N concentration significantly higher in comparison to cerradão when all species are considered (t-value = –2.48, d.f. = 51, $P < 0.017$), as well as for the most abundant species (t-value = –2.89, d.f. = 22, $P = 0.008$) and PICs analysis with all species pooled (one-sample t-test, d.f. = 19, $P = 0.028$). Mean leaf N concentration values did not differ across sites when congeneric pairs were analysed ($P = 0.06$) (Table 1). However, three of the six congeneric pairs showed higher leaf N concentration values for SDF species and none presented higher values for cerradão species (Fig. 3f). Shared species had similar leaf N concentration values in both vegetation types (Table 1). Only two out of the 11 shared species had across-site differences in leaf N concentration values (Fig. 3c).

Overall, legumes and nonlegumes did not differ in leaf N concentration (t-value = 1.395, d.f. = 51, $P = 0.169$). There was an overlap between leaf N concentration averages of legumes and nonlegumes. For legumes, leaf N concentration ranged from 1.5 to 3.7%, while for nonlegumes it varied from 1.2 to 3.6%. However, independently of vegetation type, we did not find an overlap between leaf N concentration of nitrogen-fixing (from 2.5 to 3.7%) and non-fixing legumes (from 1.5 to 2.4%), with mean value higher for the former. In addition, nitrogen-fixing legumes had higher mean leaf N concentration than non-fixing legumes (t-value = 2.62, d.f. = 6, $P = 0.040$).

DISCUSSION

Total soil N was higher in SDF in comparison to the savanna woodland, which is a pattern commonly found for many forest–savanna contact zones (Bowman & Panton 1993; Ruggiero *et al.* 2002; Schmidt & Stewart 2003; Viani 2010). The decrease in total soil N with depth in both vegetation types also corroborates the results of investigations in other forest and savannas ecosystems (Bustamante *et al.* 2004; Ometto *et al.* 2006; Mardegan *et al.* 2009) and probably reflects a decrease in litter inputs and total organic matter cycling with soil depth.

The increase in soil δ^{15}N in the deeper soil layers, independently of vegetation type, corroborates the patterns reported for tropical forests and savannas (Piccolo *et al.* 1996; Koopmans *et al.* 1997; Bustamante *et al.* 2004; Brenner *et al.* 2005; Ometto *et al.* 2006). It is because there is an increase in fractionation against ^{15}N during the mineralization of the organic matter (Högberg 1997), which leaves a decomposed organic matter with higher δ^{15}N values in the soil. In addition, foliar δ^{15}N are usually lower than soil δ^{15}N, and thus litter deposition contributes to the δ^{15}N decrease in the uppermost soil layers (Bustamante *et al.* 2004).

The higher soil δ^{15}N in the nutrient-rich SDF compared to cerradão confirms our hypothesis and is consistent with ^{15}N natural abundance pattern described for tropical savannas and monsoon forests in Australia (Schmidt & Stewart 2003). Higher nitrogen availability in SDF soils (Fig. 1, Viani 2010) may lead to higher N losses by pathways which discriminate against ^{15}N, such as denitrification, nitrification and mineralization, and lead to soil ^{15}N-enriched pools with different δ^{15}N signatures (Mariotti *et al.* 1981; Martinelli *et al.* 1999; Robinson 2001; Houlton & Bai 2009). Indeed, several investigations have revealed a positive correlation of soil N availability, nitrification and mineralization rates with soil and leaf δ^{15}N values at the community and ecosystem level (Schmidt & Stewart 2003; Kahmen *et al.* 2008; Craine *et al.* 2009). Therefore, in spite of the lack of data on N transformations in both vegetation types, our δ^{15}N results strongly suggest that processes transforming soil N are more intensive in the SDF than in the cerradão site.

Seasonally dry forest plants had higher leaf δ^{15}N compared to cerradão plants, except when congeneric pairs were compared (Table 1). A similar pattern was described for savanna and forest plants in northern Australia (Schmidt & Stewart 2003). According to these authors, higher foliar δ^{15}N of monsoon forest plants compared to savanna plants may be caused by: (i) high δ^{15}N of soil N sources as a result of high N turnover, nitrification and/or N loss; (ii) high use of NO_3^- and associated lower fractionation against ^{15}N compared to NH_4^+ use; and (iii) low contribution of mycorrhizal fungi to plant N acquisition and associated lower discrimination against ^{15}N during fungal N transfer. Except for congeneric pairs, we did not find significant difference in leaf δ^{15}N between plants growing at cerradão and SDF when the background soil δ^{15}N was discounted from leaf δ^{15}N (δ^{15}N leaf – soil, $\Delta\delta^{15}$N). Therefore, it suggests that the soil background difference in δ^{15}N is the main reason for differences in foliar δ^{15}N of cerradão and SDF plants. Nevertheless, it is difficult to assign a precise reason why there are similar $\Delta\delta^{15}$N of closely related species or of populations of cer-

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radão and SDF co-occurring species, even though it is likely that it reflects the use of the same N sources in both sites. It is because the mean soil $\delta^{15}N$ is not always a good indicator of the specific $\delta^{15}N$ of the N sources available for individual plants (Högberg 1997).

Consistent with previous studies in several ecosystems with contrasting N availability, we found a large range of leaf $\delta^{15}N$ values in both cerradão and SDF sites (Michelsen *et al.* 1996; Nadelhoffer *et al.* 1996; Bustamante *et al.* 2004; Ometto *et al.* 2006; Coletta *et al.* 2009). The N-poor cerradão site had a larger range of leaf $\delta^{15}N$ values in comparison to the SDF; however, this difference seems very small (1.6‰) to characterize a higher diversity of N use strategies in the N-poor cerradão site. In fact, the large range of leaf $\delta^{15}N$ values for both sites reinforces that diversification in N use strategies occurs not only in N-poor sites, such as the Brazilian Cerrado, but also in relatively nutrient-rich environments, like the SDF from south-eastern Brazil. At this point while we can only speculate we nonetheless believe that the data support the idea that divergence in leaf $\delta^{15}N$ values within plant communities reflects different N use strategies. This is because the occurrence of biotic and abiotic processes simultaneously complicates the interpretation of plant use of different N sources solely based on foliar $\delta^{15}N$ values (Högberg 1997; Robinson 2001). Although the $\delta^{15}N$ signature of the plant's N source is a critical driver of variability in $\delta^{15}N$ among plant species, soil isotope signatures of NH_4^+ and NO_3^- are not universal and both can vary their values significantly in different ecosystems depending on the nature of the N cycle (Kahmen *et al.* 2008).

Only two (*D. furfuracea* and *R. montana*) out of the 42 woody species had positive $\Delta\delta^{15}N$, which indicates enrichment of ^{15}N on leaves compared to bulk soil $\delta^{15}N$. The negative $\Delta\delta^{15}N$ for the remaining species is probably the result of the uptake of mycorrhiza delivered and/or mineral soil N, which are depleted in ^{15}N compared to bulk soil N or soil organic N sources (Mariotti *et al.* 1981; Robinson 2001), a pattern consistently found in different ecosystems (see Amundson *et al.* 2003; Kahmen *et al.* 2008). The cerradão species *R. montana* had the highest mean leaf $\delta^{15}N$ value, even when SDF species are considered. Interestingly, this species also showed the highest leaf $\delta^{15}N$ in the investigation of ^{15}N natural abundance among several cerrado species in central Brazil (Bustamante *et al.* 2004). Similarly, Proteaceae species from fynbos in South Africa (Stock *et al.* 1995) and savanna and monsoon forests in northern Australia (Schmidt & Stewart 2003) also had the most positive leaf $\delta^{15}N$ in the community. According to Smirnoff *et al.* (1984), Proteaceae members consistently have low nitrate reductase activity in their leaves and this might be a factor accounting for their high leaf $\delta^{15}N$ values (Bustamante *et al.* 2004). In addition, Proteaceae species are typically non-mycorrhizal plants (Lamont 1982). Mycorrhizal fungi deliver isotopically depleted N to plants (Högberg 1997; Hobbie *et al.* 2000; Evans 2001; Craine *et al.* 2009) and the difference in leaf $\delta^{15}N$ of proteaceous and non-proteaceous species with similarly low nitrate use is an indicator of the role of mycorrhizal fungi in determining leaf $\delta^{15}N$ (Schmidt & Stewart 1997). However, further investigations are needed to better understand N use strategy in *Roupala*, as its positive $\Delta\delta^{15}N$ is suggestive of the use of organic N sources.

The lack of difference between foliar $\delta^{15}N$ of legumes and nonlegumes can be attributable to the great variability in foliar $\delta^{15}N$ of nonlegume species. When legumes and nonlegumes were separated in N-fixers and non-fixers, fixers had more negative leaf $\delta^{15}N$ values (closest to 0, except for *Machaerium stipitatum*), while non-fixers have more positive leaf $\delta^{15}N$ values. Although this suggests N_2 fixation by the nitrogen-fixing species, the estimation of the contribution of N_2 fixation as a source of N based solely on the analysis of $\delta^{15}N$ values is misleading due to many other abiotic and biotic factors accounting for variation in leaf ^{15}N isotope signatures (see Högberg 1997; Evans 2001).

The higher leaf N concentration for SDF when compared to cerradão suggests differences in resource investment strategies for SDF and cerradão plants. Higher leaf N concentration is positively associated with photosynthetic capacity and growth rate (Wright *et al.* 2004; Poorter & Bongers 2006). Therefore, this result reinforces the idea that the nutrient-rich SDF is a more competitive environment, favouring species with traits that maximize growth (Viani 2010). In contrast with previous studies, we did not find differences in leaf N concentration between legumes and nonlegumes (Vitousek *et al.* 2002; Aidar *et al.* 2003; Bustamante *et al.* 2004; Nardoto *et al.* 2008). The legumes we studied form a heterogeneous group composed by nitrogen-fixing and non-fixing plants. In this sense, we did find higher leaf N concentration for nitrogen-fixing legumes in comparison to non-fixing legumes, which corroborates the idea that association with nitrogen-fixing bacteria is an efficient strategy to increase legume leaf N concentration (Mckey 1994).

In conclusion, our results support the notion that environments with higher N availability have higher soil and plant $\delta^{15}N$ values (Martinelli *et al.* 1999; Craine *et al.* 2009) and suggest that differences in foliar $\delta^{15}N$ values for cerradão- and SDF-related species are mainly determined by differences in background soil $\delta^{15}N$. Moreover, our study revealed that cerradão and SDF have a high interspecific variation in leaf $\delta^{15}N$, which suggests contrasting N use strategies among species of both communities. Nevertheless, at the species level, further detailed investigations

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of N use strategies are necessary (i) to better understand factors affecting variation in leaf $\delta^{15}N$ among species within sites and (ii) to ensure whether close related species and species co-occurring in cerradão and SDF use the same N use strategies despite differences in abundance and dynamic of N between these two vegetation types.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Leaf $\delta^{15}N$, $\Delta\delta^{15}N$ ($\delta^{15}N$ leaf – $\delta^{15}N$ soil) and N concentration (mean ± SE) of cerradão and seasonally dry forest woody plants.

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