

Specific leaf area explains differences in leaf traits between congeneric savanna and forest trees

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Summary

1. Leaf traits are commonly associated with the life history, distribution and resource requirements of a species. To improve our understanding of the ecological and physiological differences between tropical savanna and forest trees, we compared leaf traits of species native to savanna and gallery (riverine) forests in the Cerrado region of central Brazil.

2. Congeneric species pairs from 14 different taxonomic families were studied, each with a savanna species and a forest species present at the study site. Only individuals growing in savanna conditions under full sun were studied. We measured foliar nutrients, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and specific leaf area (SLA: leaf area per unit leaf mass). We used phylogenetically independent contrasts to compare savanna and forest species and to test for correlations among species traits.

3. Overall, leaves of forest species had 17% higher N concentration, 32% higher P concentration, and 37% higher K concentration, despite growing in similar soils. Concentrations of all three elements were strongly and positively correlated with SLA.

4. Forest species had 52% greater SLA, on average, than savanna species, which accounts for the higher foliar nutrient concentrations of these species.

5. Savanna species had higher $\delta^{13}\text{C}$ values than forest species, indicating higher water-use efficiency. The SLA was negatively correlated with $\delta^{13}\text{C}$, suggesting that SLA may also account for the higher water-use efficiency of savanna species.

6. There was no difference in foliar $\delta^{15}\text{N}$ between savanna and forest species, but foliar $\delta^{15}\text{N}$ was negatively correlated with soil pH.

7. These results contribute to recent studies showing that tropical savanna and forest species represent two distinct functional types, with large differences in ecology and physiology, that have important consequences for the dynamics of savanna–forest boundaries.

Key-words: isotopes, phylogenetically independent contrasts, specific leaf area, tropical forest, water-use efficiency

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Introduction

Over large areas of the tropics, savanna and forest vegetation coexist in a mosaic at landscape and regional scales. In general, forest vegetation is associated with greater nutrient or water availability (Furley 1992; Ruggero *et al.* 2002), but often this association is imperfect or non-existent (Gillison 1983; Bowman 1992, 2000; Furley 1992; Haridasan 1992; Schwartz *et al.* 1996; Fölster

et al. 2001). Several factors may explain a weak association between vegetation type and soil properties. First, savanna–forest boundaries are known to shift location in response to climate and fire regimes (Kershaw 1986; Hopkins 1992; Desjardins *et al.* 1996; Schwartz *et al.* 1996; Sanaïotti *et al.* 2002), so vegetation distribution is not expected to be always in equilibrium with soil properties. Additionally, the distribution of a forest may be limited by total nutrient stocks at the site, rather than the amount in the soil (Haridasan 1992). In the tropics, where a large fraction of ecosystem nutrient stocks resides in the vegetation, soil nutrient

concentrations might give a poor reflection of overlying vegetation. Finally, the vegetation itself can substantially affect soil nutrient availability, with soils becoming enriched under trees due to higher litter inputs and reduced fire frequency (Kellman 1979; Belsky *et al.* 1989; Mordelet *et al.* 1993). Therefore, even where vegetation is strongly associated with soil properties, it is often unclear whether the soil determines the distribution of vegetation types or *vice versa*.

The boundary between savanna and evergreen forest is characterized by a transition not only in tree density, but also in tree species (Adejuwon & Adesina 1992; Felfili & Junior 1992), with few species being common to both environments. Savanna and forest tree species differ in fire tolerance, allocation patterns, stature, and requirements for seedling establishment (Hoffmann & Franco 2003; Hoffmann *et al.* 2003, 2004), yet studies on the determinants of savanna and forest have typically not acknowledged the role of species traits in governing vegetation structure and dynamics. Furthermore, although nutrient availability is often purported to determine the distribution of savanna and forest, little is known about differences in the nutrient requirements of savanna and forest species. Hoffmann & Franco (2003) did not find a difference between savanna and forest seedlings in response to nutrient availability; however, the levels of nutrient addition in that study appear to have been too low or too short (150 days) to elicit a strong growth response overall, making observation of differences in nutrient requirements among species unlikely. Other studies suggest a tendency for higher foliar concentrations of some nutrients in evergreen forest species relative to savanna species (Fensham & Bowman 1995; Högborg & Alexander 1995; Schmidt *et al.* 1998; Schmidt & Stewart 2003). These studies revealed considerable overlap in nutrient concentrations between the two groups of species, and none controlled for soil conditions, making it uncertain whether species traits or environment were responsible for observed differences. Similarly, Haridasan (1992) did not find clear differences in mean leaf nutrient concentration between cerrado savanna and cerrado, a vegetation type commonly classified as forest (Ribeiro & Walter 2001) but which typically contains a mixture of savanna and forest tree species.

Despite the ambiguity of previous results, we expect forest species to exhibit higher leaf nutrient concentrations than savanna species due to differences in specific leaf area (SLA: leaf area per unit leaf mass). In the Cerrado region of Brazil, forest species tend to have higher SLA than savanna species, at least as seedlings (Hoffmann & Franco 2003), while nutrient concentration is strongly and positively correlated with SLA (Reich *et al.* 1997). Although the relationship between SLA and foliar nutrients appears robust across biomes and growth forms (Reich *et al.* 1997), water availability can substantially alter this relationship (Wright *et al.* 2001). For a given value of SLA, plants on drier sites can exhibit higher foliar concentrations of N and P,

possibly because higher concentrations of Rubisco permit more complete drawdown of internal leaf CO₂ concentration (Wright *et al.* 2001). This would result in higher water-use efficiency (WUE) by allowing the plant to photosynthesize effectively with lower stomatal conductance (WUE, Field *et al.* 1983; Wright *et al.* 2001). Therefore, although we expect forest species, which should be adapted to higher water availability, to have higher leaf nutrient concentrations due to their greater SLA, we also expect that, after controlling for SLA, savanna species will have higher N and P concentrations than forest species. In partial support of this hypothesis, Franco *et al.* (2005) found cerrado savanna trees to have consistently higher maximum net assimilation rates (A_{\max}) than expected from the universal relationship between A_{\max} and SLA obtained across multiple biomes (Reich *et al.* 1997), but unfortunately a direct comparison with tropical forest species was not possible. Furthermore, we expect savanna species to exhibit higher WUE than forest species and that, among species, WUE will be positively correlated with N concentration. We tested these hypotheses within a phylogenetic context for tree species of the savannas and gallery forests of the Cerrado region of central Brazil, while controlling for site conditions by selecting only individuals that established in open savanna vegetation.

We also tested for differences in foliar $\delta^{15}\text{N}$ between savanna and forest species growing under similar environments. Savanna species have been shown to have lower values of $\delta^{15}\text{N}$ than forest species in Australia (Schmidt & Stewart 2003) and Africa (Högborg & Alexander 1995). This contrasts with the widely observed pattern that plants tend to have higher $\delta^{15}\text{N}$ in dry, rather than wet, environments (Schulze *et al.* 1998; Austin & Sala 1999; Handley *et al.* 1999; Swap *et al.* 2004). In these studies, the authors attribute these trends in leaf $\delta^{15}\text{N}$ to site differences in nutrient-cycling processes, rather than to genetically determined differences between savanna and forest species. Nevertheless, foliar $\delta^{15}\text{N}$ can be strongly influenced by species traits, particularly by associations with N₂-fixing bacteria, ectomycorrhizas or vesicular-arbuscular mycorrhizas (Högborg & Alexander 1995). As it is unclear whether differences in ^{15}N concentration between savanna and forest are determined entirely by ecosystem-level differences in nutrient cycling, rather than species traits, we compare foliar ^{15}N of savanna and forest tree species under similar environments.

Materials and methods

STUDY SITE

This work was performed within the Cerrado, a region of approximately $2 \times 10^6 \text{ km}^2$ in south-central Brazil. Within the Cerrado, savanna is the dominant vegetation type in the uplands, while corridors of evergreen gallery (riparian) forests typically occur along streams.

Additionally, dry forest, ranging in phenology from deciduous to evergreen, occurs locally on upland sites throughout the region and at the transitions to the Amazonian and Atlantic rainforests.

Plant samples were collected primarily at the Ecological Reserve of IBGE (Instituto Brasileiro de Geografia e Estatística) near Brasília in the Federal District, Brazil at an elevation of approximately 1100 m. As needed, additional samples were collected at another site with intact natural vegetation located approximately 10 km from the IBGE reserve. Mean annual rainfall at the meteorological station in IBGE was 1426 mm for 1993–2002, with a distinct dry season from May to September and a mean annual temperature of 22.5 °C.

METHODS

We chose 14 savanna–forest species pairs based on availability at the study site (Fig. 1). Each pair consists of one savanna species and one forest species of the same genus; no two genera were selected from the same family. Furthermore, we selected only pairs for which both species could be found in open savanna. Although forest tree species are typically absent from cerrado savanna, long-term fire protection at the study site has permitted gallery forest species to establish and persist in the neighbouring savanna. The use of congeneric species pairs from different families ensures phylogenetic independence because it ensures the divergence into savanna and forest species has occurred independently in each genus.

We collected leaves of all 28 species in May 2004 (end of wet season). To control for site differences, we collected only from individuals growing within the savanna environment, even for those species typical of

forest. For most species these were naturally occurring individuals, but for *Hymenaea martiana* we utilized individuals planted in savanna at least 20 years previously, with no management for at least the past 15 years and perhaps longer. All species are evergreen, except for the species of *Aspidosperma*, *Tabebuia* and *Aegiphila*.

Mature leaves were collected from outer, sunlit portions of the canopy of three mature unshaded individuals per species. Leaf samples were washed with distilled water in the laboratory and dried in an air oven at 80 °C. Wet digestion using a mixture of sulphuric, nitric and perchloric acids (1 : 10 : 2) was employed for the chemical analysis. Phosphorus was determined colorimetrically, and the cations potassium, calcium, magnesium, iron, manganese, zinc and copper were determined using an atomic absorption spectrophotometer (Allen 1989).

To estimate specific leaf area, 10 leaf discs per individual were collected with a cork borer of known diameter. The leaf midrib and prominent secondary veins were avoided to reduce sample variation. The discs were dried to constant mass at 60 °C, and weighed. For species where it was possible to find additional individuals growing in open conditions, we measured the SLA of up to eight additional individuals.

A soil sample was collected from the surface layer (0–10 cm) in the immediate vicinity of each individual or group of individuals sampled. Soil pH was measured in 1 : 2.5 soil–water suspension and in 1 N KCl. Exchangeable Ca, Mg and Al were determined in soil extracts of 1 N KCl; exchangeable K and available P, Fe, Mn, Zn and Cu were determined in Mehlich's extract of a diacid mixture of 0.05 N HCl and 0.025 N H₂SO₄. The cations in soil extracts were determined by atomic absorption spectrophotometry, and P by colorimetry (Allen 1989). Total N in soil was determined by Kjeldahl digestion and distillation. Soil organic carbon was determined by the method of Walkley and Black (Allen 1989).

Analyses of isotope abundance and N concentration were performed at the Isotopic Ecology Laboratory at the Centro de Energia Nuclear na Agricultura (CENA), University of Sao Paulo, through full combustion of the samples under helium continuous flow in an elemental analyser (Carlo Erba, CHN-1110, Milan, Italy) coupled to a isotope ratio mass spectrometer (Thermo Finnigan, Delta Plus, Bremen, Germany). The isotopic ratios for carbon and nitrogen are expressed as deviations, in parts per thousand, from the PDB limestone and from atmospheric air nitrogen international standards, respectively, by:

$$\delta X(\text{‰}) = 1000[(R_{\text{sample}}/R_{\text{standard}}) - 1]$$

where R_{sample} and R_{standard} are the ratios $^{13}\text{C} : ^{12}\text{C}$ or $^{15}\text{N} : ^{14}\text{N}$. CO₂ and N₂ gases resulting from the sample combustion were analysed in duplicate, with an analytical error of 0.3 and 0.5‰, respectively.

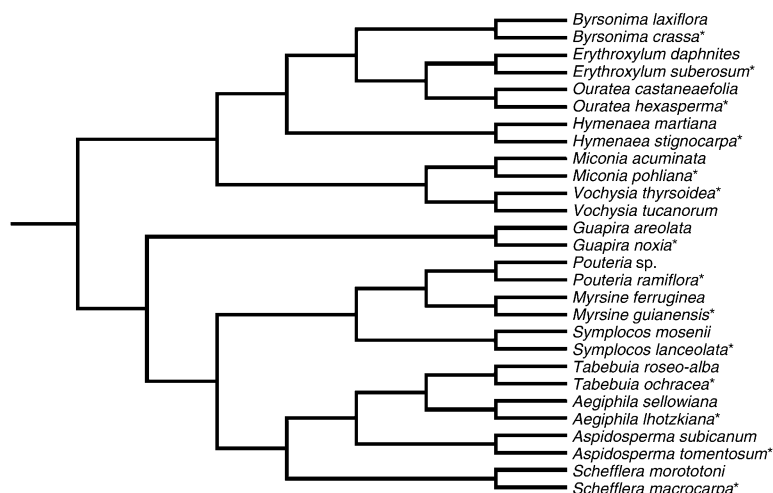


Fig. 1. Phylogenetic relationships of the study species. Savanna species are marked with asterisks. Each genus belongs to a separate family: *Byrsonima* (Malpighiaceae), *Erythroxylum* (Erythroxylaceae), *Ouratea* (Ochnaceae), *Hymenaea* (Leguminosae – Caes.), *Miconia* (Melastomataceae), *Vochysia* (Vochysiaceae), *Guapira* (Nyctaginaceae), *Pouteria* (Sapotaceae), *Myrsine* (Myrsinaceae), *Symplocos* (Symplocaceae), *Tabebuia* (Bignoniaceae), *Aegiphila* (Verbenaceae), *Aspidosperma* (Apocynaceae), *Schefflera* (Araliaceae).

ANALYSIS

We used a factorial ANOVA to test for effects of genus and plant type (savanna vs forest) on leaf traits, with genus as random factor and plant type as a fixed factor. For comparisons between plant types, this is equivalent to a paired *t*-test with species being paired within genera (Sokal & Rohlf 1995). Nutrient concentrations and SLA were log₁₀-transformed prior to analysis, due to heterogeneity of variances. This transformation was not possible for the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data because of negative values, so instead we performed an ANOVA weighted by the reciprocal of the sample variance of a species.

Subsequently, analysis of covariance was performed to compare the same leaf traits, with SLA as the covariate. This test was performed to determine whether differences in nutrient concentration or isotope ratios between savanna and forest species can be explained entirely by differences in SLA, or whether differences remain after controlling for SLA. In this analysis, the correlation between SLA and other leaf traits is based largely on relationships among genera, which in this case do not guarantee phylogenetic independence. To determine whether correlations among traits are supported after accounting for phylogenetic relationships, we re-examined these relationships using phylogenetically independent contrasts (Felsenstein 1985).

The phylogenetic relationships among the 14 study genera were obtained from the angiosperm supertree provided by Davies *et al.* (2004) using the PHYLOMATIC utility (Webb & Donoghue 2004). Based on the resulting tree (Fig. 1), we used the PDAP 1.00 (Midford *et al.* 2002) package of MESQUITE ver. 1.05 (Maddison & Maddison 2004) to test for correlated evolution among traits using the approach of Felsenstein (1985). In this analysis, all branches of the phylogeny were scaled to a length of 1.

Results

Overall, leaves of forest species had 17% higher N concentration, 32% higher P concentration and 37% higher K concentration than leaves of savanna species (Table 1). Savanna species had higher mean values of $\delta^{13}\text{C}$ and N : P ratio than forest species (Table 1). The concentrations of other nutrients, as well as $\delta^{15}\text{N}$, did not differ between plant types when compared with a factorial ANOVA including genus as the second factor (Table 1).

Forest species had significantly greater SLA than savanna species in all but two of 14 genera (Fig. 2). Overall, the SLA of forest species was 52% greater than that of savanna species (Table 1). Across all species, foliar concentrations of N, P, K, Fe and Cu were positively correlated with SLA, while N : P ratio was negatively correlated with SLA (Fig. 3). In all cases except Fe, differences in SLA were sufficient to explain differences in mean concentrations between savanna

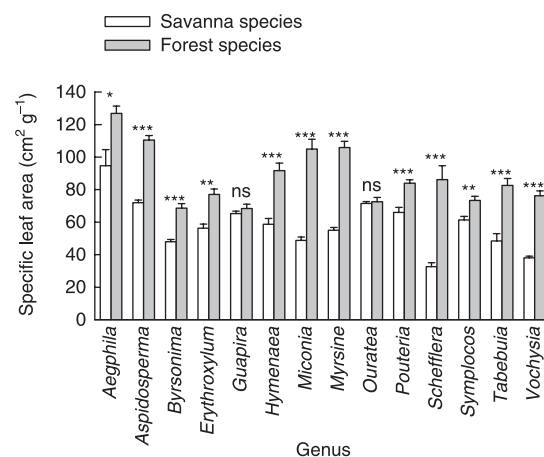


Fig. 2. Mean specific leaf area (\pm SE) of congeneric savanna and forest species. Significance levels determined by *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

Table 1. Mean (\pm SE †) specific leaf area (SLA) and nutrient and isotope concentrations of leaves of savanna and forest trees

Parameter	Savanna species	Forest species	P_{type}^\ddagger	$P_{\text{genus}}^\ddagger$
SLA ($\text{cm}^2 \text{g}^{-1}$)	57.3 (4.0)	87.1 (4.8)	$<<0.001$	0.16
N (%)	1.48 (0.16)	1.73 (0.14)	0.003	0.0001
P (%)	0.067 (0.0072)	0.089 (0.0077)	0.002	0.006
K (%)	0.57 (0.078)	0.79 (0.12)	0.04	0.015
Ca (%)	0.55 (0.061)	0.78 (0.13)	0.08	0.015
Mg (%)	0.16 (0.015)	0.18 (0.022)	0.35	0.0031
Fe (mg kg^{-1})	107.4 (10.8)	95.5 (5.9)	0.37	0.062
Mn (mg kg^{-1})	214 (51)	115 (25)	0.08	0.31
Zn (mg kg^{-1})	24.3 (1.3)	26.5 (2.1)	0.45	0.09
Cu (mg kg^{-1})	7.43 (2.19)	9.01 (2.92)	0.09	0.0002
N : P ratio	22.6 (1.1)	20.5 (1.9)	0.02	0.002
$\delta^{13}\text{C}$ (‰)	-27.76 (0.25)	-28.44 (0.23)	0.02	0.14
$\delta^{15}\text{N}$ (‰)	0.25 (0.50)	-0.53 (0.77)	0.34	0.18

† Standard errors based on variation among species means.

$^\ddagger P_{\text{type}}$, significance level for comparison between savanna and forest species; P_{genus} , significance level for comparisons among genera.

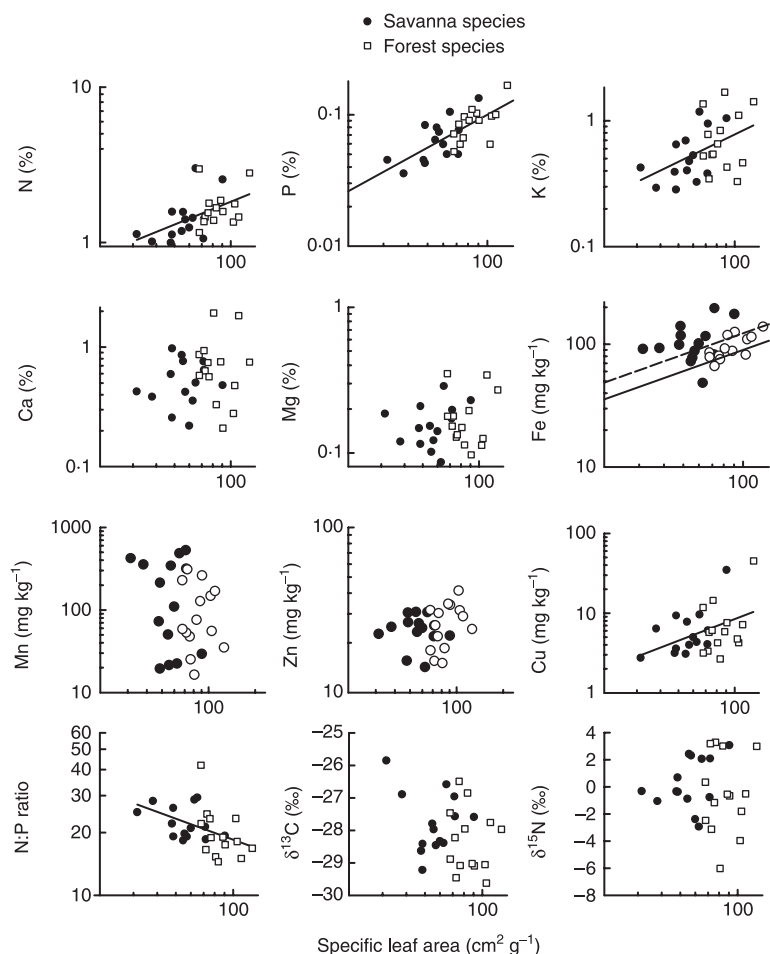


Fig. 3. Relationship between specific leaf area (SLA) and nutrient content for savanna and forest species. Where no regression line is present there is no significant correlation between nutrient concentration and SLA. Where a single regression line is present the correlation is significant ($P < 0.05$), but the relationship does not differ between savanna and forest species. For iron, savanna species had a higher concentration (upper line) than forest species (lower line) after accounting for SLA.

and forest species, as there was no significant difference between savanna and forest species once SLA is taken into account with analysis of covariance. In the case of Fe, savanna species had higher concentrations than forest species after factoring out SLA (Fig. 3).

Although forest species had higher concentrations of several nutrients, savanna species tended to have higher nutrient content per leaf area. This difference was significant for N (2.6 vs 2.0 g m⁻², $P = 0.001$); Mg (297 vs 213 mg m⁻², $P = 0.01$); Fe (19 vs 11 mg m⁻², $P = 0.0007$); Zn (4.5 vs 3.1 mg m⁻², $P = 0.003$); and Cu (1.20 vs 0.97 mg m⁻², $P = 0.03$, data not shown). For all elements except Cu, these differences can be explained by a significant negative correlation between SLA and nutrient content per area ($P < 0.05$, data not shown).

Phylogenetically independent contrasts confirmed most of the trends observed in the comparisons among species (Fig. 4). The contrasts for mass-based concentrations of P, K and N, as well as N : P ratio, were significantly and positively correlated with the contrast

for SLA, although Cu was only marginally correlated with SLA ($P = 0.057$), and Fe was not ($P = 0.44$). When examined with phylogenetically independent contrasts, nutrient content on a leaf-area basis was negatively correlated with SLA for N, P, Ca, Mg and Fe ($P < 0.05$), but not for K or Cu (data not shown). The contrast for $\delta^{13}\text{C}$ was negatively correlated with that for SLA ($r^2 = 0.43$, $P = 0.02$; Fig. 4), although this relationship was largely dependent on a single point corresponding to the contrast between the two *Schefflera* species. When either of these taxa was removed from the analysis, the relationship was no longer significant.

Leaf $\delta^{13}\text{C}$ was not correlated with N ($r^2 = 0.005$, $P = 0.73$) or P ($r^2 = 0.012$, $P = 0.57$) when tested across species, nor when tested with phylogenetically independent contrasts ($r^2 < 0.08$, $P > 0.16$). When tested across all species there was a weak positive correlation between leaf $\delta^{15}\text{N}$ and total N (Fig. 4, $r^2 = 0.17$, $P = 0.03$), and between $\delta^{15}\text{N}$ and P (Fig. 4, $r^2 = 0.20$, $P = 0.009$). Both these relationships became non-significant when tested with phylogenetically independent contrasts ($r^2 < 0.12$, $P > 0.07$). $\delta^{15}\text{N}$ was negatively correlated with soil pH and positively related with available Al^{3+} (Fig. 5). These relationships were significant regardless of whether individual values or species means were used. Nevertheless, soil pH does not account entirely for observed species differences; when tested with ANCOVA with soil pH as a covariate, there was significant variation among species ($F_{24,51} = 3.18$, $P = 0.003$).

The study individuals occurred in acidic (mean pH = 5.0 in H₂O) and dystrophic soils typical of cerrado savannas. The soils on which the study individuals of the savanna species occurred did not differ from soils on which the forest species occurred for N ($P = 0.11$), K ($P = 0.08$), Ca ($P = 0.29$), Mg ($P = 0.21$), Fe ($P = 0.82$), Mn ($P = 0.47$), Zn ($P = 0.74$), Cu ($P = 0.09$), pH ($P = 0.19$) and C ($P = 0.13$). However the forest species did tend to occur in soils of higher P (1.58 vs 1.15 p.p.m., $P = 0.006$).

Discussion

As expected, on a mass basis forest species had higher foliar concentrations of N, P and K than savanna species due to their higher SLA, combined with positive correlations between SLA and each of these three nutrients. However, contrary to predictions, savanna species did not have higher concentrations of any of these nutrients after factoring out SLA as a covariate (Fig. 3). In contrast, Wright *et al.* (2001) found that species from drier sites had higher N and P concentrations for a given SLA than species from wetter sites. In that study, comparisons were performed between sites with annual rainfall of 387 and 1220 mm, which probably represents a higher range of water availability than the different landscape positions typically occupied by our study species. Also, we controlled for resource availability by sampling only individuals that established in open savanna, whereas this was not

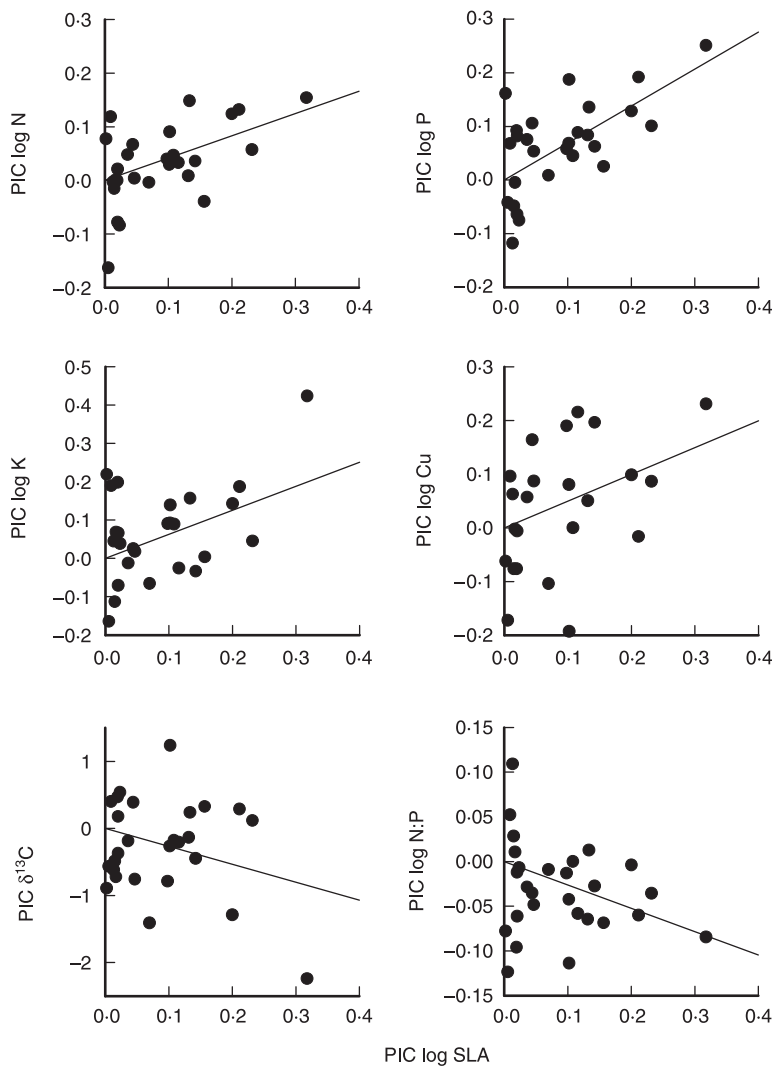


Fig. 4. Relationships between phylogenetically independent contrasts (PIC). All relationships shown have significant correlations ($P < 0.05$).

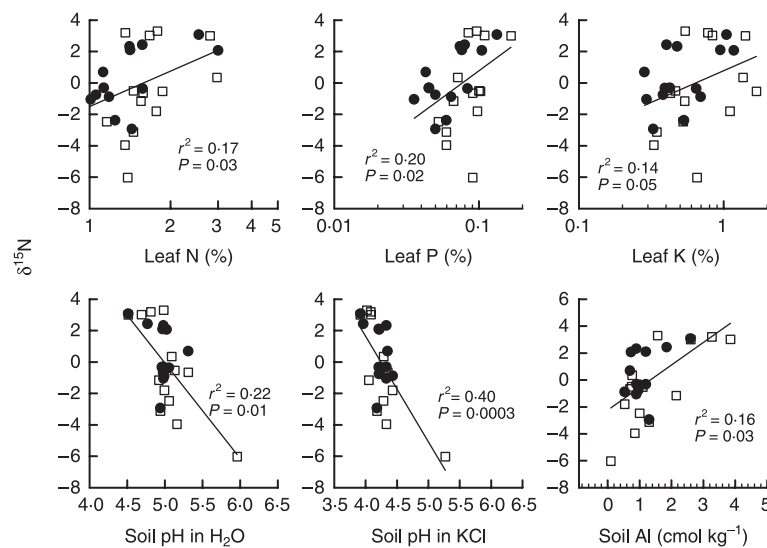


Fig. 5. Interspecific variation in $\delta^{15}\text{N}$. Filled circles, savanna species; open squares, forest species. All relationships were also statistically significant when tested among individuals, rather than among species as shown here.

possible in the study of Wright *et al.* (2001). Unfortunately we do not have corresponding data for forest species in their typical habitat, so it is unclear how this would affect the relationship between SLA and nutrient concentration. Finally, although savanna species tend to occupy drier sites, they typically do not exhibit substantial water stress during the prolonged dry season, as evidenced by high rates of transpiration and high predawn water potentials (Meinzer *et al.* 1999; Franco 2002). In the Cerrado, where upland soils are typically many metres deep and mean annual rainfall is well over 1000 mm, ample water is available to savanna trees due to their deep root systems, even after several months without precipitation. In short, there may be little selective pressure to increase WUE by increasing leaf nutrient concentrations, as water availability does not appear to strongly limit photosynthesis of established trees.

Nevertheless, the significantly higher values of $\delta^{13}\text{C}$ among savanna species indicate that these species have higher WUE (Farquhar & Richards 1984; Farquhar *et al.* 1988; Ehleringer *et al.* 1993), although, contrary to our predictions, this did not arise from higher investment in leaf N. In fact, $\delta^{13}\text{C}$ was not correlated with the concentration of N or any other nutrient, contrary to predictions. Instead, the higher $\delta^{13}\text{C}$ of savanna species appears to arise from the negative correlation between $\delta^{13}\text{C}$ and SLA, although the latter was weak and statistically significant only when examined with phylogenetically independent contrasts. Others have found similar relationships between $\delta^{13}\text{C}$ and SLA, but across precipitation gradients that confounded the relationship (Schulze *et al.* 1998; Lamont *et al.* 2002). Here, however, the relationship is independent of precipitation, indicating that leaf structure rather than water availability might explain differences in WUE between savanna and forest species.

All the individuals studied were in savanna vegetation, which was possible because long-term fire suppression has allowed forest species to establish in savanna. Of the 12 soil variables measured, only P concentration was higher under forest species relative to savanna species, which might explain the lower foliar N : P ratio of forest species. This difference could be important as the mean N : P ratio was >20 (Table 1), indicating that P is more limiting than N to plant growth (Güsewell 2004). However, there was a negative relationship between N : P ratio and SLA, suggesting that differences in SLA, rather than nutrient availability, may be responsible for differences between species types.

For the remaining soil characteristics there was no significant difference in the soils underlying savanna and forest species. This lack of a difference reflects our sampling criteria that limited sampling to individuals occurring in savanna conditions, and therefore does not indicate that savanna and forest species have similar soil preferences. Similarly, the finding that savanna and forest trees are constrained by the same functional

relationship between SLA and nutrient concentration does not suggest that their nutrient relations are similar. On the contrary, low SLA is a common trait in nutrient-poor environments, probably because it permits a longer leaf life span and increased nutrient-use efficiency (Reich *et al.* 1992). However, SLA is also under selection according to light and water availability (Gutschick 1999; Niinemets 2001), so the high irradiances and seasonal drought typical of savanna environments are likely to have contributed to the evolution of low SLA of savanna species. The SLA and nutrient concentrations tend to be correlated with litter nutrient concentrations (Wright & Westoby 2003), decomposition rate (Diaz *et al.* 2004), and palatability (Cornelissen *et al.* 2004), so the differences in leaf traits between savanna and forest species are likely to have important implications for carbon nutrient cycling in the respective habitats.

Despite the differences in nutrient concentrations between savanna and forest trees, there was no clear difference in $\delta^{15}\text{N}$ that might have indicated differences in N relations between these two groups. Other studies comparing savanna and forest sites have shown forest species to have higher $\delta^{15}\text{N}$ (Höglberg & Alexander 1995; Schmidt & Stewart 2003), so the lack of a difference here suggests that differences found by others were due to ecosystem processes rather than species-level differences.

There was, however, a strong negative relationship between soil pH and leaf $\delta^{15}\text{N}$, independent of plant type. Soil pH is known to have large effects on N cycling in ecosystems, with nitrification being inhibited by acidic conditions (Ste-Marie & Pare 1999), and soil NH_4^+ concentration sometimes becoming particularly high at low pH, relative to NO_3^- (Bigelow & Canham 2002). This is relevant because Garten (1993) found soil NH_4^+ to have a higher $\delta^{15}\text{N}$ than soil NO_3^- , and that leaf $\delta^{15}\text{N}$ was strongly related to soil NH_4^+ concentration. If similar effects of pH occur in cerrado soils, the more acidic soils would have a relatively higher abundance of isotopically heavy NH_4^+ , which should be reflected in higher $\delta^{15}\text{N}$. Unfortunately we do not have corresponding data on soil $\delta^{15}\text{N}$ and NH_4^+ and NO_3^- concentrations to examine whether this is a plausible explanation for the negative relationship between soil pH and $\delta^{15}\text{N}$. Differences in the importance of mycorrhizas has been used to explain intersite variation in foliar $\delta^{15}\text{N}$ (Hobbie *et al.* 2000), but it seems unlikely to explain the negative relationship with soil pH. We expect that mycorrhizas would become relatively more important under the acidic soils where Al^{3+} is more abundant. As N obtained from mycorrhizas is relatively depleted in ^{15}N (Evans 2001), the opposite relationship with pH would be expected.

Despite the significant relationship between foliar $\delta^{15}\text{N}$ and soil pH, the role of species-specific differences in N relations should not be dismissed, as there was significant variation among species after taking soil pH into account with ANCOVA. This interspecific

variation is consistent with the findings of Bustamante *et al.* (2004), who reported $\delta^{15}\text{N}$ values for eight of the savanna species studied here. When tested among these species, there was a strong correlation between our values and theirs ($r^2 = 0.82$, $P = 0.002$, data not shown).

Regardless of the factor responsible for these consistent differences in $\delta^{15}\text{N}$ across species, it does not appear to be highly conserved at the genus level, as evidenced by a lack of significant differences among genera (Table 1). In contrast, variation among genera was responsible for much of the interspecific variation in the concentration of most nutrients (Table 1). Therefore these traits are conserved within genera, with some genera consistently exhibiting high concentrations of an element while others exhibit low values. Interestingly, however, this was not the case for SLA, indicating that this trait was determined more strongly by the habitat of a species than by its taxonomic affinity. Therefore the tendency of some genera to have high N concentrations, for example, is not due to these genera having consistently higher values of SLA. This contrasts with the comparison between savanna and forest species, where the higher foliar N concentration of forest species can be largely explained by their greater SLA. Instead, both species within a genus tend to occupy similar positions relative to the overall relationship between N and SLA. If one species has a higher N concentration than expected based on SLA, then the other species in the genus is likely to have a higher-than-expected N concentration. For *Guapira*, *Aegiphila*, *Erythroxylum* and *Schefflera*, both species in each genus had higher N concentration than expected based on SLA, while N concentrations of *Miconia*, *Ouratea*, *Aspidosperma*, *Byrsonima*, *Myrsine*, *Symplocos* and *Pouteria* were lower than expected. Only *Tabebuia* and *Hymenaea* countered this trend, whereby the N concentration of one species was higher than expected based on its SLA, while its congener was lower than expected. Similar trends emerged for all other minerals except Fe and Mn. This suggests that, while rather large shifts in leaf nutrient concentrations have arisen within genera due to divergence in SLA between savanna and forest species, other aspects of their nutrient economy may have been conserved at the genus level. We do not have additional information to suggest whether this reflects conservatism of symbiotic associations, nutrient-uptake capacity, allocation patterns, internal recycling or some other factor, but any of these could be important.

Much remains to be understood about soil–vegetation relationships at savanna–forest boundaries. It has been widely recognized that nutrients play an important role in determining the distribution of tropical savanna and forest, and that the vegetation itself may reinforce the disparity in soil nutrient concentrations due to the higher litter accumulation and reduced fire frequency in forests relative to savanna (Kellman 1989; Bowman 2000). However, little attention has been given to the possibility that species characteristics,

not just total biomass and site conditions, may play an important role in soil–vegetation relationships at the savanna–forest boundary. The data presented here reveal fundamental differences in leaf traits and nutrient relations of savanna and forest species. Further work is needed to elucidate how these differences reflect nutrient requirements and species distributions, and how they influence nutrient and carbon cycling in these two extensive tropical biomes.

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