Research paper

Leaf-level photosynthetic capacity dynamics in relation to soil and foliar nutrients along forest–savanna boundaries in Ghana and Brazil

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Forest–savanna boundaries extend across large parts of the tropics but the variability of photosynthetic capacity in relation to soil and foliar nutrients across these transition zones is poorly understood. For this reason, we compared photosynthetic capacity (maximum rate of carboxylation of Rubisco at 25 C° (V_{cmax}^{25}), leaf mass, nitrogen (N), phosphorus (P) and potassium (K) per unit leaf area (LMA, N_{area}, P_{area} and K_{area}, respectively), in relation to respective soil nutrients from 89 species at seven sites along forest–savanna ecotones in Ghana and Brazil. Contrary to our expectations, edaphic conditions were not reflected in foliar nutrient concentrations but LMA was slightly higher in lower fertility soils. Overall, each vegetation type within the ecotones demonstrated idiosyncratic and generally weak relationships between V_{cmax}^{25} and N_{area}, P_{area} and K_{area}. Species varied significantly in their $V_{cmax}^{25} \leftrightarrow N_{area}$ relationship due to reduced investment of total N_{area} in photosynthetic machinery with increasing LMA. We suggest that studied species in the forest–savanna ecotones do not maximize V_{cmax}^{25} per given total N_{area} due to adaptation to intermittent water availability. Our findings have implications for global modeling of V_{cmax}^{25} and forest–savanna ecotone productivity.

Keywords: carboxylation capacity, leaf traits, nitrogen, photosynthesis, tropical vegetation transitions.

Introduction

Forest–savanna boundaries represent the ecotone between tropical forest and savanna biomes, and extend across large parts of South America, Africa, Australia and Asia (Torello-Raventos et al. 2013, Veenendaal et al. 2014, Lloyd et al. 2015). These ecotones are particularly sensitive to ongoing environmental change as they are border regions between contrasting biomes, reflecting both local and regional variation in abiotic conditions (Oliveras and Malhi 2016). On one hand, increasing CO_2 may favor the expansion of forests into the savanna vegetation (Bond and Midgley 2012), and fire

exclusion policies may reinforce this advance (Staver et al. 2011). On the other hand, an intensification of the duration and intensity of dry season and of general climate variability, and an increase in maximum temperatures may favor the expansion of drought-tolerant savanna vegetation into the forest (Hely et al. 2006). Changes in floristic composition can in turn have important implications on the biodiversity, ecosystem functioning and carbon balance of these ecotones.

To date, forest–savanna boundaries have received little attention from a functional traits perspective, and comprehensive characterization of leaf economic, chemical and structural trait

distributions along the transitions is still lacking. Trait-based analyses are critical in explaining plant strategies, ecosystem functioning and species assembly mechanisms (Kraft et al. 2008). Functional traits associated with the leaf economic spectrum (leaf chemistry, structure and photosynthesis) (Wright et al. 2004) have proved to be valuable in modeling nutrient fluxes, changes in ecosystem productivity and shifts in vegetation boundaries with land-use and climate change (Yang et al. 2016, Fyllas et al. 2017a, 2017b). Therefore, understanding the diversity and variability of functional traits along the forest–savanna ecotones could provide vital insights into the mechanism governing the ecology of these transitions.

Photosynthetic capacity, expressed as maximum rate of carboxylation per area (V_{cmax}), is one of the key functional traits that determine individual plant fitness (Smith et al. 2004). This parameter has been successfully modeled at the biochemical level (Farquhar et al. 1980) and is widely used to simulate photosynthesis in terrestrial biosphere models (Kattge et al. 2009). Photosynthetic capacity is widely regarded to be limited by leaf nutrient content in the tropics, particularly by nitrogen (N) (Bahar et al. 2016, Norby et al. 2017). The $V_{cmax} \leftrightarrow N$ relationship is considered to be linear and invariant in most terrestrial biosphere models (Luo et al. 2004, Prentice et al. 2015). However, an increasing number of studies in tropical regions are observing either a variable $V_{cmax} \leftrightarrow N$ relationship (Domingues et al. 2015) or do not find any relationship (Coste et al. 2005, Dusenge et al. 2015). Lloyd et al. (2013) have suggested that, because of the many roles that foliar N can play (e.g., anti-herbivory and apoplastic immune defense, cell wall proteins (Burns et al. 2002, Wang and Dong 2011, John et al. 2017)), it is possible that $V_{cmax} \leftrightarrow N$ relationships appear to be insignificant or weak, especially when numerous species across biomes are considered. In addition to leaf N, foliar phosphorus (P) and/or potassium (K) can have a significant effect on photosynthesis along forest–savanna ecotones (Bloomfield et al. 2014, Domingues et al. 2015, Lloyd et al. 2015). Potassium is thought to be a particularly important element in these drought-prone environments as it is essential for plant osmotic processes and plays a vital role in long-distance water transport (Wang et al. 2013, Anschütz et al. 2014).

In addition to direct leaf-level nutrient limitation, V_{cmax} may be indirectly influenced by some aspects of soil fertility (Ali et al. 2015). Correlation between soil nutrients relevant for photosynthesis and corresponding foliar nutrient concentrations have been observed across Amazonia (Fyllas et al. 2009, Quesada and Lloyd 2016). However, Bloomfield et al. (2014) observed a lower slope between leaf and soil phosphorus ([P]) along the forest–savanna transition in Australia than in the Amazon. This finding was speculated to be because of the inability of studied Australian forests to utilize P efficiently, as evolutionarily they are adapted to P-limited soils. Indeed, exploring the link between soil fertility and canopy nutrient stocks can provide us with additional insights into floristic adaptations to abiotic factors.

Many forest–savanna ecotones are situated on old, nutrient-depleted soils, where soil nitrogen ([N]), potassium ([K]) and/or [P] can limit functioning of the ecosystem (Haridasan 2008, Bond 2010). Hence, exploring relationships between soil and leaf nutrient concentrations is particularly important when studying photosynthesis dynamics. These links have been addressed along the forest–savanna ecotones in the Amazon (Lloyd et al. 2015), however the findings were difficult to interpret as sites in this study were situated along extensive rainfall as well as fertility gradients. To our knowledge, only one study by Domingues et al. (2015) investigated leaf gas exchange dynamics within forest–savanna ecotone in West Africa without the complexity of rainfall variation.

Species' ecological strategies can provide additional insights into variability of photosynthesis (Niinemets et al. 1998, Eamus 1999). For example, greater constructions costs of evergreen leaves (Eamus 1999) enable them to maintain photosynthesis even during the dry season, while deciduous species maximize carbon gain during the wet season (Tomlinson et al. 2013). Nevertheless, this generally accepted variability of photosynthetic capacity among the species of different leaf habit was shown not to be the case in the Brazilian Cerrado, where deciduous and evergreen species did not differ in leaf trait values (Cianciaruso et al. 2013). The authors suggested that species of forest–savanna ecotones employ different ecological strategies not to maximize carbon gain, but to cope with strongly pronounced environmental pressures. In addition to deciduousness, the potential ability of certain Fabaceae species to fix N (Batterman et al. 2013) can also be advantageous in sites with strong seasonality; during drought, additional N can enable greater water-use efficiency (Adams et al. 2016). It is particularly important to investigate the effects of these ecological strategies on gas exchange in forest–savanna ecotones as these zones are often dominated by deciduous legumes (Lock 1989).

In this study, we addressed the above-discussed research gaps using a novel and large dataset comprising of V_{cmax} values and key nutrient concentrations for 89 tree species distributed along two forest–savanna ecotones, one in the Palaeotropics (Ghana) and another in the Neotropics (Brazil). We asked the following questions: are there significant differences in V_{cmax} along the forest–savanna transitions? Are these differences linked with soil and, consequently, foliar nutrient concentrations? What role do taxonomy and ecological strategies play in regulating V_{cmax}?

Specifically, we hypothesized that:

H1: edaphic conditions vary in a consistent direction along the forest–savanna transitions. Specifically, savanna soils exhibit lower [N], [P] and [K] values than nearby forests (Lloyd et al. 2015).

H2: differences in soil fertility along the transitions are reflected in foliar nutrient concentrations and leaf mass per unit area (LMA) (Lloyd et al. 2015).

H3: foliar nutrient concentrations have an effect on V$_{cmax}$. Specifically, P has a significant positive effect on V$_{cmax}$, especially in savanna plots (Domingues et al. 2015); K shows a negative relationship with V$_{cmax}$, especially in savanna plots

(Lloyd et al. 2015); and leaf N is positively linked with V$_{cmax}$ in all study plot types.

H4: differences in V$_{cmax}$ are reflected in plant ecological strategies. Specifically, (4a) evergreen species have lower V$_{cmax}$ than deciduous species (Eamus 1999); (4b) pioneer species have higher V$_{cmax}$ than shade-bearers (Silvestrini et al. 2007); and (4c) N$_2$-fixing species have higher foliar N (Adams et al. 2016) and, consequently, higher V$_{cmax}$.

Materials and methods

Study sites

The forest–savanna gradient in the Palaeotropics consisted of three one-hectare plots situated in close proximity (<10 km separation) in Kogyae Strict Wildlife Reserve (7°15′ N, 1°04′ W), central Ghana. The vegetation in the area is classified as dry semideciduous forest (Hall and Swaine 1981). The climate is classified as equatorial savanna with dry winter (Aw) (Kottek et al. 2006) with a mean annual precipitation (MAP) of 1030 mm year^{-1} and mean annual temperature of 26.4 °C and maximum midday temperature of 36 °C. The main wet season lasts from April through June with a secondary wet season from September to November. Soils in the plots are classified as haplic nitisols and arenosols (Table 1).

The forest–savanna gradient in the Neotropics consisted of four one-hectare plots in or near the Bacaba Municipality Reserve, Nova Xavantina, (14°41′ S; 52°20′ W), Brazil. The predominant vegetation in the area is cerrado sensu stricto, which is described as continuous grassy layer intercepted by trees with up to 60% canopy cover (Ribeiro and Walter 1998). The study plots were situated in patches of gallery and transitional (cerradão) forests and short savanna vegetation on shallow, rocky soils (cerrado rupestre). The non-forest plots (Table 1) were situated within 4 km of each other and the forest plot was 40 km from the others. The climate of the area is Aw with a MAP of 1635 mm year^{-1} and mean annual temperature of 24.9 °C (de Farias et al. 2016). The region exhibits strong seasonality, with the rainy season between October and March and a very strong dry season between May and September. The soils of the study area are ferralsols, plinthosols and leptosols (Table 1).

The study plots were classified into three different vegetation types within both forest–savanna ecotones: forest, transition and savanna (two in Brazil) plots. This classification was based on differences in species diversity, grass cover and fire occurrence and consistent with classification used by Oliveras and Malhi (2016) and Torello-Raventos et al. (2013).

Data collection

Data collection was conducted in March 2014 in Brazil and in October 2015 in Ghana. These periods were representative of the usual climate for the areas. In total we sampled 89 angiosperm tree species from 36 families (see Table S1 available as Supplementary Data at *Tree Physiology* Online) that constituted ~80% of the plot basal area (tree diameter at breast height >10 cm). For each species within a plot, we sampled three mature and canopy-emergent trees (total of 298 trees); and within each tree we randomly selected three mature (but not senescing) leaves from one fully sunlit branch, yielding a total of 831 leaf samples for LMA, N_{area}, P_{area} and K_{area} and 713 for gas exchange traits (no data were available for *Buchenavia capitata*, *Aspidosperma macrocarpon* and *Syagrus flexuosa*). The cut branch was immediately placed and recut under water.

Leaf gas exchange sampling

Leaf gas exchange traits were measured using an open flow gas exchange system LI-6400XT (Li-Cor Inc., Lincoln, NE, USA). Three leaves from each branch were sampled for light-saturated rate of net CO_2 assimilation at ambient (400 ppm) CO_2 (A_{sat}). One individual tree per species within each study plot was sampled for photosynthetic response to variation in substomatal CO_2 concentration, C_i, by generating A–C_i curves. These were measured by changing CO_2 concentration to the following sequence: 400, 300, 200, 100, 50, 400, 600, 800, 1200, 1500 and 2000 μmol m^{-2} s^{-1}. The photosynthetic photon flux density was set to 2000 μmol m^{-2} s^{-1} and block temperature was kept closest to ambient and constant throughout the sampling period at 30 °C. Measurements of other photosynthetic traits were beyond the scope of this paper.

The A–C_i curve fitting method and extraction of V_{cmax} values followed the procedure described in detail by Domingues et al. (2010). Using this method, 85 measurements (one for each sampled species) were derived for V_{cmax} and R_{day}, respiration in the light. To increase the number of V_{cmax} measurements we further estimated additional photosynthetic capacity values based on the V_{cmax} estimation from the one-point method described by De Kauwe et al. (2016):

$$V_{cmax,a} = (A_{sat} + R_{day}) \frac{(C_i + K_m)}{(C_i - \Gamma^*)} \tag{1}$$

where Γ^* is the CO_2 compensation point in the absence of day respiration (μmol mol^{-1}). The R_{day} values were calculated following Domingues et al. (2010) and available at the species level. K_m is the Michaelis–Menten constant, given by

$$K_m = K_c \left(1 + \frac{O_i}{K_o}\right) \tag{2}$$

where K_c and K_o are the Michaelis constants for CO_2 and O_2, respectively (μmol mol^{-1}), and O_i is the intercellular concentration of O_2 assumed to be 210 mmol mol^{-1}. The K_c, K_o and Γ^* values were calculated according to equations listed in De Kauwe et al. (2016) and Bernacchi et al. (2001).

Estimated V_{cmax} values were in good agreement with V_{cmax} calculated from A–C_i curves ($r^2 = 0.96$; see Figure S1 available

Table 1. Key study site characteristics. Abbreviations: mean annual precipitation (MAP); basal area of trees (BA; >10 cm DBH); total soil phosphorus ([P]); nitrogen ([N]); carbon ([C]); calcium ([Ca]); potassium ([K]); magnesium ([Mg]); soil cation exchange capacity (CEC). Soil nutrient concentrations are for the soil depth of 0–0.3 m except for CRP01 plot, where data was only available for 0–0.2 m (*). Soil chemistry data were provided by ForestPlots database Lopez-Gonzalez et al. (2011) and Moore et al. (2018).

Region	External plot code	Study vegetation classification	Forest type	MAP (mm)	BA (m² ha⁻¹)	Soil type	[P] (mg kg⁻¹)	[N] (%)	[C] (%)	[Ca] (mg kg⁻¹)	[K] (mg kg⁻¹)	[Mg] (mg kg⁻¹)	CEC (mmol⁺ kg⁻¹)	Sand (%)	Clay (%)	Silt (%)
Ghana																
	KOG01	Forest	Dry forest	1030	17.5	Haplic nitisol	67.2	0.05	0.71	378.8	42.5	75.5	26.3	82.4	2.3	15.3
	KOG03	Transition	Transitional forest	1030	13.5	Haplic arenosol	74.6	0.05	0.67	307.9	35.6	78.6	22.8	79.7	3.3	17.0
	KOG04	Savanna	Woody savanna	1030	12.4	Haplic arenosol	81.9	0.04	0.62	237.1	28.6	81.8	19.4	76.9	4.3	18.7
Brazil																
	VCR02	Forest	Tall forest	1635	12.8	Plinthosol	160.5	0.09	1.15	96.3	40.6	30.9	11.8	66.4	23.6	10.0
	NXV02	Transition	Tall closed woodland	1635	16.1	Ferralsols	129.5	0.08	1.19	50	46.5	27.5	18.1	75.3	17.8	6.9
	NXV01	Savanna 1	Savanna woodland	1635	7.5	Ferralsols	107.5	0.05	0.78	19.5	39.9	7.6	12.0	85.5	11.1	3.4
	CRP01	Savanna 2	Savanna woodland	1635	14	Lithic leptosol	171.4*	0.12*	2.7*	303.7*	73.9*	79.7*	9.9*	–	–	–

as Supplementary Data at *Tree Physiology* Online) with an overall positive bias of 3.4 µmol m⁻² s⁻¹ and root mean square error of 5.26 µmol m⁻² s⁻¹.

Finally, to make our data and findings comparable to the wider literature of photosynthetic capacity variability we scaled the measured and estimated values of V_{cmax} to a reference temperature of 25 °C following Sharkey et al. (2007) and further refer to it in text as V_{cmax}^{25}.

Leaf structure and chemistry determination

Leaves, or all leaflets if compound, were collected immediately following the gas exchange measurements, had petioles and rachis removed, and were scanned at 300 pixels per inch resolution with a digital flatbed scanner (Canon LiDE 110, London, UK) and weighed. Leaves were then placed in an oven at 70 °C and dried until they reached constant mass. Total leaf lamina area (cm²) was calculated from scanned images using NIH ImageJ (http://rsbweb.nih.gov/ij/) with a custom MATLAB script (https://github.com/bblonder/leafarea). Dried leaf mass was divided by leaf area to calculate LMA. Leaf mass per area values for leaves from Brazil were analysed and presented in Neyret et al. (2016).

Dried leaf samples were transferred to the laboratory for further nutrient analysis. Plant material were pulverized and digested in a sulfuric–salicylic acid mixture and selenium (Novozamsky et al. 1983) before colorimetric determination of N and P concentrations using a continuous flow analyser (SKALAR SAN plus system, Breda, The Netherlands). Potassium levels were measured using flame atomic emission spectroscopy (Walinga et al. 1989). Foliar nutrients were expressed per area basis (divided by LMA).

Nitrogen investments in the major photosynthetic compartments

We estimated the proportion of total leaf N invested in Rubisco, P_r, based on work by Niinemets and Tenhunen (1997) from V_{cmax}^{25} values (Eq. (3)). The proportion of foliar N in Rubisco, P_r, is expressed as:

$$P_r = V_{cmax}^{25}/6.25\,N_{area}\,V_{cr} \qquad (3)$$

where V_{cr} is the specific activity of Rubisco (20.5 qmol CO_2 [g Rubisco]⁻¹ s⁻¹ at 25 °C, Jordan and Ogren 1984), N_{area} is the total leaf nitrogen and the value of 6.25 converts nitrogen content to protein content.

Area- vs mass-based analysis

We analyse and present trait data on a per area (cf. per mass) basis. This was done because photosynthetic function is proportional to area, as only a few cell layers of mesophyll are responsible for the majority of light interception and photosynthesis rather than all of the leaf volume (Evans and Poorter 2001, Poorter et al. 2009). There are also significant mathematical

issues related to interpreting relationships among traits on a per mass basis, i.e., when they have been divided by LMA (Lloyd et al. 2013, Osnas et al. 2013). Most relevantly, this includes photosynthesis being much more variable on a mass than on an area basis due to additional resource investments in non-photosynthetic functions. This results in greater regression covariance and in 'spurious correlations' among traits. Lloyd et al. (2013) also noted that when comparing different sites and/or functional groups, mass-based photosynthesis and N relationships are potentially confounded by many factors not directly related to photosynthetic metabolism. Finally, area-based photosynthetic capacity is useful for the parameterization of terrestrial biosphere models, which utilize area-based values only (e.g., Fyllas et al. (2014)). Mass correction of photosynthesis related traits are as appropriate as area based; however, they might be more suitable when considering carbon economy, functioning and/or growth of an entire plant or when defensive and structural functions of N and LMA are discussed.

Species grouping

All tree species in Ghana were classified into three ecological guilds according to their light requirements for germination and seedling establishment: pioneers, non-pioneer light demanders and shade bearers (Hawthorne, 1995) (see Table S1 available as Supplementary Data at *Tree Physiology* Online). Pioneers have the greatest light requirements for germination and establishment, non-pioneer light demanders require light to develop beyond sapling stage and shade bearers can germinate and survive in the shaded parts of the forest. Due to lack of ecological data this classification was not available for Brazil. Additionally, the study considered 57 species with deciduous leaf habit (semideciduous and brevideciduous included) (17 in Ghana and 40 in Brazil) and 26 evergreen species (five in Ghana and 21 in Brazil) (see Table S1 available as Supplementary Data at *Tree Physiology* Online). Finally, seven species of Fabaceae family in Brazil and three in Ghana were identified as potentially capable of biological nitrogen fixation via symbiotic association with rhizobia according to Sprent (2009).

Statistical analyses

All statistical analyses were performed in RStudio version 1.1.453 (RStudio Team 2018). Differences in soil nutrients between the countries were tested using Student's *t*-test, and among the study plots using one-way ANOVA followed by post hoc Tukey's HSD test in the 'agricolae' package (de Mendiburu 2017) (H1). Pearson's Product–Moment Correlation (*r*) was used to test the relationship between soil and community-weighted (see Appendix 1 available as Supplementary Data at *Tree Physiology* Online) foliar nutrients (H2).

Hypotheses 3 and 4 were tested using linear mixed-effect models (LMMs) (package 'lme4' (Bates et al. 2015)) as they allowed to account for a hierarchical nested structure of our

dataset. Additionally, LMMs can effectively resolve the phylogenetic non-independence of data that, in our case, stems from sampling leaves from the same taxonomic units (families, species and trees).

Model assumptions were checked using protocols outlined in Zuur et al. (2009). Generally, studied traits did not follow a normal distribution due to positive or negative skewness. In most cases, squared-root transformation applied to a response variable in the LMMs resulted in model residuals conforming to normal distribution. In a small number of cases, we transformed the variables with a Box-Cox method using 'bcPower' function in 'car' package (Fox and Weisberg 2011).

We tested the effect that foliar nutrient had on the V_{cmax}^{25} values using LMMs with taxonomic units as random effects (Family/Species/Tree) (H3). The relationships were investigated at three levels: throughout the entire dataset, within each ecotone and within each study plot. We report t, P values and marginalized r^2 for fixed effects (derived using 'MuMin' package (Barton 2018)), and Akaike information criterion (AIC). To assess if a combination of foliar nutrients was more likely to explain V_{cmax}^{25} variability than individual nutrients, we compared the model structures using a goodness of fit test (function 'anova') and made the decision for the best fit model using AIC and chi-squared test (χ^2) values (Tang et al. 2014). This model structure was also used to test the effect that LMA and N_{area} have on P_r.

We tested the differences in LMA, foliar nutrient concentrations and V_{cmax}^{25} among species of different leaf deciduousness, guilds and ability to fix N (H4) with a similar model structure as for H3. In this case, we added study plot as a separate random effect to account for the uneven number of species of different ecological strategies in each site. Model significance was derived using 'anova' function with Satterthwaite's method implemented in 'lmerTest' package (Kuznetsova et al. 2017). Post-hoc Tukey's comparisons were derived using 'lsmeans' package (Lenth 2016).

Finally, we performed a variance partitioning analysis to examine the factors that best explain the variance of each studied trait (Fyllas et al. 2009). The LMM for this analysis did not include any fixed effects but incorporated study sites and taxonomic scales as random effects. The study plot was nested separately from taxonomic units to allow families and species to occur in multiple plots.

Results

Variations in leaf and soil nutrients

The soils (0–30 cm) in the Ghana transect were slightly poorer in [N] ($t_{24.86} = 3.69$, $P < 0.01$) and in [P] ($t_{17.08} = 6.59$, $P < 0.001$) and not significantly different in [K] ($t_{24.37} = 1.57$, $P = 0.13$) (Table 1). Savanna 1 plot in Brazil was found on soils of significantly lower [P] ($F_{2,15} = 121.5$, $P < 0.001$) and [N]

$(F_{2,15} = 8.76, P < 0.01)$ than closely situated transition and forest plots. There was no difference in soil fertility among the plots within the Ghana transect ($P = 0.19, P = 0.75, P = 0.09$ for [N], [P] and [K], respectively).

Overall, mean foliar nutrient concentrations were significantly different among the different study plots types ($P < 0.01$; Table 2). On average, N_{area} values were higher in Brazil, while P_{area} and K_{area} were significantly greater in the Ghana plots. Within the Brazil transect, Savanna 1 plot exhibited leaves with the highest nutrient concentrations and Forest plot had particularly low P_{area} values (0.07 g m^{-2}). The average LMA value across the study sites was 124.93 g m^{-2} (± 3.12) with the lowest and the highest values present in Brazilian Forest and Savanna 1 plots, respectively. Leaf mass–area ratio increased moving from forest to savanna in both transects, but foliar nutrients showed no consistent trends.

Correlation between [P] and P_{area} was strong and negative ($r = -0.84, P < 0.05$) and driven by the Brazilian Forest and Savanna 2 plots (Figure 1). There was no relationship between [N] and [K] and foliar nutrients when expressed on area basis. Foliar nutrient concentrations expressed on mass basis and plotted against soil nutrient concentration demonstrated very similar patterns (see Figure S2 available as Supplementary Data at *Tree Physiology* Online) with P being the only nutrient showing significant correlation with soil nutrients.

Variations in photosynthetic capacity

Photosynthetic capacity (V_{cmax}^{25}) values ranged from 4.06 to 88.72 μmol m^{-2} s^{-1}, with the mean value across all study sites being 34.45 μmol m^{-2} s^{-1}. Mean V_{cmax}^{25} was significantly different among the study plots ($F_{156.52} = 3.88, P < 0.01$; Figure 2) with the lowest values in the Brazilian Forest plot (23.91 $\pm$ 1.1 μmol m^{-2} s^{-1}) and the highest in the Brazilian Savanna 1 plot (38.83 $\pm$ 1.2 μmol m^{-2} s^{-1}). When the Brazilian Forest plot was excluded from the analysis, there was no significant difference in V_{cmax}^{25} among the study plots

($F_{151.61} = 1.98, P = 0.08$). Remarkably, the mean V_{cmax}^{25} was not significantly different between the two transects in Ghana and Brazil ($F_{78.39} = 0.79, P = 0.37$) despite the differences in soil and leaf nutrient status.

Relationships between photosynthetic capacity and foliar nutrients

We observed a large spread of $V_{cmax,a}^{25}$ values in relation to the foliar nutrient concentrations, which resulted in often weak and idiosyncratic to scale associations between the traits (Figure 3). Photosynthetic capacity was significantly affected by both N_{area} and P_{area} but not by K_{area} when the entire dataset was used for the analysis (Table 3). In the Ghana transect, N_{area} was the only foliar nutrient that had a significant (and positive) relationship with V_{cmax}^{25} ($r^2 = 0.10, t = 3.99, P < 0.001$). In Brazil, on the other hand, all nutrients showed significant positive relationships with the V_{cmax}^{25} ($P < 0.05$). Within transects, the transition plot in Ghana was the only study site where there were consistent significant positive relationships between N_{area}, P_{area} and K_{area} and V_{cmax}^{25} values ($P < 0.05$), while Ghana Forest and Brazil Savanna 2 plot did not show any significant relationships between foliar nutrients and V_{cmax}^{25}.

When foliar nutrients were assessed individually, in most cases P_{area} was the best explanatory variable to V_{cmax}^{25} (lowest AIC values and highest r^2 values; Table 3). Model fit was not improved by including more than one nutrient with two exceptions (see Table S2 available as Supplementary Data at *Tree Physiology* Online). In the model encompassing data from both ecotones, including all three nutrients resulted in a significantly improved explanatory power of V_{cmax}^{25} ($\Delta AIC = 7.3, \chi^2 = 11.34, P < 0.01, r^2 = 0.08$). Including N_{area} and P_{area} in the model using data from the Ghana transition plot also improved the model fit ($\Delta AIC = 4, \chi^2 = 5.99, P < 0.05, r^2 = 0.34$). Nevertheless, r^2 values were generally low with more than half of significant relationships having r^2 less than 0.1 (Table 3).

Table 2. Mean leaf mass per area, LMA (g m^{-2}), and area based foliar nutrient concentrations (g m^{-2}) across the study plots in Ghana and Brazil. SE represent standard errors (± 1). The denominator degrees of freedom (*Den. d.f.*) and F values are for mixed effect models comparing trait averages among the plots. Letters indicate results of post hoc tests. Significance levels: ***$P < 0.001$; **$P < 0.01$.

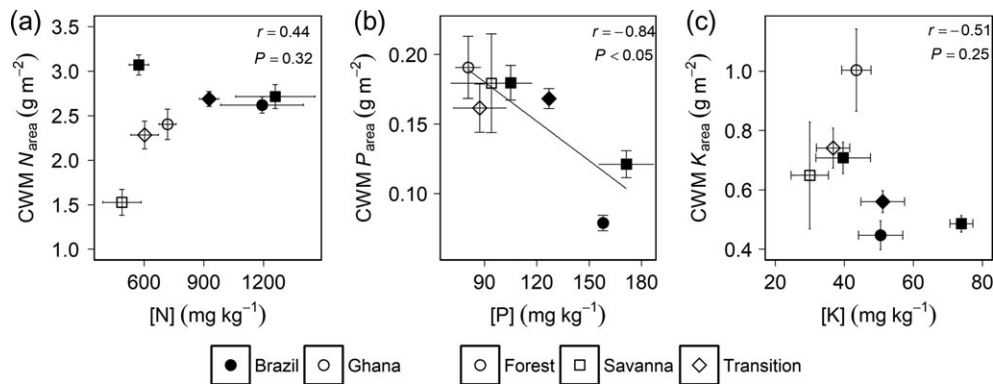| Country	Plot	LMA (g m^{-2})			N_{area} (g m^{-2})			P_{area} (g m^{-2})			K_{area} (g m^{-2})		
		Mean	SE	Post-hoc	Mean	SE	Post-hoc	Mean	SE	Post-hoc	Mean	SE	Post-hoc
Ghana	Forest	119.64	3.29	ab	2.21	0.06	ab	0.19	0.01	bc	0.82	0.03	c
	Transition	130.53	4.00	ab	2.33	0.06	bc	0.17	0.01	bc	0.73	0.03	c
	Savanna	132.07	4.62	ab	1.66	0.04	a	0.21	0.01	c	0.80	0.04	abc
Brazil	Forest	103.33	2.31	a	2.57	0.06	bcd	0.07	0.00	a	0.48	0.03	a
	Transition	122.96	2.71	a	2.63	0.05	bc	0.16	0.00	bc	0.54	0.02	ab
	Savanna 1	141.02	2.40	b	3.03	0.05	e	0.18	0.00	c	0.70	0.02	bc
	Savanna 2	124.98	2.52	ab	2.57	0.05	cd	0.12	0.00	b	0.45	0.01	a
	F		3.28			10.61			11.36			6.60	
	Den. d.f.		187.66			178.00			175.45			170.56	
	P		**			***			***			***	

Figure 1. The relationship between community weighted (CWM; Appendix 1) foliar and soil nutrient concentrations (top 20 cm; mg kg^{-1}) with standard errors: (a) nitrogen (N); (b) phosphorus (P); and (c) potassium (K). Symbols indicate vegetation types in Ghana and Brazil. P and r values are for Pearson's correlations.
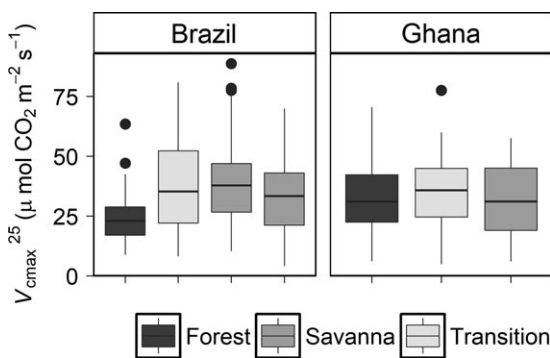

Figure 2. Area-based photosynthetic capacity (V_{cmax}^{25}) among the study plots. The box boundaries represent 25th and 75th percentiles; the median (solid line); whiskers: 10th and 90th percentiles; outliers (dots).

Within-leaf N allocation

We calculated the average proportion of total leaf N that was invested in Rubisco, P_r, to further explore the effect of N_{area} on V_{cmax}^{25}. Threefold variation of P_r was observed across the study plots with significantly higher investment of N in Rubisco in Ghana transect than in Brazil ($F_{53.69} = 4.68$, $P < 0.05$). The proportion of total N_{area} that was invested in Rubisco and bioenergetics, decreased significantly with increasing LMA ($t = -6.72$, $r^2 = 0.09$, $P < 0.001$; Figure 4a) and N_{area} ($t = -9.14$, $r^2 = 0.16$, $P < 0.001$; Figure 4b). In particular, the upper envelope of values showed a strong trend, with high P_r only occurring in low LMA and low N_{area} leaves. In low N_{area} leaves up to 30% of N was invested in Rubisco, but in high N_{area} leaves only 10% was invested. Across the datasets, mean P_r was 0.1 in Brazil and 0.13 in Ghana.

Role of ecological strategies

Foliar nutrient concentrations and V_{cmax}^{25} were not significantly different between evergreen and deciduous species, or among species of different light requirements for germination and establishment (see Table S3 available as Supplementary Data at

Tree Physiology Online). Leaf mass per area was significantly greater in evergreen species in Brazil ($F_{50.42} = 5.19$, $P < 0.05$), but not in Ghana. Species that are potentially able to fix N had significantly lower K_{area} ($F_{57.65} = 5.59$, $P < 0.05$) than species that do not fix N in Ghana (see Table S3 available as Supplementary Data at *Tree Physiology* Online), but N_{area}, P_{area}, V_{cmax}^{25} or LMA were not significantly different between these two groups.

Role of taxonomy

Variance partitioning analysis indicated that majority of the variance in V_{cmax}^{25} (37.1%) occurred within trees, while LMA, N_{area} and P_{area} mostly varied among species. Foliar potassium showed almost 50% of variance summed at intraspecific level (Figure 5).

We further investigated the $V_{cmax}^{25} \leftrightarrow N_{area}$ relationship within and among individual species. We found that this relationship was extremely variable (see Figure S3 available as Supplementary Data at *Tree Physiology* Online) with some species (8% of all species) having a strong positive V_{cmax}^{25} and N_{area} and others showing strong negative (17% of all species) or no relationship at all. The slope between V_{cmax}^{25} and N_{area} for individual species decreased with increasing LMA ($P < 0.05$; Figure 6), indicating that species with higher LMA have weaker $V_{cmax}^{25} \leftrightarrow N_{area}$ relationships.

Discussion

The overall objective of this study was to improve the limited understanding of photosynthetic capacity dynamics within and among forest–savanna ecotones in relation to key nutrients. Our study benefits from a large dataset of measured and derived V_{cmax}^{25} values (for 713 leaves from 85 species) in two forest–savanna vegetation transects in Ghana and Brazil. We found that, despite significant differences in leaf chemistry, V_{cmax}^{25} was less variable among the study sites than expected. Among the possible explanations for these patterns we considered differences
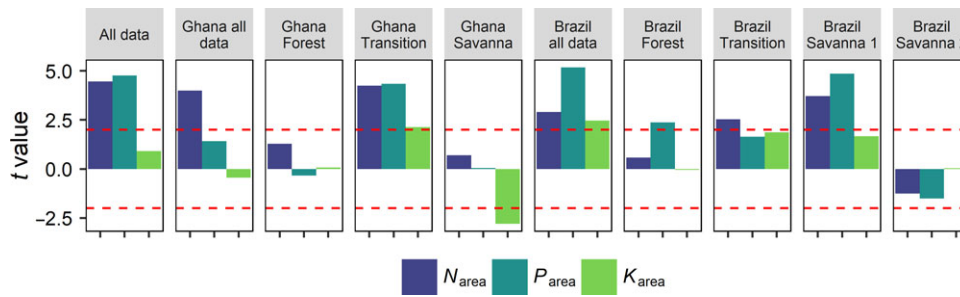
Figure 3. Comparison of foliar nutrient effects on photosynthetic capacity (V_{cmax}^{25}) within plots, ecotones and across the dataset. The effect size is t value of a mixed effect model (Table 3). The dashed lines show statistically significant effects at $\alpha = 0.05$.

Table 3. Summary of linear mixed-effects models testing relationships between photosynthetic capacity (V_{cmax}^{25}) and area based foliar nitrogen (N_{area}), phosphorus (P_{area}) and potassium (K_{area}). Family, species and tree were treated as random effects. Akaike information criterion values (AIC) are comparable horizontally. AIC values in bold indicate nutrient providing the best fit for the model. Significance levels: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s. = $P > 0.05$.

|	n	$V_{cmax}^{25} \sim N_{area}$				$V_{cmax}^{25} \sim P_{area}$				$V_{cmax}^{25} \sim K_{area}$			
		t	r^2	P	AIC	t	r^2	P	AIC	t	r^2	P	AIC
All data	576	4.46	0.05	***	1827.4	4.77	0.06	***	**1820.1**	0.91	0.00	n.s.	1843.9
Ghana all data	195	3.99	0.10	***	**641.9**	1.41	0.02	n.s.	650.4	−0.44	0.00	n.s.	655.5
Ghana Forest	82	1.29	0.03	n.s.	267.6	−0.34	0.00	n.s.	**265.0**	0.08	0.00	n.s.	268.7
Ghana Transition	61	4.24	0.26	***	202.4	4.34	0.28	***	**197.7**	2.13	0.09	*	212.4
Ghana Savanna	52	0.70	0.01	n.s.	183.7	0.05	0.00	n.s.	181.0	−2.80	0.16	*	**177.6**
Brazil all data	381	2.90	0.02	**	1186.1	5.17	0.11	***	**1163.8**	2.47	0.02	*	1185.6
Brazil Forest	51	0.58	0.01	n.s.	146.8	2.37	0.12	*	**138.3**	−0.05	0.00	n.s.	145.6
Brazil Transition	78	2.53	0.07	*	264.4	1.64	0.03	n.s.	**262.4**	1.87	0.05	n.s.	264.3
Brazil Savanna 1	168	3.71	0.08	***	514.8	4.86	0.20	***	**501.0**	1.67	0.02	n.s.	523.1
Brazil Savanna 2	84	−1.25	0.02	n.s.	266.2	−1.50	0.04	n.s.	**259.8**	0.04	0.00	n.s.	264.2
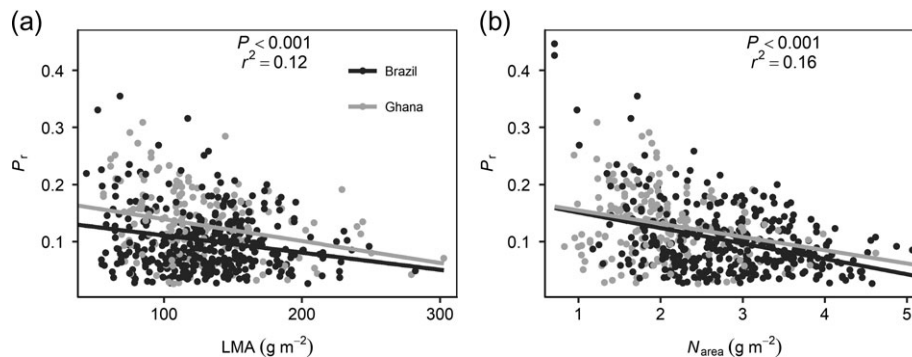
Figure 4. Fraction of total leaf nitrogen invested in Rubisco and bioenergetics, P_r, in relation to (a) leaf mass per area (LMA) and (b) leaf area-based nitrogen (N_{area}) in Ghana and Brazil.

in N partitioning between photosynthetic machinery and leaf structure, and species ecological strategies. Together, our findings question the assumption that foliar nutrient concentrations are good predictors of photosynthetic capacity at the leaf level.

Soil and canopy nutrients

Soil [P] values in the Ghana transect were at the lower end of the pan-Amazonian values reported by Quesada et al. (2010) for soils across Amazonia, whereas the values for most of the Brazil

plots were in the middle range. Soil [N] was particularly low across the study sites (average 0.6 mg g^{-1}), most likely because of high sand content in the soil (average 77.7%) (Weil and Brady 2016). Soil [K] values across our study plots were similar to those reported by Lloyd et al. (2015) for the forest–savanna ecotones in Brazil.

We expected that savanna soils will exhibit lower [N], [P] and [K] values than nearby forest and transition plots, but this was the case only in Brazil (Savanna 1 plot). This finding suggests
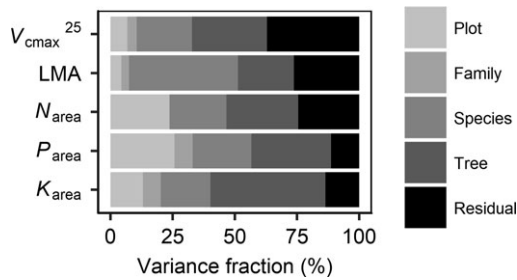
Figure 5. Partitioning of the total variance for photosynthetic capacity ($V_{cmax}{}^{25}$), leaf mass per area (LMA), and area-based nitrogen (N_{area}), phosphorus (P_{area}) and potassium (K_{area}) into taxonomic (family, species, tree, residual) and environmental (plot) components.
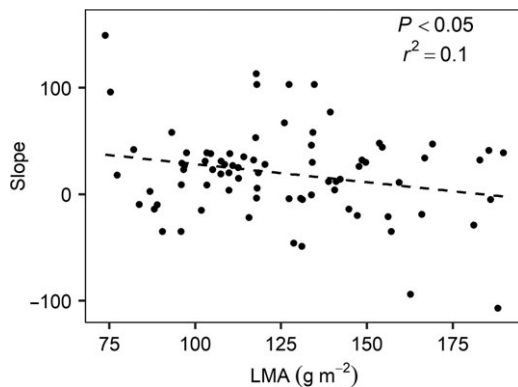

Figure 6. Slope value derived from linear regressions between photosynthetic capacity ($V_{cmax}{}^{25}$) and total leaf nitrogen (N_{area}) for each studied species (see Figure S3 available as Supplementary Data at *Tree Physiology* Online) vs species average leaf mass per area (LMA).

that at least in Ghana, local edaphic conditions were of lesser importance in determining the patchy distribution of forests and savannas as suggested by other studies on forest–savanna transitions (Gautier and Spichiger 2004, Silva et al. 2006, Veenendaal et al. 2014, Lloyd et al. 2015). In addition to edaphic factors, water availability in the soil (dependent on soil structure and drainage) and fire frequency can form and maintain the boundaries of studied ecotones (Bueno et al. 2018).

Our observations on soil and leaf nutrient concentrations do not support the assumption that soil environment is the primary determinant of foliar nutrient concentrations in forest–savanna boundaries. This is in contrast to what has previously been observed across lowland tropical forests in Amazonia (Quesada and Lloyd 2016).

The absence of significant correlations between soil and leaf nutrient concentrations could be explained by irregular water supply as decline in soil moisture impedes nutrient uptake by plants (Hu and Schmidhalter 2005). Barbosa et al. (2014) found that foliar *N* and *P* decreased in species dominating South African semi-arid savannas in response to uneven water availability. Moreover, soil and leaf *P* showed a significant negative relationship, which was driven by the Forest plot in Brazil, where

total soil *P* concentration was high (0.16 g mg^{-1}) but foliar concentrations were particularly low (0.07 g m^{-2}). One of the possible explanations for this observation is reduction of bioavailable soil *P* for plants due to frequent soil waterlogging (Steffens et al. 2005), which is characteristic of plinthosol (Sehgal et al. 1998). Additionally, high amount of clay in the soil (23.6%) might restrict *P* movement in the soil and hamper the nutrient uptake (Wiersum 1962).

It is also likely that the lack of relationship between soil and leaf nutrient levels observed in our study was due to different allocation of resources among plant organs. Dry semideciduous forests of Ghana are known to allocate more than 50% of carbon to woody production, which is significantly lower in wetter sites (Moore et al. 2018). The respective proportion of nutrient allocated to wood rather than to leaves could also increase. While nutrient levels of wood, roots and leaves are thought to be correlated (Liu et al. 2010), stressful environments can have a strong effect on these relationships. In semi-arid environments especially, wood plays an important role in nutrient storage and supply (He et al. 2015). Additionally, in environments of intermittent water supply nutrients in the wood, such as *N* and *K*, are essential for osmoregulation; *N*-based compounds are used to mobilize starch for osmotic adjustment (Martin et al. 2014), and *K* plays an important osmotic role in cambial cell expansion (Fromm 2010).

Foliar nutrient constraints on photosynthetic capacity

The studied forest–savanna ecotones had particularly low $V_{cmax}{}^{25}$ values (average 34.45 µmol m^{-2} s^{-1}) when compared with other biomes with different edaphic conditions including lowland tropical and temperate forests, coniferous trees and crops (Kattge et al. 2009, Bahar et al. 2016). Observed $V_{cmax}{}^{25}$ values were similar to values reported for 'long-grass savanna woodlands' and dry semideciduous forests in West Africa (Domingues et al. 2010, 2015).

Idiosyncratic relationships between $V_{cmax}{}^{25}$ and foliar nutrients observed here have been reported in other studies along forest–savanna transitions (Domingues et al. 2010, 2015, Bloomfield et al. 2014). In West Africa and Australia $V_{cmax}{}^{25}$ of trees growing in savanna was more limited by leaf *N* and forest species by *P*. In our study *P* had a slightly stronger effect on $V_{cmax}{}^{25}$ than *N* in most cases. This finding is in agreement with suggestions that *P* can modify, co-limit or limit $V_{cmax}{}^{25}$ more than *N* (Domingues et al. 2010, Norby et al. 2017, Walker et al. 2017). Interpretation of the $V_{cmax}{}^{25} \leftrightarrow P$ relationship is, however, much more difficult than with *N*. It is thought that *P* can co-limit regeneration of ribulose 1,5 biphophate (RuBP) or decrease activation of Rubisco (Norby et al. 2017).

It is possible that the overall lack of significant relationship between $V_{cmax}{}^{25}$ and K_{area} (except the Savanna plot in Ghana) was due to indirect effects of *K* on plant functioning. While *K* is known to have a direct positive effect on stomatal and

chloroplast functioning (Tränkner et al. 2018), in sites of intermittent water supply and drought conditions, due to the role that it plays in osmotic adjustment, K enables more efficient water retention in plant tissues, improves cell membrane stability and plant hydraulic conductance (Wang et al. 2013). Indeed, Lloyd et al. (2015) found that it was the soil rather than leaf K concentrations that were linked with photosynthetic rates in forest–savanna ecotones across the Amazon Basin.

Despite the significant trends, in most cases area-based foliar nutrient concentrations were not good predictors of the V_{cmax}^{25} values, as very little or none of the variation was explained (the highest r^2 value across the data set was 0.28; Table 3). Similarly, weak or no significant relationships were found between V_{cmax}^{25} and leaf N or P in the TROBIT (Tropical Biomes in Transition) project (Domingues et al. 2010), which spanned a rainfall gradient in West Africa. The same lack of relationship was observed in other tropical forests too, such as montane cloud and rainforests (Coste et al. 2005, van de Weg et al. 2012, Dusenge et al. 2015).

A few explanations have been proposed for the lack of significant $V_{cmax}^{25} \leftrightarrow N$ relationships. Dusenge et al. (2015) suggested that V_{cmax}^{25} was not related to N_{area} because of differences in within-leaf N allocation to photosynthetic machinery vs light harvesting compounds in pioneer and climax species. This explanation is not consistent with our findings, as classification of species into pioneer and shade-tolerant categories did not have a statistically significant effect on the $V_{cmax}^{25} \leftrightarrow N_{area}$ relationship. Coste et al. (2005) suggested that this lack of relationship is because of a proportionally greater investment of N in leaf structure in tropical rainforest species of high LMA. This suggestion is particularly fitting to our findings because the modeled fraction of N allocated towards photosynthetic machinery (Niinemets and Tenhunen 1997) declined with increasing N_{area} and LMA across the study sites (Figure 6). This pattern was observed previously: for example, the fraction of N allocated to photosynthesis decreased with increasing LMA across *Quercus* species while that to membrane-associated proteins increased (Takashima et al. 2004). Higher LMA species require greater N allocation towards structure to increase major vein allocation and mesophyll cell layers (Milla-Moreno et al. 2016, John et al. 2017).

High LMA species are characteristic of arid and semi-arid regions as denser leaves have lower water requirements under dry conditions and are more resistant to wilting (Poorter et al. 2009). The mean LMA value observed in our study, 124.9 $\pm$ 2.3 g m^{-2}, is significantly greater than that reported for tropical and temperate forests in a global study (73 and 81 g m^{-2}, respectively) (Poorter et al. 2009). As discussed above, the weak relationship between leaf total N_{area} and V_{cmax}^{25} observed here is potentially due to requirement for higher investment of N in high LMA leaves, possibly because of adaptation to intermittent water availability. This pattern was observed in Australia by Prentice et al. (2014) who found that the relationship between V_{cmax}^{25} and N_{area} is weaker in drier climates.

In addition to photosynthetic process and leaf structure, N is used for other functions. For example, up to 15% of total leaf N can be invested into cyanogenic glycosides, alkaloids and/or glucosinolates, defensive compounds against herbivory (Burns et al. 2002). These compounds can be particularly important in forest–savanna ecotones, as up to 17% of foliar biomass produced annually in Cerrado can be consumed by leaf-cutter ants alone (Costa et al. 2008). van de Weg et al. (2012) attributed the weak relationship between total N_{area} and V_{cmax}^{25} to large investment of leaf N in cyanogenic glycosides.

Finally, the lack of strong relationships between V_{cmax}^{25} and foliar nutrients observed in our study can be partially explained by variability in stomatal conductance. Despite V_{cmax}^{25} being indicative of biochemical rather than stomatal limitations on gas exchange, the relationship between V_{cmax}^{25} and stomatal conductance was significant ($r^2 = 0.26$, $P < 0.001$; data not shown). Stomatal limitation on V_{cmax}^{25} might be emphasized when performing photosynthesis measurements on cut branches (in situ measurements were not feasible). Nevertheless, we allowed cut branches to rehydrate and for stomatal conductance to stabilize after removal from the tree. This methodology generally provides reliable estimates of gas exchange (Dang et al. 1997, Cavaleri et al. 2008).

Effects of taxonomy and ecological strategies on photosynthetic capacity

N₂-fixation   As the soils in our study sites were poor in [N], we expected that N_2-fixing plants would have higher concentrations of N_{area} and, consequently, higher V_{cmax}^{25}, which was not the case. Nardoto et al. (2008) suggested that in most cases legumes within mature forests of Amazonia do not fix N, even when physiologically capable of doing so. Additionally, in both transects N fixation ability did not correlate with V_{cmax}^{25}. These findings are in agreement with global meta-analysis by Adams et al. (2016) who found that N-fixers, especially in semi-arid climates, do not have higher photosynthesis rates than non-fixing plants.

Deciduousness   Our expectation for higher V_{cmax}^{25} in deciduous species was not supported by the results. Cianciaruso et al. (2013) suggested that leaf habit does not constitute different functional groups in forest–savanna ecotones as a number of strong abiotic and biotic pressures, such as fire and herbivory, increases the plasticity of traits. Additionally, it is thought that deciduous and evergreen species differ in water uptake from the soils (Hasselquist et al. 2010) as well as crown structural traits (Esteso-Martinez et al. 2006). It is possible that the role of deciduousness is not to maximize carbon gain but to alleviate the effects of intermittent water supply. Clearly, more focused studies are required to uncover the relationship among foliar

nutrient concentrations, photosynthesis and leaf habit in forest–savanna ecotones.

Variance of photosynthetic capacity and foliar nutrients in different taxonomic levels

A variance partitioning analysis indicated high levels of trait variance within species and within trees, rather than among species. This is also supported by a low phylogenetic control over photosynthetic and leaf chemistry traits (see Appendix 1 available as Supplementary Data at *Tree Physiology* Online) and other studies (Oliveras et al., Messier et al. 2017). These findings suggest the importance of studying variability of photosynthetic traits at lower taxonomic levels. In many cases, considering species life history strategy was useful in explaining observed patterns in the trait values. For example, *Tachigali vulgaris*, a pioneer with one of the highest V_{cmax}^{25} values across the studied ecotone in Brazil (see Figure S4 available as Supplementary Data at *Tree Physiology* Online), is an extremely fast-growing and successful species in terms of recruitment in Cerrado (Morandi et al. 2016). *Bombax buonopozense*, a deciduous pioneer species with the highest V_{cmax}^{25} values in Ghana, is known for its adaptation to harsh savanna environments, specifically droughts and fires (Cardoso et al. 2016). *Bombax buonopozense* is also known for high *N* investment in herbivory defenses (Iroka et al. 2016), which could be a possible explanation for the lack of $V_{cmax}^{25} \leftrightarrow N_{area}$ relationship observed in this species (see Figure S3 available as Supplementary Data at *Tree Physiology* Online). The low V_{cmax}^{25} and nutrient values from *Vitellaria paradoxa*, a tree native to the savanna zone in Ghana, could be explained by high nutrient requirements for fruiting, as each tree can produce around ~20 kg year^{-1}. This species is also known for its slow growth and long juvenile phase. Despite low values for studied traits this species is one of the most abundant trees across the West African landscapes.

Conclusions

The proximity of the study plots within each region enabled us to investigate variation of V_{cmax}^{25} in different vegetation formations and its links with foliar nutrient concentrations without the added complexity of variation in climate, temperature or altitude. We observed a large scatter of V_{cmax}^{25} values in relation to N_{area}, P_{area} and K_{area}, which reflects different allocation of resources among leaf structure and photosynthetic machinery, multiple ecological strategies and phenological adaptations among species. Further, we can speculate that for species existing in forest–savanna ecotones it is not necessary to maximize photosynthetic capacity for a given leaf *N* to survive. A more important selective pressure may be the ability to cope with irregular availability of water and long periods of drought, which might require greater investment in *N*-osmolytes to leaf turgor maintenance. This work has demonstrated that variability in V_{cmax}^{25} values as well as $V_{cmax}^{25} \leftrightarrow N_{area}$ relationships along the forest–savanna ecotones is large, and that the leaf *N*–photosynthesis paradigm employed in many biosphere models needs to be revised in the context of tropical ecosystems.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

Conflict of interest

None declared.

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