Oecologia (2015) 179:585–597
DOI 10.1007/s00442-015-3354-y

ECOSYSTEM ECOLOGY - ORIGINAL RESEARCH

Effects of precipitation regime and soil nitrogen on leaf traits in seasonally dry tropical forests of the Yucatan Peninsula, Mexico

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Received: 9 September 2014 / Accepted: 15 May 2015 / Published online: 27 May 2015

Abstract Leaf traits are closely associated with nutrient use by plants and can be utilized as a proxy for nutrient cycling processes. However, open questions remain, in particular regarding the variability of leaf traits within and across seasonally dry tropical forests. To address this, we considered six leaf traits (specific area, thickness, dry matter content, N content, P content and natural abundance ^{15}N) of four co-occurring tree species (two that are not associated with N_2-fixing bacteria and two that are associated with N_2-fixing bacteria) and net N mineralization rates and inorganic N concentrations along a precipitation gradient (537–1036 mm per year) in the Yucatan Peninsula, Mexico. Specifically we sought to test the hypothesis that leaf traits of dominant plant species shift along a precipitation gradient, but are affected by soil N cycling. Although variation among different species within each site explains some leaf trait variation, there is also a high level of variability across sites, suggesting that factors other than precipitation regime more strongly influence leaf traits. Principal component analyses indicated that across sites and tree species, covariation in leaf traits is an indicator of soil N availability. Patterns of natural abundance ^{15}N in foliage and foliage minus soil suggest that variation in precipitation regime drives a shift in plant N acquisition and the openness of the N cycle. Overall, our study shows that both plant species and site are important determinants of leaf traits, and that the leaf trait spectrum is correlated with soil N cycling.

Keywords Ecosystem function · Phosphorus · Stable isotopes · Soil nutrient cycling

Introduction

Foliage traits such as leaf structure and nutrient content are key determinants of plant physiology and biogeochemical cycles (Reich and Oleksyn 2004; Wright et al. 2004; Osnas et al. 2013). Considerable attention has been given to the close relationships among specific leaf area (SLA; leaf area per dry mass), leaf thickness, leaf dry matter content (LDMC; the ratio of leaf dry mass to fresh mass), leaf N content (LNC_{mass}) and the natural abundance of stable N isotopes in leaves [hereafter referred to as stable N isotope composition (δ^{15}N)] with terrestrial N cycling (Amundson et al. 2003; Orwin et al. 2010; Laughlin 2011). Leaf traits are also influenced by local adaptation and global acclimation to environmental heterogeneity (Reich 2014; Maire et al. 2015). For example, it is well known that leaf traits vary with climate; plants inhabiting drier sites have lower SLA, and greater LDMC and nutrient content than plants at wet sites (Cunningham et al. 1999; Wright et al. 2001, 2005; Santiago et al. 2004). Such relationships show that plants adjust their leaf morphology and chemical composition in response to climatic conditions. Overall, regional and global leaf trait variations along gradients of climate and soil fertility could become an important metric for the study of global patterns of terrestrial ecosystem function such as nutrient cycling (Reich et al. 1997; Craine et al. 2009; Campo et al. 2014).

Communicated by Gerardo Avalos.

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Knowledge of leaf morphology and chemistry across many ecosystem types is essential for global modeling of plant responses to climate change, but a comprehensive, quantitative evaluation of the relationship between variation in leaf traits and climate has been studied relatively little in water-limited ecosystems—especially seasonally dry tropical forests (Hulshof et al. 2014), which occupy large areas of tropical regions (Miles et al. 2006). In these forests, a major constraint on plant and ecosystem functioning is strong water seasonality (Campo et al. 2001; Giraldo and Holbrook 2011). Although most tropical forests have some degree of seasonality, predictable changes in environmental factors are likely to influence traits that are important for trees' ability to withstand seasonal drought in such ecosystems as seasonally dry tropical forests (see Gotsch et al. 2010). The present study analyses patterns of intra-specific variation in the leaf traits of four dominant species that co-occur along a precipitation gradient in the Yucatan Peninsula, Mexico. In addition, while regional-scale comparisons of precipitation tend to depict the effects of insufficient water availability at drier locations, several studies have demonstrated that precipitation gradients may impact functional leaf traits via their effects on nutrient availability as well (Cunningham et al. 1999), i.e., due to accelerated nutrient loss associated with increased precipitation (e.g., N leaching; Schuur and Matson 2001; Nardoto et al. 2008). Thus, we also examine how the variation in leaf traits relates to soil N availability. These relationships were explored for two tree species that are not associated with N_2-fixing bacteria and two species associated with N_2-fixing bacteria (e.g., species in the Fabaceae family) to determine differences among species in their N economy. By holding species constant across three sites, we examine the degree to which traits vary from a semiarid to subhumid precipitation regime. Rates of precipitation vary from 537 mm in the driest site to 1036 mm in the wettest site along our gradient, but air temperature, topography, parent material and vegetation (forest type and successional status) are all similar across the sites; as a result, these factors can be eliminated as possible causes of variation in leaf attributes and we focus our findings on differences in water availability.

Since many regions that include seasonally dry tropical forests are predicted to experience a decrease in annual precipitation (Meir and Pennington 2011), we examined the patterns of intra-specific variation in leaf attributes in the regional context of the Yucatan Peninsula by addressing the following specific questions:

1. Do leaf traits differ among species along the precipitation gradient of the Yucatan Peninsula?
2. Do the leaf traits of individual species differ among sites?
3. What are the relationships between leaf traits and soil N availability?

We hypothesized that the relationships among leaf traits and rates of precipitation are quantitatively similar at diverse sites. Alternatively, leaf traits correlations may occur, but may vary among species or along the precipitation gradient, potentially reflecting differences in N availability among sites.

We used three approaches to address these questions. First, we measured SLA, leaf thickness, LDMC, $\mathrm{LNC}_{\mathrm{mass}}$, leaf P content ($\mathrm{LPC}_{\mathrm{mass}}$) and natural abundance ^{15}N values in foliage of four dominant tree species, *Bursera simaruba*, *Gymnopodium floribundum*, *Piscidia piscipula* and *Lysiloma latisiliquum*, at each of the three seasonally dry tropical forest sites, which vary in mean annual precipitation from 537 to 1036 mm per year (Table 1). We chose these traits because they reflect aspects of leaves' nutrient economy and adaptations to water stress. Second, we examined soil N availability and other properties through measurements of natural abundance ^{15}N in soils, total N (organic plus inorganic N) and inorganic N concentration in soils, and rates of net N mineralization, and explored relationships with the six leaf traits at each of the three sites. Although $\mathrm{LPC}_{\mathrm{mass}}$ and natural abundance ^{15}N in foliage are rarely included in the leaf trait spectrum, these attributes provide information about the species' response to the precipitation gradient as well as the soil N cycle (Niklas et al. 2005). On the other hand, due to the link between leaf and soil δ^{15}N values, leaf δ^{15}N values have been shown to relate positively to rates of soil N cycling (Amundson et al. 2003; Templer et al. 2007). In addition, since natural abundance ^{15}N in leaves is initially dependent on the signature of soil organic N from which the N made available to plants is derived, the offset between soil and plant signatures is considered an index of the short-term enrichment or depletion of ^{15}N available to plants and a potentially better index of short-term processes than δ^{15}N in leaves alone (Amundson et al. 2003; Craine et al. 2009). Last, we also examined the relationship between the natural abundance ^{15}N isotopes in leaves and soils, and the difference between leaf and soil δ^{15}N [interpreted as a proxy for plant N sources along the precipitation gradient (Amundson et al. 2003)].

Materials and methods

Study sites

The study was carried out at three mature seasonal dry tropical forest sites in the Yucatan Peninsula, located along a strong north-to-south mean annual precipitation (MAP) gradient, where Chicxulub is the furthermost northern site

Table 1 General characteristics of the three studied forest sites

	Semiarid site	Intermediate site	Sub-humid site
Local name	Chicxulub	X'matkuil	Hobonil
Coordinates	21°17′N, 89°36′W	20°52′N, 89°36′W	20°00′N, 89°02′W
Altitude (m)	8	22	36
Climate[a]	BS1	Aw0	Aw1
Mean annual temperature (°C)[b]	26.4	26.6	26.2
Mean annual precipitation (mm $year^{-1}$)[b]	537	993	1036
Precipitation/potential evapotranspiration (mm mm^{-1})[b]	0.48	0.75	0.95
Mean monthly precipitation in the dry season (mm $month^{-1}$)[b]	23.6	30.5	45.2
Vegetation[c]			
Aboveground biomass (Mg ha^{-1})	47.4 ± 7.2	48.8 ± 5.4	65.6 ± 9.7
Total root biomass (Mg ha^{-1})	22.1 ± 1.2	21.0 ± 1.5	19.3 ± 0.2
Soil[d]			
Density (g cm^{-3})	0.61 ± 0.02	0.71 ± 0.03	0.80 ± 0.01
Sand (%)	74.5 ± 1.0	68.3 ± 1.7	58.4 ± 1.5
Silt (%)	1.0 ± 0.0	1.1 ± 0.1	1.7 ± 0.3
Clay (%)	21.7 ± 1.0	24.9 ± 1.2	39.9 ± 1.7
pH (H_2O)	8.1 ± 0.05	8.0 ± 0.05	7.6 ± 0.07

[a] Climate following the modified Köppen system (García 1998)

[b] Cuevas et al. (2013)

[c] Roa-Fuentes et al. (2012)

[d] J. Campo, submitted

and receives the lowest amount of precipitation (semiarid site), X'matkuil (intermediate site) is in the middle and Hobonil (sub-humid site) is located the furthest south and receives the highest rates of precipitation (Table 1).

Long-term climate data from weather stations across the Yucatan region show that all three sites are characterized by a distinct period of low precipitation [5–7 months with precipitation below 100 mm (Roa-Fuentes et al. 2012)]. The three sites strongly differ in the ratio of precipitation to potential evapotranspiration and in mean monthly precipitation during the dry season, which modulates plant water use throughout the year (Eamus et al. 2001). Mean annual temperature at the three sites is close to 26 °C, with less than a 5 °C difference between the coolest and the warmest months; all sites are semiarid to sub-humid, and support either tropical very dry or dry forest in the Holdridge life zone system (Holdridge et al. 1971).

Soils in the Yucatan Peninsula are derived from Tertiary limestone in a typical karstic landscape of flat rock outcrops and shallow depressions [karstic environment sensu Bautista et al. (2011)], and generally fall into two groups: shallow black soils (Lithic Usthortens) surrounding rock outcrops, and deeper red soils (Lithic Haplustolls) at slightly lower relief. Soils at the dry end of the gradient (i.e., semiarid site) are mainly black soils; with increased MAP there is also an increase in the cover area of red soils, which are the predominant soils at sub-humid site. Organic debris and limestone fragments make up a large portion of the soil matrix. They also contain amorphous metal oxides and more stable secondary minerals, as well as some 2:1 layer minerals such as illites (mica), talc and chlorite inherited from impurities in the weathered calcite and dolomite (Shang and Tiessen 2003).

Study species

At each forest site, sixteen 12 × 12-m plots with 8-m buffers between them were established in 2000 for an N addition experiment (Bejarano et al. 2014a). In all plots, species identity, density, frequency and basal area [derived from diameter breast height (DBH) measurements, minimum DBH $= 2.5$ cm, and tree density] of individuals were used to calculate the Curtis and McIntosh (1950) importance value (IV). These IVs were used to select the four most important tree species (i.e., dominant tree species both) with DBH ≥ 5 cm both at each site and across all sites: two non-N_2-fixing species, *Bursera simaruba* (L.) Sarg. (Burseraceae) and *Gymnopodium floribundum* Rolfe) (Polygonaceae), and two potential N_2-fixing associated species, *Piscidia piscipula* (L.) Sarg. and *Lysiloma latisiliquum* (L.) Benth; all the examined species are deciduous. All species are henceforth referred to by genus name only.

Leaf sampling

Six healthy, mature trees (one in each of the six unfertilized plot) of each of the four tree species with comparable DBH (10–11 cm) from each forest were randomly chosen for leaf sampling. On each tree, 20 twigs from the top, middle and bottom crown positions ($n = 60$ per tree total) were marked with aluminum tags for leaf sampling. Leaves of each species were collected from the three sites midway through the rainy season (September) in 2010 and 2011. Only mature and fully expanded leaves were selected from the three different canopy positions. The leaves were mixed evenly within each tree and stored in water-saturated conditions following the protocol proposed by Garnier et al. (2001).

Leaf measurements and analysis

Forty leaves were randomly selected from each tree for the complete set of leaf measurements. The area of each leaf was estimated using a leaf area meter (LI 3100C) immediately following collection. Leaf thickness was measured in four places between major veins with a digital micrometer (Mitutoyo 293). LDMC (mg dry mass g^{-1}) was determined after drying at 70 °C for 48 h, and was calculated as the ratio of leaf dry mass and previously determined fresh mass. The SLA ($cm^2\ g^{-1}$) was calculated as the ratio of leaf area to leaf dry mass, excluding the petioles and rachises for the compound-leaved species.

Forty oven-dried leaves per tree were ground, and a sub-sample was taken for the micro-Kjeldahl procedure and for determining natural abundance ^{15}N in leaves. The concentrations of total N and P in the leaves (LNC_{mass} and LPC_{mass}, respectively) were determined from acid digestion in concentrated H_2SO_4 (Anderson and Ingram 1993) using an NP elemental analyzer (Bran+Luebbe). Natural abundance ^{15}N analysis was carried out in the Stable Isotope Laboratory of Boston University, where leaves were combusted in a Euro EA elemental analyzer and the combustion gases were separated on a gas chromatography column, passed through a GV Instruments diluter and reference gas box, and analyzed for ^{15}N content on a GVI IsoPrime isotope ratio mass spectrometer.

Soil sampling and analysis

Soils at each forest site were sampled twice to determine N concentration midway through the rainy season (in September) of 2010 and 2011 (i.e., at the same time of leaf collection). On each sampling date, four cores of the upper part of the soil profile (0–10 cm in depth) below the organic layer were taken at 20 cm from the trunk under the canopy of each selected species. The four samples collected for each tree were combined in the field and stored at 4 °C for up to 48 h until processing. In the laboratory, the composite soil samples were homogenized and sieved using a 2-mm mesh and a sub-sample was dried at constant weight for moisture determination. The remaining soil was used to determine natural abundance ^{15}N and N concentrations. The soils collected at this time were also used in net N mineralization assays.

The concentration of total soil N was determined via acid digestion in concentrated H_2SO_4 using an NP elemental analyzer (Bran+Luebbe). We measured inorganic N concentrations (NO_3–N + NH_4–N) and rates of net N mineralization after each soil sampling date using a 15-day aerobic incubation method (Robertson et al. 1999). A sub-sample of soil (15 g) was extracted with 100 mL of 2 M KCl prior to the start (2 days after sample collections) of the incubation to determine initial concentrations of NH_4^+ and NO_3^- (Robertson et al. 1999). The soil KCl solution was shaken for 1 h and allowed to settle overnight (8 h). A 20-mL aliquot supernatant was transferred into sample vials and frozen for later analysis (initial inorganic N concentration). A second sub-sample was wetted to field water holding capacity with distilled water, maintained at field capacity moisture (by gravimetry) and incubated at 25 °C for 15 days before extraction with KCl (final inorganic N concentration). An analysis of both initial and final inorganic N concentrations was done with an autoanalyzer (Bran+Luebbe) system using procedures to determine NO_3–N + NO_2–N, which were reported as NO_3–N, and the salycilate–hypochlorite procedure for NH_4–N. Net N mineralization was determined from the difference in inorganic N at the beginning and end of the incubation, and results were expressed in terms of mean daily inorganic N production.

Natural abundance ^{15}N in soils was determined in the Stable Isotope Laboratory of Boston University. We calculated the difference between the natural abundance ^{15}N in leaves and soils ($\delta^{15}N_{leaf} - \delta^{15}N_{soil}$) as a means of assessing the enrichment factor (see Amundson et al. 2003). Natural abundance δ^{15}N values in leaves close to 0 ‰ were interpreted as an indicator of plants with access to N_2 via N fixation; in contrast, δ^{15}N values different from 0 ‰ were interpreted as an indication that plants receive the majority of their N from other sources (Robinson 2001).

Statistical analyses

Leaf trait values were transformed using natural logarithms before analyses to adjust to normal distribution. Leaf traits and soil N did not vary significantly between years at each of the three sites (Table 2). Therefore, we combined the results from these 2 years, and only report the effects of species and of sites. Thus, differences in leaf traits across species and sites were tested with a two-way ANOVA

Table 2 Variation between years in leaf N and P contents of dominant tree species (non-N_2-fixing species, *Bursera simaruba*, *Gymnopodium floribundum*; and potential N_2-fixing species, *Piscidia piscipula* and *Lysiloma latisiliquum*) in seasonally dry tropical forests at Yucatan Peninsula, Mexico ($n = 36$ trees per species)

	Year 1	Year 2	*F*	*P*
Leaf N content per dry mass (mg N g^{-1})				
Bursera sp.	18.6 ± 0.65	18.9 ± 0.80	0.063	0.804
Gymnopodium sp.	18.4 ± 0.92	16.7 ± 1.08	1.355	0.252
Lysiloma sp.	25.6 ± 0.79	24.2 ± 0.93	1.386	0.247
Piscidia sp.	23.0 ± 0.63	23.4 ± 0.62	0.204	0.654
Leaf P content per dry mass (mg P g^{-1})				
Bursera sp.	1.76 ± 0.13	1.48 ± 0.15	2.09	0.157
Gymnopodium sp.	1.46 ± 0.11	1.39 ± 0.11	0.239	0.628
Lysiloma sp.	2.44 ± 0.14	2.01 ± 0.19	3.65	0.064
Piscidia sp.	2.05 ± 0.16	1.90 ± 0.12	1.22	0.273

using means taken across the 2 years. Relationships among leaf attributes were investigated by calculating correlation matrices based on species means and by principal component analysis (PCA). We also used PCA to analyze total leaf trait spectrum variation (including SLA, thickness, LNCmass, and LPCmass) as a component. The leaf trait spectrum and rates of net N mineralization were used to examine bivariate relationships. Student's *t*-test was used to determine whether the $\delta^{15}N$ mean for each species was significantly different from 0 ‰. The package smatr 3.2.2 (Warton et al. 2011) was used in R to determine whether the numerical values of the slope and intercept differed. We used the package vegan 2.0-3 (Oksanen et al. 2011) to carry out the PCA. All statistical analyses were carried out using the R 2.13.1 1 program (R Development Core Team 2011).

Results

Leaf traits

Regional patterns

Leaf traits differed significantly across the precipitation gradient (Fig. 1; Table 3). The range of variation across sites was 19 % in the case of SLA (116–138 $cm^2\ g^{-1}$) and 33 % in the case of thickness (204–271 μm). SLA increased from the semiarid site to the sub-humid site (Fig. 1a), whereas leaf thickness showed the opposite pattern (the highest leaf thickness was found at the semiarid site, while the lowest appeared at the sub-humid site) (Fig. 1c). In contrast, differences in LDMC across sites were low and non-significant (range 362–377 mg g^{-1}) (Fig. 1e).

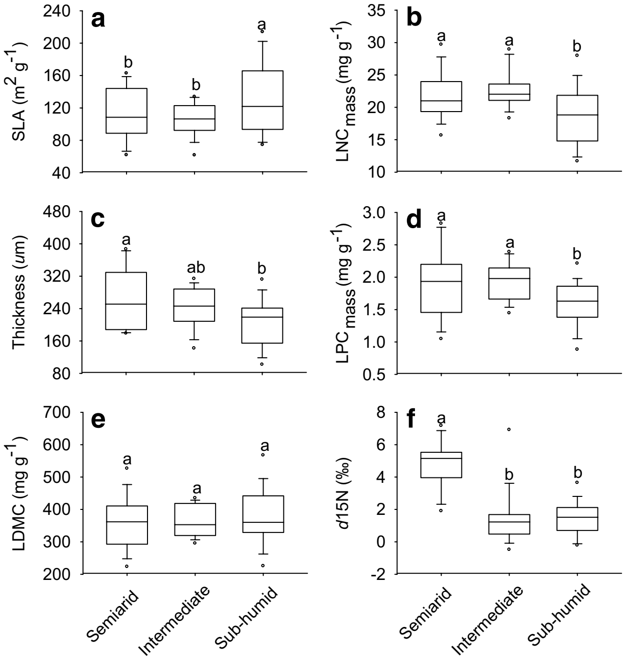

Fig. 1 Specific leaf area (*SLA*) (**a**), leaf N content per mass unit (*LNC$_{mass}$*) (**b**), leaf thickness (**c**), leaf P content per mass unit (*LPC$_{mass}$*) (**d**), leaf dry matter content (*LDMC*) (**e**) and leaf stable N isotope composition ($\delta^{15}N$; *d15N*) values (**f**) of dominant tree species in seasonally dry tropical forests along a precipitation gradient at Yucatan Peninsula, Mexico. Sites are ordered from the driest (semiarid), to wetter (intermediate) and the wettest (sub-humid). *Bottom of the box* 25th percentile, *top of the box* 75th percentile, *whiskers* smallest and largest values of the data set, *horizontal line within the box* median value. *Different letters* indicate significant differences across sites ($n = 6$)

Leaf nutrient contents differed among sites by 20 % in the case of LNC$_{mass}$ (range 18.8–22.8 mg N g^{-1}), and by 23 % in the case of LPC$_{mass}$ (1.58–1.95 mg P g^{-1}). LNC$_{mass}$ and LPC$_{mass}$ decreased with increased precipitation, and their values were the lowest at the sub-humid site (Fig. 1b, d) (Table 3). Natural abundance ^{15}N in leaves displayed more marked changes across sites, varying by a factor of 3 (range 1.5–4.7 ‰). Also, natural abundance ^{15}N in leaves declined with increased MAP, but not in a pattern analogous to the one observed for leaf N and P content (Fig. 1f). In the case of the natural abundance ^{15}N in leaves, the semiarid site had the most positive leaf $\delta^{15}N$ values. A post hoc comparison test indicated that leaves from the intermediate and sub-humid sites constituted a statistically homogeneous group.

Differences among species

Species effects in SLA were due to differences between N_2-fixing and non-N_2-fixing trees (Fig. 2a; Table 3). In addition, leaf thickness was consistently lower in *Gymnopodium* sp. (white circles, Fig. 2b; Table 3). We found a gradient in LDMC in the direction of *Lysiloma* sp. > *Gymnopodium* sp. > *Piscidia* sp. > *Bursera* sp. (Fig. 2c). The corresponding ANOVA indicated that this gradient is highly significant (Table 3) and paired comparisons using the Tukey–Kramer honest significant difference test showed that *Lysiloma* sp. leaves consistently sustained lower levels of water ($P < 0.05$ in all sites), while *Bursera* sp. leaves have the high water content ($P < 0.05$ in all sites). The leaves of *Gymnopodium* sp. and *Piscidia* sp. constituted an intermediate, statistically homogeneous group ($P > 0.05$).

Table 3 Species and site effects on leaf traits of dominant tree species in seasonally dry tropical forests along a precipitation gradient at Yucatan Peninsula, assuming the interaction site × species was not significant ($n = 6$ trees per site)

Variable	Source of variation		Total model	
	Site	Species	$F_{1,35}$	$r^2_{\text{adj.}}$
Specific leaf area (SLA)	8.45**	45.4***	14.5***	0.72
Leaf thickness	14.0***	30.3***	12.7***	0.64
Leaf dry matter content (LDMC)	0.67 NS	29.9***	30.3***	0.55
Leaf N content per dry mass (LNC_{mass})	37.1***	64.3***	28.9***	0.81
Leaf P content per dry mass (LPC_{mass})	17.7***	40.2***	16.6***	0.71
Stable N isotope composition ($\delta^{15}N$) in leaf	34.0***	9.41**	19.0***	0.74
$\delta^{15}N_{leaf} - \delta^{15}N_{soil}$	40.4***	7.78**	11.8***	0.69

NS Non-significant; ** $P < 0.01$; *** $P < 0.001$

Leaf N and P contents differed among species (Fig. 2d, e; Table 3), largely reflecting differences in nutrient requirements between potential N_2-fixing trees (21.8 ± 0.7 mg N g^{-1} and 1.77 ± 0.05 mg P g^{-1}; mean ± 1SE) and non-N_2-fixing trees (17.7 ± 1.7 mg N g^{-1} and 1.53 ± 0.04 mg P g^{-1}). Mean $\delta^{15}N$ values measured in the leaves of potential N_2-fixing trees were significantly more depleted in ^{15}N in comparison to values in leaves of non-N_2-fixing trees (by 1.2 ‰) (Fig. 2f; Table 3). Among tree species, *Piscidia* sp. had the lowest mean leaf $\delta^{15}N$ (1.1 ± 0.4 ‰), and *Gymnopodium* sp. had the highest (3.5 ± 0.6 ‰). Although the average leaf $\delta^{15}N$ value for *Bursera* sp. and *Lysiloma* sp. did not differ (2.7 ± 0.7 and 2.8 ± 0.4 ‰, respectively), *Lysiloma* sp. and *Piscidia* sp. appeared to be fixing N_2 (with leaf $\delta^{15}N$ values between 0 and 2 ‰) at both intermediate and sub-humid sites.

Relationships among leaf traits

Pairwise comparisons between SLA and LDMC showed a common slope ($P = 0.69$) indistinguishable from 1 ($r^2 = 0.95$; 95 % CI = 0.93–1.32) for all tree species across the three forests; i.e., the lamina surface area scaled isometrically with leaf dry mass. The slope for individual species decreased following the order *Piscidia* sp. (1.03; $r^2 = 0.52$, $P = 0.001$) > *Bursera* sp. (0.94; $r^2 = 0.55$, $P < 0.001$) > *Gymnopodium* sp. (0.85; $r^2 = 0.66$, $P < 0.001$) > *Lysiloma* sp. (0.77; $r^2 = 0.40$, $P < 0.01$). Numerical y-intercept values were different among species

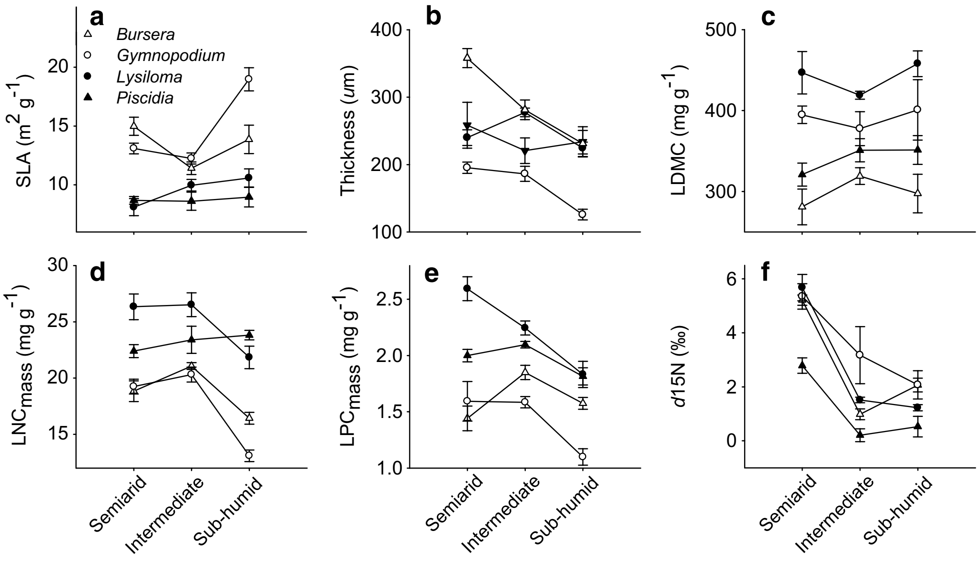

Fig. 2 SLA (**a**), leaf thickness (**b**), LDMC (**c**), LNC_{mass} (**d**), LPC_{mass} (**e**) and leaf $\delta^{15}N$ values (**f**) of dominant tree species in seasonally dry tropical forests along a precipitation gradient at Yucatan Peninsula. Sites are ordered from the driest (semiarid), to wetter (intermediate) and the wettest (sub-humid). *White symbols* Non-N_2-fixing species (*circle Gymnopodium* sp., *triangle Bursera* sp.); *black symbols* potential N_2-fixing species (*circle Lysiloma* sp., *triangle Piscidia* sp.). Data are means ± 1 SE, ($n = 6$). For abbreviations, see Fig. 1

Table 4 Pearson correlation coefficients for leaf traits of dominant tree species (non-N_2-fixing species: *Bursera* sp. and *Gymnopodium* sp., and potential N_2-fixing species: *Piscidia* sp. and *Lysiloma* sp.) in seasonally dry tropical forests at Yucatan Peninsula

Leaf traits	SLA							
	Total ($n = 72$)	Sites			Species			
		Semiarid ($n = 24$)	Intermediate ($n = 24$)	Sub-humid ($n = 24$)	*Bursera* sp. ($n = 18$)	*Gymnopodium* sp. ($n = 18$)	*Lysiloma* sp. ($n = 18$)	*Piscidia* sp. ($n = 18$)
Leaf thickness	−0.74***	−0.70**	−0.74***	−0.72***	−0.69**	−0.77**	−0.79**	−0.82***
LDMC	−0.46**	−0.42*	−0.38*	−0.55**	−0.84***	−0.49*	−0.48*	−0.33 NS
δ^{15}N in leaf	0.11 NS	−0.34 NS	0.16 NS	0.33 NS	−0.30 NS	−0.29 NS	−0.33 NS	−0.17 NS
LNC$_{\text{mass}}$	−0.51***	−0.63**	−0.15 NS	−0.56**	−0.31 NS	−0.56*	−0.26 NS	−0.39 NS
LPC$_{\text{mass}}$	−0.53***	−0.54**	−0.29 NS	−0.59**	−0.40 NS	−0.41 NS	−0.12 NS	−0.33 NS

For abbreviations, see Table 3

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 5 Total (inorganic plus organic) and inorganic N (NO_3^- and NH_4^+) concentrations, net N mineralization, natural abundance of stable ^{15}N isotopes in soils (mean ± SE) beneath non-N_2-fixing species (*Bursera* sp. and *Gymnopodium* sp.) and potential N_2-fixing species (*Piscidia* sp. and *Lysiloma* sp.) in seasonally dry tropical forests at Yucatan Peninsula ($n = 6$ trees per specie)

Parameter	Sites	Species			
		Bursera sp.	*Gymnopodium* sp.	*Lysiloma* sp.	*Piscidia* sp.
Total N (mg g^{-1})	Semiarid	28.7 ± 1.6	28.0 ± 1.3	29.6 ± 1.2	24.5 ± 1.6
	Intermediate	12.9 ± 3.7	11.6 ± 3.1	23.1 ± 1.7	23.1 ± 1.3
	Sub-humid	11.1 ± 1.8	8.4 ± 0.7	20.8 ± 1.6	28.7 ± 1.9
NO_3-N (μg g^{-1})	Semiarid	52 ± 9	36 ± 1	62 ± 8	66 ± 8
	Intermediate	15 ± 6	24 ± 6	30 ± 4	27 ± 4
	Sub-humid	13 ± 4	12 ± 2	47 ± 9	41 ± 13
NH_4-N (μg g^{-1})	Semiarid	43 ± 2	35 ± 4	35 ± 3	44 ± 2
	Intermediate	12 ± 2	10 ± 1	18 ± 3	23 ± 5
	Sub-humid	12 ± 2	10 ± 1	32 ± 4	13 ± 2
Net N mineralization (μg g^{-1} day^{-1})	Semiarid	21 ± 1	21 ± 2	30 ± 5	34 ± 2
	Intermediate	14 ± 1	9 ± 1	18 ± 2	15 ± 2
	Sub-humid	14 ± 2	12 ± 2	30 ± 4	27 ± 2
Soil δ^{15}N (‰)	Semiarid	6.48 ± 0.28	6.47 ± 0.31	6.54 ± 0.25	5.42 ± 0.28
	Intermediate	3.88 ± 0.32	4.40 ± 0.44	3.26 ± 0.48	4.03 ± 0.35
	Sub-humid	4.52 ± 0.29	4.67 ± 0.31	2.69 ± 0.21	3.41 ± 0.22

($P < 0.001$); the post hoc comparison indicates that the *y*-intercept value was higher for *Bursera* sp. compared to other species (i.e., *Gymnopodium, Lysiloma* sp., and *Piscidia* sp.). The *y*-intercept for *Gymnopodium, Lysiloma* sp., and *Piscidia* sp. constituted a statistically homogeneous group ($P = 0.93$).

Correlation analysis showed that SLA had a negative relationship with leaf thickness, LPC$_{\text{mass}}$, LNC$_{\text{mass}}$, and LDMC for the total data set, reflecting consistent leaf traits at the semiarid and sub-humid sites (Table 4). In contrast, the relationship between SLA and leaf δ^{15}N was non-significant in the total data set and in the data for each site. The negative relationship of SLA with LDMC was consistently significant across species. However, the relationships between SLA and nutrient contents were inconsistent across species.

Soil N availability to plants

Overall, total and inorganic N concentrations in soils and rates of net N mineralization decreased with increased precipitation and reflected differences between soils under potential N_2-fixing trees and those under non-N_2-fixing trees at the wetter sites ($P < 0.001$; Table 5). Our study shows that δ^{15}N in soil, like plant δ^{15}N, decreases with increased MAP, with seasonally dry tropical forests at sub-humid sites having the lowest values ($P < 0.01$), and the magnitude of the difference between plant and soil

Table 6 Difference between the natural abundance of stable ^{15}N isotopes in plants and soils beneath non-N$_2$-fixing species (*Bursera* sp. and *Gymnopodium* sp.) and potential N$_2$-fixing species (*Piscidia* sp. and *Lysiloma* sp.) in seasonally dry tropical forests at Yucatan Peninsula ($n = 6$ trees per specie)

	Sites	Species			
		Bursera sp.	*Gymnopodium* sp.	*Lysiloma* sp.	*Piscidia* sp.
$\delta^{15}N_{leaf} - \delta^{15}N_{soil}$ (‰)	Semiarid	−0.99 ± 0.20	−0.87 ± 0.12	−0.33 ± 0.17	−0.26 ± 0.20
	Intermediate	−0.20 ± 0.15	0.35 ± 0.16	0.87 ± 0.35	1.05 ± 0.39
	Sub-humid	0.54 ± 0.21	0.10 ± 0.21	1.47 ± 0.04	0.79 ± 0.25

Table 7 Eigenvalues, cumulative percent variation, and eigenvectors of the first three principal components (*PC*s) for the six leaf traits that best predicted the leaf trait spectrum; for other abbreviations, see Table 3

	PC1	PC2	PC3
Eigenvalue	2.694	1.197	0.984
Cumulative percent variation	47.32	66.28	82.87
Eigenvectors			
SLA	−0.51	0.01	−0.02
Leaf thickness	0.45	−0.51	0.15
LDMC	0.17	0.80	−0.23
LNCmass	0.50	0.05	−0.02
LPCmass	0.51	0.13	−0.01
δ^{15}N in leaf	−0.02	0.28	0.96

($\delta^{15}N_{leaf} - \delta^{15}N_{soil}$) changing from negative to positive with greater precipitation (Table 6).

N concentrations and rates of net N mineralization in soils differed among species, with the soils beneath potential N$_2$-fixing trees showing higher values and rates than those beneath non-N$_2$-fixing trees ($P < 0.01$; Table 5). Also, natural abundance ^{15}N in soils beneath non-N$_2$-fixing trees was greater than in soils beneath potential N$_2$-fixing trees, reflecting differences between both groups at the sub-humid site ($P < 0.05$). Consistently, $\delta^{15}N_{leaf} - \delta^{15}N_{soil}$ was higher for potential N$_2$-fixing trees (0.5 ± 0.4 ‰ for *Piscidia* sp., 0.7 ± 0.6 ‰ for *Lysiloma* sp.) than for non-N$_2$-fixing trees (−0.1 ± 0.4 ‰ and −0.2 ± 0.4 ‰, for *Gymnopodium* sp. and *Bursera* sp., respectively) (Tables 3, 6).

Relationships among leaf traits and N availability to plants

Leaf thickness and nutrient contents increased with increasing net N mineralization ($r^2 = 0.28$, $P = 0.02$, for leaf thickness; $r^2 = 0.39$, $P < 0.001$, and $r^2 = 0.32$, $P = 0.007$, for LNC$_{mass}$ and LPC$_{mass}$, respectively). In contrast, SLA was negatively correlated with rates of soil N mineralization ($r^2 = -0.29$, $P = 0.02$). In the case of LDMC, the data did not fit a significant trend with variation in N supply ($r^2 = 0.16$, $P = 0.19$). Leaf δ^{15}N was positively correlated with soil N mineralization ($r^2 = 0.40$, $P < 0.001$), and with δ^{15}N in soils ($r^2 = 0.89$, $P < 0.001$), indicating that the general patterns of N availability can be assessed using leaf δ^{15}N alone.

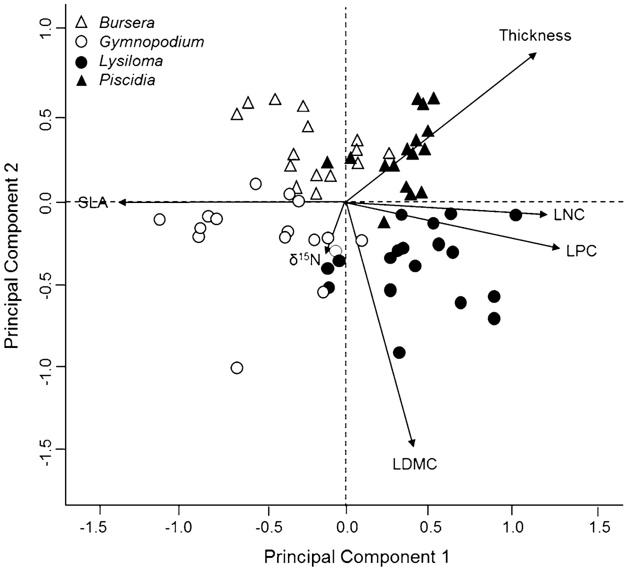

Fig. 3 Principal components (PC) analyses biplots of the leaf trait data for dominants tree species. *White symbols* Non-N$_2$-fixing species (*circle Gymnopodium* sp., *triangle Bursera* sp.); *black symbols* potential N$_2$-fixing species (*circle Lysiloma* sp., *triangle Piscidia* sp.)

Across the entire data set, the first principal axis summarized 47 percent of the variation among traits (Table 7; Fig. 3). SLA, LNC$_{mass}$, and LPC$_{mass}$ had the highest correlation scores on this axis. The second axis accounted for 19 percent of the variation. LDMC and leaf thickness explained the second-largest fraction of the explained variation in leaf traits. Species found at the negative end of the PC1 exhibited high SLA values (non-N$_2$-fixing, *Gymnopodium* sp. and *Bursera* sp.). By contrast, species found at the positive end of this spectrum (potential N$_2$-fixing, *Lysiloma* sp. and *Piscidia* sp.) exhibited high LNC$_{mass}$ and LPC$_{mass}$.

There was a significant positive relationship between rates of net N mineralization and leaf trait spectrum at all sites (Fig. 4). The linear bivariate relationship between net

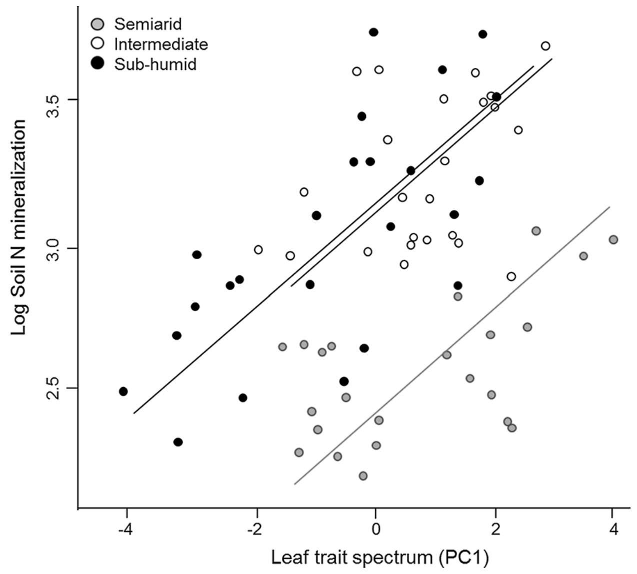

Fig. 4 Linear regression between net N mineralization in soils and leaf trait spectrum (PC1) ($n = 72$). *Gray circles* semiarid site, *white circles* intermediate site, *black circles* sub-humid site

N mineralization and the leaf traits spectrum across sites showed a common slope of 0.18 ($P = 0.10$; CI, 0.15–0.24). There were clear difference in the numerical values of the y-intercept, with lower soil N mineralization appearing at a given point in the leaf trait spectrum for the site with the lowest water availability (semiarid site, 2.46), and, by contrast, no differences between the sites with intermediate and high water availability (intermediate site, 3.16 and sub-humid site, 3.10).

Discussion

Regional patterns

Our results show that leaf traits are affected by variation in precipitation regime in seasonally dry tropical forests, a type of forest that has not been studied as extensively as tropical moist forests (e.g., Santiago et al. 2004; Poorter and Bongers 2006; Baltzer and Thomas 2010; Wright et al. 2010; and others). Ranking variations among co-occurring species among forests suggest flexibility in leaf trait adjustment in response to water availability or other factors. For example, leaf $\delta^{15}N$ declined with increased MAP for both potential N_2-fixing and non-N_2-fixing species. This pattern of decrease in leaf $\delta^{15}N$ with greater precipitation is similar to results reported in global data sets (Craine et al. 2009), and could be related to our observed changes in N supply (from both net N mineralization and soil $\delta^{15}N$, two ways of quantifying aspects of the supply of N to plants) across the Yucatan Peninsula. Overall, we cannot determine whether such intraspecific variation in this and almost all other leaf traits along the precipitation gradient (except LDMC, which was invariable) is attributable to genotypic differences, or whether it reflects plant acclimation to changes in precipitation regime (cf. Lambers et al. 2008), or both. Regardless of the explanation for intraspecific variability, it is important to highlight the fact that leaf trait varies significantly with species identity and with the sites in Yucatan's tropical forests. In any case, our results show that, in addition to interspecific variation in leaf traits leading to predictable patterns in leaf attributes in the seasonally dry tropics (Campo and Dirzo 2003; Prior et al. 2003; Powers and Tiffin 2010; Renteria and Jaramillo 2011), an environmentally induced, phenotypic response to variable amounts of moisture may also influence leaf characteristics (Gotsch et al. 2010). Aside from these intra- and interspecific variations, our research on the consequences of spatial variation in precipitation regime in the Yucatan Peninsula allows us to highlight the importance of including studies from a wide range of species and habitats within biomes (Holbrook et al. 1995; Hulshof et al. 2014), in order to derive broader applicable relationships between ecosystem function and leaf traits.

Across sites and species, we observed isometric scaling of SLA and LDMC, both morphological characteristics that underlie leaves' persistence and function in seasonally dry environments (Niklas et al. 2007; Giraldo and Holbrook 2011). We hypothesize that deciduous tree species co-occurring along precipitation gradients have different mechanisms to keep pace with increases in mature SLA across species, and to avoid reducing SLA in stressful environments; more research on such mechanisms should be conducted. On the other hand, our results did not support a positive and invariant relationship between SLA and LNC_{mass} (Shipley et al. 2005), two important traits well correlated with C gain and maximum assimilation rate by plants (Reich et al. 1997). Our data indicate that forest trees at the semiarid site (i.e., under strong water limitation) build thicker leaves (with low SLA) with high leaf N content to reduce water loss during photosynthesis (Wright et al. 2001; Pringle et al. 2011)—with low losses of leaf mass due to herbivory [2.4–5 % (Campo and Dirzo 2003)] and levels of herbivory that are lower than average for plants from other seasonally dry tropical forests [6.7–9.2 % (see Dirzo and Domínguez 1995)]. Moreover, the high leaf N content in dry habitats may have been advantageous because it allows plants to photosynthesize at high rates, taking advantage of the higher light availability (Mooney et al. 1978; Cunningham et al. 1999).

Differences between potential N_2-fixing and non-N_2-fixing trees

Differences between potential N_2-fixing and non-N_2-fixing species were generally greater than differences among sites, and both functional groups were separated by principal components analysis (Fig. 4). The leaves of potential N_2-fixing species were typically more "sclerophyllous" (e.g., thicker and with higher LDMC and lower SLA) than those of non-N_2-fixing species, suggesting that potential N_2-fixing trees reduce both water loss and susceptibility to desiccation and herbivory (Campo and Dirzo 2003) by constructing thicker leaves. Also, there are large differences in N content between both functional groups, reflecting the more efficient N assimilation process (McKey 1994) and the greater nutrient requirements of potential N_2-fixing plants as compared to non-N_2-fixing plants (Crews 1999; Lambers et al. 2008). In contrast to this difference in nutrient economy between both functional groups, the N:P ratio in leaves (calculated on a molar basis) was very consistent (27 ± 0.8 for potential N_2-fixing trees, and 26 ± 1.0 for non-N_2-fixing trees). Despite this invariant nutrient stoichiometry, our study provides general evidence that a dichotomy between potential N_2-fixing and non-N_2-fixing species can be inferred from leaf traits.

Although potential N_2-fixing species do not comprise the majority of species in seasonally dry tropical forests in the Yucatan Peninsula, they contribute the largest component of woody biomass to Yucatan vegetation [67 % of the total aboveground biomass (Ceccon et al. 2002)]. Since potential N_2-fixing leaves are characteristically more sclerophyllous than those from the counterpart set of non-fixing plants, and sclerophyllous leaves are usually associated with slow growth rates (Lambers and Poorter 1992), other factors must confer a competitive advantage to potential N_2-fixing species in these seasonally dry tropical forests. The most probable factor is the greater N and P contents in leaves that could support higher growth rates (Aerts and Chapin 2000). High N and P content in leaves could be related not only to water economy, but also to plant growth if primary productivity in the ecosystem is limited by nutrients, as occurs in seasonally dry tropical forests on the Yucatan Peninsula (Campo and Vázquez-Yanes 2004).

The relationships found in our study between natural abundance ^{15}N in leaves and precipitation regime for both potential N_2-fixing trees and non-N_2-fixing trees (Fig. 2f) are similar to those found by Austin and Vitousek (1998), Amundson et al. (2003), and Houlton et al. (2006). As in those studies, leaf ^{15}N values decreased with increased precipitation, suggesting an increasing N-limited condition with increasing precipitation, and an increased fraction of ecosystem N losses being depleted in^{15}N at the semiarid site (which has the largest N pools in soils). The high level of ^{15}N in leaves from the semiarid site suggests that trees taking up inorganic N relatively enriched in ^{15}N as a result of conditions at this site might experience relatively high rates of gaseous N loss through NH_4^+ volatilization and/or gaseous loss during nitrification and denitrification, as compared to the rates of gaseous N loss and N leaching loss at the wetter sites. Although no study has yet compared gaseous losses of N along this precipitation gradient, we found the highest soil $\delta^{15}N$ values at the dries site (Table 5), which could reflect increasing rates of N cycling associated with losses of ^{15}N-depleted inorganic N that lead to gradual ^{15}N enrichment of the remaining bulk soil N. Consequently, our study suggests that the N cycle may display greater openness with decreased precipitation in this seasonally dry tropical forest [an ecosystem that experiences high denitrification rates (Matson and Vitousek 1995)].

Across sites, the ^{15}N enrichment factor ($\delta^{15}N_{leaf} - \delta^{15}N_{soil}$) of potential N_2-fixing and non-N_2-fixing trees became negative with decreased precipitation, suggesting a climate-driven switch in plant N acquisition. Negative values are considered to result from plant uptake of inorganic soil N, which is depleted in ^{15}N compared to soil organic N (Mariotti et al. 1981). Despite this regional pattern in plant N nutrition, there is also a large variability in $\delta^{15}N_{leaf} - \delta^{15}N_{soil}$ among different species within each site, which could reflect differences in NO_3 vs. NH_4 uptake among tree species at each site. Previous studies have reported that increased NH_4 uptake leads to a gradual increase in $\delta^{15}N_{leaf} - \delta^{15}N_{soil}$ values (Miller and Bowman 2002; Houlton et al. 2007). Although the $\delta^{15}N_{leaf} - \delta^{15}N_{soil}$ values for the species investigated here varied across sites, potential N_2-fixing trees consistently had higher values than non-N_2-fixing trees, suggesting a lower NO_3:NH_4 uptake ratio. In summary, functional groups and also species within functional groups differed in δ^{15}Nleaf − δ^{15}Nsoil values, probably reflecting differences in N acquisition, but these differences varied across sites with distinct precipitation regimes, reflecting differences in soil N abundance and mineralization rates (see Table 5).

In this study, the average leaf N:P ratio (calculated on a molar basis) was lower than 31 (26 for all sites). This value has been suggested to be indicative of N-limited ecosystems (Koerselman and Meuleman 1996). Interestingly, on average there is a depletion of N within plant tissues with increased precipitation (Fig. 1b), suggesting a strong reduction in N availability at the sub-humid site. Accordingly, soil N availability decreases with increased precipitation, and N in the leaves of the potential N-fixing *Piscidia* sp. is higher at the sub-humid site than at the semiarid site (Fig. 2d), suggesting N acquisition by N fixation. This finding is consistent with the argument that the forests of the Yucatan Peninsula could be N-limited ecosystems and that this limitation increases with MAP. Moreover, the mean leaf N:P ratio in the species that we studied is closer to the lower end of the global range for tropical forests [18–170 (McGroddy et al. 2004)], and is similar to N:P ratios reported for other seasonally dry tropical forests in the Yucatan Peninsula [27 (Campo et al. 2014)]. The consistently low mean value of the N:P ratio in leaves indicates a relatively poor N content in the leaf tissues of seasonally dry tropical forests in the Yucatan Peninsula. The hypothesis of a possible N limitation in Yucatan forests could be verified with a study of primary production at the ecosystem level and an analysis of how N-limited forests respond to a release from such a limitation, as in our N-addition experiment. We do not have data on plant responses (in terms of leaf traits) to N enrichment in these seasonally dry tropical forests; however, N limitation for microbial activity in these ecosystems has been confirmed from studies of litter decomposition (Bejarano et al. 2014a) and soil C and N dynamics (Bejarano et al. 2014b), mainly at the subhumid site. Given the potential for tropical forest function subsequent to the massive impact of an increase in N deposition from the atmosphere on tropical ecosystems (Dentener et al. 2006), the topic of the present study warrants subsequent investigation.

Although the limited evidence to date indicates that the contribution of Fabaceae to N inputs by N_2 fixation in seasonally dry tropical forests is modest at the ecosystem scale (Gei and Powers 2014), N isotope values in leaves of *Lysiloma* sp. and *Piscidia* sp. from the intermediate and subhumid sites suggests that these trees derived a substantial portion of their N demand from atmospheric N_2 (Högberg 1997). Our evidence is consistent with previous studies indicating high symbiotic N_2 fixation rates by Fabaceae species in seasonally dry tropical forests (Freitas et al. 2010). At the ecosystem scale, the contribution of N inputs from symbiotic biological N_2 fixation depends on the abundance of N_2-fixing plants [generally high in these tropical forests (Pennington et al. 2009)], but also on the variability of water availability across years and between seasons (Gei and Powers 2015).

Author contribution statement L. L. R. F. and J. C. conceived and designed the experiments. L. L. R. F. and P. H. T. performed the data acquisition. L. L. R. F. and J. C. analyzed the data. L. L. R. F. and J. C. wrote the manuscript; P. H. T. participated in drafting and editing the article.

Acknowledgments We would like to thank José Erales Villamil and Carlos Nah for access to study sites. We thank Rommel Mo Aldana and Nicolas Salinas for their help in the field, and Enrique Solís for his help in the laboratory. This study was supported by PAPIIT-UNAM, no. 220610 and CONACYT 154754 grants. We are grateful to three anonymous referees for valuable and constructive criticism on earlier drafts of this paper.

Conflict of interest The authors declare no conflict of interest with the organizations that sponsored the research.

Ethical standard The experiments presented in this manuscript comply with the current laws in Mexico, where the experiments were performed.

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