Biome-specific effects of nitrogen and phosphorus on the photosynthetic characteristics of trees at a forest-savanna boundary in Cameroon

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**Abstract** Photosynthesis/nutrient relationships of proximally growing forest and savanna trees were determined across several sites within an ecotonal region of Cameroon (Africa). Although area-based foliar nitrogen concentrations were typically lower for savanna trees there was no difference in area-based photosynthetic rates between tree characteristics of the two vegetation formation types. Overall savanna trees appear to use N more efficiently than their forest counterparts. On the other hand, area-based phosphorus concentrations were – on average - slightly lower for forest trees and with a dependency of photosynthetic characteristics on foliar P only evident for savanna trees. Compared to many other tropical regions, soils of the study area were notably high in P. Thus the generality of the results in relation other woody plants of the terrestrial tropics remains unclear.
Introduction

Forests and savannas are the dominant vegetation types of tropical regions (Walter and Mueller-Dombois 1971) but differ fundamentally in their structural characteristics and species composition (Torello-Raventos et al. 2013). The tree species associated with forest versus savanna differ in numerous physiological characteristics such as fire survivorship (Hoffmann et al. 2009; Ratnam et al. 2011) and leaf traits (Hoffmann et al. 2005). We have, however, an incomplete knowledge on how the species differ in photosynthesis characteristics, e.g. in relation to the control of rates of photosynthesis by nutrient availability. Although tropical forests are more productive and comprise larger carbon stocks than tropical savannas, forest species often have lower maximum photosynthetic carbon assimilation rates per area of leaf (Domingues et al. 2010; Hoffmann et al. 2005). This is a finding similar to when the leaves of evergreen tropical forests are compared with those of temperate deciduous forests (Kattge et al. 2009; Meir et al. 2002) but a mechanistic explanation for such differences is lacking.

Although it has been widely demonstrated that photosynthetic capacity depends on the leaf nitrogen content (Evans 1989; Field and Mooney 1986), in the tropics, where soils are old and weathered, P limitation may be more typical (Reich and Oleksyn 2004; Reich et al. 2009) and with likely links to stand level productivity (Mercado et al. 2011; Quesada et al. 2012). On the basis of several lines of evidence, it has, however, also been suggested that, in contrast to tropical forests, savannas may be more likely to be limited by N than P (Lloyd et al. 2009).

Our earlier work from West Africa has demonstrated that both Rubisco activity and electron transport activity of African savanna and forest trees can potentially be limited by either N or P; this depending on their relative concentrations in the leaf tissue (Domingues et al. 2010).

But interpretations of forest/savanna differences in that paper were complicated by the savanna
measurements coming from a wide range of precipitation regimes with soil types that were extremely diverse.

Moreover, because trees from more arid regions had higher photosynthetic capacities at any given level of N and/or P than those from more mesic areas, no simple model emerged as generally applicable to all species examined. As the results represent a transect spanning a large climatic gradient, they may, while representative for species of the investigated forest and savanna types have been systematically biased in relation to site specific climatic conditions. Insight into the plasticity of photosynthetic traits among forest and savanna species occurring under similar climatic conditions is therefore crucial to understand the role of leaf physiology when forest changes into savanna or vice versa.

Here we report on work from a naturally occurring forest/savanna mosaic in Central Cameroon where we investigate photosynthetic and associated foliar trait characteristics of trees and shrubs for forest and savanna species growing in close proximity and thus the same climatic regime for which some areas of savanna are currently transforming into forest. We aim to test the hypotheses that under similar climatic conditions in a zone of transition:

a) Scaling between foliar nitrogen and phosphorus is different for forest and savanna species;

b) Photosynthetic nutrient efficiencies of savanna species are greater than those of the forest species;

c) Species growing in savannas show more indications of nitrogen limitation than forest species (which would in turn, be more likely to be limited by phosphorus).
Material and Methods

Study location

Measurements were made during the end of the 2007 wet season (October/December) at the Mbam-Djerem National Park, central Cameroon (Fig. 1). The area encompasses a transitional zone between the Guinea-Congo/Sudan formations (Maisels 2004; White 1983) where savannas co-exist with tall canopy forest and gallery forest in a mosaic characterised by relatively sharp boundaries (Mitchard et al. 2009). Mean annual precipitation is estimated at about 1.6 m a⁻¹ (Hijmans et al. 2005).

Study plots

Measurements were made in seven permanent one-hectare plots chosen to contain three recognizable vegetation groupings on the basis of their structure and species composition, classified by Torello-Raventos et al. (2013) as follows: (i) “long-grass savanna woodland” (three plots denoted MDJ-02, MDJ-04 and MDJ-08, these all having a fractional grass cover of greater than 0.1 and a height exceeding 1.0 m with woody strata fractional canopy cover (ς) between 0.25 and 0.50 and with an average height (H) between 6 and 12 m for trees with a diameter at breast height (D) greater than 0.1 m); (ii) three plots within the broad “forest” groupings of Torello-Raventos et al. (2013) (H > 12 m, ς > 0.5) and there being one “axylale-rich forest” (MDJ-01) one “shrub-rich forest” (MDJ-03) and one “tall forest” (MDJ-07). Finally, we included (iii) the “transitional forest” of Torello-Raventos et al. (2013) represented by a single plot MDJ-05. This is an area which was once savanna, but which had recently been invaded by forest species as described for our study area (Mitchard et al. 2011). For the interested reader, photographs of this site as well as MDJ-04 (long grass savanna) and the forested MDJ-01 and MDJ-03 are provided in Fig. 6 of Torello-Raventos et al. (2013).
Site characterisation

Biodiversity indices and measurements of plot structure were determined from measurements as detailed in Torello-Raventos et al. (2013). Soil sampling and associated measurements were made as described in Quesada et al. (2010) and Veenendaal et al. (2014).

Gas exchange characteristics

Data were obtained from 196 leaves fully exposed to the sun sampled from 69 individuals representing 42 species of adult perennial C3 trees and shrubs (Appendix A). Within each plot the quantitatively dominant species were selected, and measurements made for photosynthetic capacity ($A$-$C_i$ curves), leaf nutrients and leaf mass per unit area ($M_a$). Methodological details follow Domingues et al. (2010), with a simple modification introduced for estimation of the two key photosynthetic capacity parameters ($V_{\text{max}}$, the maximum rate of carboxylation and $J_{\text{max}}$, the maximum rate of electron transport) optionally incorporating a mesophyll conductance term ($g_m$) (Warren 2008) into the parameter estimation routine. The parameter $g_m$ is difficult to estimate from CO$_2$ response curves and the approach adopted in the present work followed two steps. A curve fit based on CO$_2$ concentrations at the intercellular air spaces ($C_i$) as reported in Domingues et al. (2010) was performed first to generate initial values of the photosynthetic capacity parameters ($V_{\text{max}}$-$C_i$ and $J_{\text{max}}$-$C_i$). Next, a second curve fit was performed incorporating $g_m$ in order to calculate CO$_2$ concentrations at the sites of carboxylation ($C_c$) using the $V_{\text{max}}$-$C_i$ and $J_{\text{max}}$-$C_i$ values as a starting point for the iteration process. To make our data comparable, the parameter fits for $V_{\text{max}}$ and $J_{\text{max}}$ were scaled to a reference temperature (25°C) as described in Bernacchi et al. (2001).

Usually three replicates (leaves) were sampled from each individual plant sampled in this study, and up to three, but sometimes one or two individuals of the same species were sampled at a given plot (Appendix A). When possible measurements were taken directly from tree
branches, but often branches were detached from trees and smaller stems were then immediately
recut under water.

Statistical and modeling analysis

For statistical comparisons of leaf traits among plots, species averages within each plot were
computed after first taking averages from replicated samples of individual plants. Statistical
inferences on the relationships of photosynthetic capacity parameters and associated leaf traits
(nutrients and/or structure) were based on both simple and multiple linear regressions using
values derived from determinations on individual leaves. Data were log_{10} transformed before
standardized major axis (SMA) (Warton et al., 2006) analyses but not before the application of an
area version of a dual-limitation model of nitrogen and phosphorus introduced by Domingues
et al. (2010) and here employed on an area basis viz.

\[
Q_{\max} = \min \left\{ \frac{a_N + b_N[N]}{a_P + b_P[P]} \right\}, \quad (1)
\]

where \(Q_{\max}\) is either \(V_{\text{max}}\) or \(J_{\text{max}}\), \(a_N\) and \(a_P\) are intercepts and \(b_N\) and \(b_P\) are slopes empirically
derived from fitting the model to the data. Model comparisons were based on evaluations of
Akaike information criteria (AIC) and Bayesian information criteria (BIC). Bootstrapping
analysis (Chernick and LaBudde 2011) were applied in order to derive confidence intervals for
parameters originated from the application of the dual-limitation model (Eq. 1) All statistical
analysis was conducted using the statistical environment R (R Development Core Team 2011).

Results

Assignment of species to the forest or savanna guilds

As described in detail by Torello-Raventos et al. (2013), species found within the forest-savanna
ecotone can usually be classified as belonging to ‘forest’ or ‘savanna’ based on their observed
distribution, although a small degree of overlap inevitably occurs. This is illustrated in Fig. 2
where the distributions of tree/shrub species ($D > 0.1$ m) are represented using a Venn-Euler
diagram. Here, the number of plant species found in more than one vegetation type is
represented numerically and proportionally by the areas of intersection among the circles. There
were only 8 out of the 164 species observed in the seven study plots that occurred in both forest
and savanna - see also Table E1 of the Supplementary Information of Torello-Raventos et al.
(2013). The transitional forest (MDJ-05) did, however, contain many savanna species, and several
species occurring in neither nearby forest nor nearby savanna plots.

Stand properties

Consistent with Fig. 2 a larger variety of families, genera and species were found at the forest
sites (Table 1). Not surprisingly, the forest plots also had larger stem density and larger basal area
(Table 1). The transitional forest plot MDJ-05 had the highest stem density but that added to a
low total basal area (Table 1). That plot also showed a relatively large number of dead standing
savanna trees (data not shown).

The measured upper layer soil physical and chemical properties (0.0-0.3 m) also varied
substantially amongst plots but not consistently between the two main study vegetation types
(Table 1). For example, effective cation exchange capacity (the sum of exchangeable bases plus
aluminium) was highest at plot MDJ-01 (forest) and MDJ-08 (savanna) and with other plots for
both forest and savanna having only about one third of those values while total soil phosphorus
varied between 307 and 977 μg g$^{-1}$ for the forest plots and 316 and 997 μg g$^{-1}$ for the savanna
plots. One upper layer measured soil parameter found to have a significant difference ($t$-test
assuming unequal variances) between forest and savanna was the C:N ratio ($p = 0.02$), this being
higher for the savanna plots.
Leaf traits

A partitioning of the measured trait variation between plots, species, individual trees, and a residual component (this representing the average variation between leaves within any given tree plus any experimental error) is shown in Fig. 3. For leaf mass per unit area ($M_a$) and nitrogen per unit area $N_a$, this shows that most of the variation not due to plot location was attributable to species identity with the proportion of variation between trees of the same species and ‘residual variation’ (i.e. attributable to within tree variability and experimental error) being relatively small. By contrast, for $P_a$ and the light/CO$_2$ saturated assimilation rate ($A_{\text{max,}a}$) most of this variation was within species or within individual trees themselves (Fig. 3). In view of this inconsistent pattern of variation among traits, we undertook all analyses on a ‘leaf-wise’ basis rather than deriving individual tree means or some sort of (often cross-plot) species average value.

Despite often considerable overlap between leaf attributes found in forest versus savanna, some differences are striking (Fig 4). For example, forest leaves typically had a lower $M_a$, higher $N_a$, a lower $P_a$ and a higher ratio of nitrogen to phosphorus (N:P). Also shown in Fig. 4 are the equivalent data for forest and savanna from the ZOT component of the West African study of Domingues et al. (2010). This shows some interesting differences, the statistically significant of which are evaluated – along with a comparison for the Cameroon forest species with South American forest - in Table 2. Taken together, Table 2 and Fig. 4 show several intra- and cross-continental differences.

Focussing first on the current study (Comparison 1) we find forest ($F$) leaves to have a higher N content than savanna ($S$) leaves, and this is both on an area and a mass basis. The effect of vegetation formation type ($\mathcal{V}$) on foliar phosphorus contents was, however, rather small; though with significantly higher for $F$ on an area basis. Despite the differences between $F$ and $S$ in both $N_a$ and $P_a$ (which we also point out were of opposite sign), there was no effect of $\mathcal{V}$ on $A_{\text{max,}a}$ (Table 2: Comparison 1).
Also in Table 2 (Comparison 2) we compare the significance of differences between forest leaves from this study in Cameroon with the earlier study from West Africa. Noting that the methodology was the same in the two locations, we find West African ZOT forest leaves to have had significantly lower $M_a$, $N_a$ and $A_{sat}$ than for Cameroon, but with about the same $P$ and $N$ and $A_{sat}$ on a mass basis.

The corresponding comparison for savanna also showed West African leaves to have a lower $M_a$, but with their nitrogen concentration, and on a leaf mass basis, photosynthetic rates basis no lower than for Cameroon. Nevertheless, because of the lower $M_a$ in West Africa, $A_{sat,m}$ were higher and with this also being associated with higher $P_m$ and $N_m$ (Table 2).

Finally, to help put our study in a broader perspective we compared our Cameroon forest data with that obtained from the forests of the Amazon Basin (Fyllas et al. 2009; Fyllas et al. 2012). That study classified Amazonian sites into two soil fertility groups on the basis of their soil chemical properties and demonstrated that ‘low nutrient soil’ forests have lower leaf N and P on both an area and mass basis, and also with higher N:P ratios than those of our Cameroon study area (Table 2). On the other hand, leaves from sites classified as ‘high nutrient soil’ by Fyllas et al. (2009) were very similar in composition to our Cameroon sites on both a mass and area basis. We therefore conclude that the African forest species sampled here are quite similar in their nitrogen and phosphorus concentrations to trees occurring on the more fertile soils of the Amazon Basin. As $M_a$ are, on average, similar, this is true on both an area and mass basis and with African forests studied here differing from Amazon Basin forests on lower nutrient status soils mostly in terms of a higher leaf phosphorus status.

Bivariate relationships

Concentrating again on the Cameroon data, Figure 5 shows the associations between $A_{sat,m}$, $M_s$, $N_s$ and $P_s$ where - noting that all savanna species were deciduous – see (Schrodt et al. 2014) we
have differentiated forest species according to their leaf habit (evergreen vs. deciduous).

Working, at the same sites in Cameroon as well as in West Africa, Schrodt et al. (2014) found this division justified on the basis of a multivariate analysis of a range of leaf traits (cation and carbon contents as well as $M_e$, $N_m$ and $P_m$).

Although the relationship between $A_{max,a}$ and $M_e$ was not significant for either of the forest habit types, we found that for the deciduous savanna species ($S_d$), there was a statistically significant positive relationship ($p=0.038$) with a (common) SMA slope of 0.13 μmol CO$_2$ g$^{-1}$ DW s$^{-1}$ but with a clear difference in intercept as compared to the two forest types (Fig. 5a). That is to say, for any given $M_e$ savanna species ($S_d$) tend to have a lower $A_{sat,a}$ than either of the forest habit types. When examined as a function of $N_a$, all three habitat groupings showed statistically significant relationships ($p < 0.10$) with $S_d$ having a photosynthetic rate about 2.5 μmol CO$_2$ m$^{-2}$ s$^{-1}$ greater than the forest species at any given $N_a$ (common slope of 5.90 μmol CO$_2$ g$^{-1}$ N s$^{-2}$)

Relationships between $A_{sat,a}$ and $P_a$ were significant only for $S_d$ (slope = 74 μmol CO$_2$ g$^{-1}$ P s$^{-1}$; $p < 0.05$) and with data for the forest evergreen leaves ($F_e$) even suggesting a negative relationship - albeit a non-significant one (Fig. 5c). The $N_a; P_a$ relationship was statistically significant for both deciduous types, with a clear difference in elevation with $S_d$ typically having a $P_a$ about 0.04 g m$^{-2}$ greater than their deciduous forest counterparts ($F_d$) at any given $N_a$ (Fig. 5d).

For all four bivariate relationships investigated there were no clear indications of trees sampled from transitional vegetation being distinct from those of either the (non-transitional) forest or savanna vegetation types. Overall, we may conclude from Fig. 5 that the strongest relationship is between $A_{sat,a}$ and $N_a$ and with the deciduous savanna species emerging as being markedly different from both forest species types, in particular, exhibiting a substantially higher mean $A_{sat,a}$ for any given $N_a$. In addition, unlike the forest species, there is also a dependence of savanna species $A_{sat,a}$ on $P_a$. At any given $N_a$ there was more P in the savanna leaves (Fig 5d).

Variations in Rubisco and electron transport capacities in relation to N and P
Although one would ideally like to model variations in both $V_{cmax}$ and $J_{max}$ in terms of the partial pressure of CO$_2$ in the chloroplast ($C_c$) this requires some reliable measure of the leaf “internal” conductance ($g_m$). Nevertheless, for the study here, both $V_{cmax}$ and $J_{max}$ ended up being estimated directly from the $A-C_i$ curve with the associated kinetic constants for $g_m = \infty$ applied (Von Caemmerer 2000). We made this decision on the basis of a) there being no significant relationship between our curve-fitting derived estimates of $g_m$ and traits previously considered to co-vary with it (e.g. $M_i$ or $\delta^{13}C$; (Niinemets 1999); b) there being no consistent differences in apparent $g_m$ between vegetation types and c) there was little systematic difference observed between $C_c$ and $C_i$ (intercellular spaces) based estimates of these photosynthetic parameters (Fig. S1).

For these $C_i$ analyses of the nutrient dependencies of $V_{cmax}$ and $J_{max}$ we also included data from the West African transect ZOT in Ghana (Domingues et al. 2010) so as to increase both the sample size and the variation of N and P observed.

Details of area-based photosynthesis-nutrient relationships as found for simple OLS linear models and the more complex dual-limitation model of Eq. (1) are shown in Table 3. For $F_i$ the best fit according to the Akaike’s Information Criterion (AIC) was the simple linear model wherein $V_{25}$ is a simple function of $N_i$ ($r^2=0.17$, $p=0.002$) and with the Bayes Information Criterion (BIC) – similar to the AIC but with more severe penalties for extra terms – giving the same rank. Of the linear models a simple dependence of $V_{25}$ on $N_i$ also gave the best fit according to the BIC, ($r^2=0.21$, $p=0.001$) but the alternative dual-limitation model of nitrogen and phosphorus (Domingues et al. 2010) being marginally better according to the AIC ($r^2=0.23$, $p=0.001$). Note, however, that in this model the $P_a$ term is negative, suggesting an inhibitory effect of phosphorus on $V_{25}$. Overall, the results for the two forest types were similar: so when combined there was, not surprisingly, an increase in the correlation coefficient values for the $N_i$ based models with the AIC suggesting the dual-limitation model ($r^2 = 0.29$) to be marginally superior to the simple $N_i$ based linear model which was unambiguously favoured when
considering the BIC. Note that in none of the forest cases was there any indication for a role for 

\( P_a \) as a modulator of \( V_{25} \) when considered on its own \((r^2 \lesssim 0.01)\), and with \( P_a \) having only a 
marginal influence when considered in conjunction with \( N_a \).

In contrast, for \( S_d \) it was found that \( P_a \) was nearly as good a predictor as \( N_a \) when 
considered on its own \((r^2 = 0.18 \text{ \textit{vs}}. 0.19)\) and with the linear model fits including both terms 
being significantly better when either \( N_a \) or \( P_a \) were considered on their own. Overall, the dual-
limitation model was, nevertheless, found superior to the OLS models according to both the 
\( AIC \) and \( BIC \) \((r^2=0.30, p < 0.001)\). Although a simple combination of the forest and savanna data 
suggest that the dual-limitation model is not the best when looking for a common (cross-biome) 
relationship– in this case it being surpassed by a model containing linear functions of \( N_a \) and \( P_a \) 
and their interaction term \( N_a \) and \( P_a \) – a simple analyses of \( AIC/BIC \) and/or the residual sum of 
squares (RSS) according to a procedure outlined in Lloyd et al. (1989) also shows that this 
combined (forest + savanna) model provides an inferior fit compared to when forest (i.e. \( F_d \) and 
\( F_e \) together) and savanna (\( S_d \)) are considered separately \((p < 0.001)\). That is to say, although for 
the forest species \( V_{25} \) showed a simple dependency upon \( N_a \) for \( S_d \) an additional role for \( P_a \) is 
clearly implicated.

A similar picture emerges when models for \( J_{25} \) are sought with little evidence of a role for 
\( P_a \) as a modulating factor for either \( F_d \) or \( F_e \) and with \( N_a \) effects apparently much more important 
for these two forest types (Table 4). As for \( V_{25} \) there is, however, a clear indication of a role for 
\( P \) for \( S_d \), and with the dual-limitation model giving the best fit. Likewise, when all data are 
combined, then comparisons of either \( AIC, BIC \) or \( RSS \) with the individual models shows that in 
any analyses of their \( J_{25} \) nutrient dependencies, forest and savanna species need to be considered 
separately.

Fitting separate relationships for both forest and savanna, the resulting goodness of 
model fit is shown for both \( V_{25} \) and \( J_{25} \) in Fig. 6. This shows that in all cases, model predictions 
involved a much smaller degree of variation than suggested by the observations. An examination
of model residuals in terms of the predictor variables $N_a$ and $P_a$ along with a range of other potentially confounding covariates such as $M_a$ and area-based cations (see Figs S2 and S3) did not however, suggest reasons for concern in terms of any trait specific systematic bias for either $V_{25}$ or $J_{25}$.

For both $V_{25}$ and $J_{25}$ the observed relationships with $N_a$ and $P_a$ are shown in Fig. 7. Here for forest we have shown the fitted lines for the modelled simple linear $N_a$ dependencies for both $V_{25}$ and $J_{25}$ but with the dual-limitation model predictions presented for $S_d$. This differentiation has been made on the basis of a bootstrapping analysis (Chernick and LaBudde 2011) of the savanna dual-limitation model results which showed that for both $V_{25}$ and $J_{25}$ the (apparently negative) $V_{25}$ and $P_a$ term was not significantly different from zero (see Table S1 in supplementary material). For $V_{25}$ our model suggests that savanna leaves with $N_a < 2.5$ g m$^{-2}$ have a higher carboxylation capacity than forest leaves (Fig. 7a).

Consistent with the simple linear $V_{25} = fN_a$ model being applied for the forest species, no fitted lines are shown for the forest species for the $P_a$ relationships of Figs 6b and 6d. In both cases, the bootstrapping analysis did, however, show the savanna co-limitation model $P_a$ slope to be significant with a 0.95 confidence interval for $V_{25}$ of 190 - 375 μmol CO$_2$ g$^{-1}$ P s$^{-1}$ with the equivalent range being 246 - 539 μmol CO$_2$ g$^{-1}$ P s$^{-1}$ for $J_{25}$.

Also shown for all four panels of Fig. 7 are fitted relationships from the original application of the dual-limitation model of (Domingues et al. 2010) to a wide range of West African tree species and location, including with a much drier climate (grey lines). In all cases the “West African” slopes are much steeper than found here for Cameroon, where the analysis has been confined to two forest-savanna transition zones and with differences at high $N_a$ and $P_a$ being particularly marked.
The question of whether the productivity of tropical forests and woodlands is controlled by the availability of nitrogen or phosphorus has long been under debate (Alvarez-Clare et al. 2013; Sollins 1998; Vitousek et al. 2010). Tropical soils are highly diverse and they are generally (but not always) old, highly weathered and low in cation exchange capacity (Jordan and Herrera 1981; Peh et al. 2011; Proctor 1987; Quesada et al. 2011; Silver 1994; Uchima and Gilman 1981; Wright et al. 2011; Young 1980). Many authors have hypothesised that growth over much of the tropics, in contrast to the temperate zone, is controlled by the availability of phosphorus rather than nitrogen, and there have been a number of experimental studies designed to test that hypothesis (Burslem et al. 1995; Gunatilleke et al. 1997; Miramanto et al. 1999; Newbery et al. 2002; Palmiotto et al. 2004). The results have been equivocal, partly because (with few exceptions, see for example Tanner et al. (1998) and Alvarez-Clare et al. (2013)) the experiments have been conducted on seedlings which are still in the processes of establishing, and partly because there appears to be inherent variation in the behaviour of different species, perhaps reflecting a varied evolutionary history and different selection pressures during the Late Glacial Maximum when tropical forests retreated into refugia (Anhuf et al. 2006; Prentice et al. 2011). In the present work we were able to evaluate the relative importance of N and P in the control of photosynthesis of a sample of many species of mature trees in two contrasting types of vegetation which were growing under identical climatic conditions in Central Africa.

The pattern of variation in the photosynthetic data showed that the area-based nitrogen content of leaves, $N_a$, was strongly a species-specific attribute, whilst for phosphorus it was more the case that $P_a$ varied within individuals of the same species (Fig. 3). Using as our individual unit of variation the individual leaf (rather than the tree or species), the best-fitting relationship between the photosynthetic parameters $V_{c(max)}$ and $J_{max}$ for both evergreen and deciduous forest
trees was a simple linear relationship with \( N_a \) and there was no relationship at all when a linear model with phosphorus content was tested. In contrast, the savanna species (all of which were deciduous) showed significant relationships with \( P_a \) as well as with \( N_a \) – albeit with a different nitrogen dependence to that found for the forest species. For the savanna species the best model was the dual-limitation model which may be considered as one application of Liebig’s Law of the Minimum, whereby photosynthesis is controlled not by the total amount of nutrients available, but by the one most limiting to carbon uptake. One might speculate that \( V_{\text{c,max}} \) would be more limited by \( N \) because of the importance of nitrogen in ribulose-1,5 bisphosphate carboxylase/oxygenase (Rubisco) whilst \( J_{\text{max}} \) would be more limited by \( P \), because of the importance of phosphorus intermediates in energy flow during photosynthesis (Morcuende et al. 2007). But this is not so in our data, presumably because carboxylation and electron transport are so tightly coupled \textit{in vivo} \( \) (see also Bloomfield et al. (2014))

With leaves intercepting light on a per-unit area basis and spurious correlations possible when two unrelated area-based entities are transformed to a mass basis – the so called ‘lulu effect’ (Lloyd et al. 2013) - we see for the current paper no practical reason to analyse our photosynthesis-nutrient dependencies on per-unit mass basis (see also Osnas et al. (2013)).

Nevertheless, for the purposes of illustration our area-based analysis is repeated on a mass basis as part of the Electronic Supplementary Material. This shows - in addition to the inevitable higher correlations associated with a ‘common-element’ correlation (Lloyd et al. 2013) – that for the forest species it is more often than not the more complex models involving not only both \( N_m \) and \( P_m \), but also their interaction that have the lowest \( AIC \) and/or \( BIC \) (Tables S2 and S3). This is as opposed to the simple forest species linear nitrogen dependency for both \( V_{25} \) and \( J_{25} \) for the area-based fits. Overall there results are consistent with the assertion that in multivariate cases a simple area-to-mass conversion can easily give rise to variables not actually associated with the dependent variable appearing to be functionally linked \( \) (Lloyd et al. 2013). We also note that whilst the area-based models showed little bias in their residuals when examined as a
function of $M_a$, N:P and a range of area based leaf-nutrient measures (Figs S2 and S3), this was not the case for the mass-based models for which there was a bias towards positive residuals at low $M_a$ (Figs S4 and S5).

With our earlier analyses using the formulation of Eqn. 1 having actually focussed on mass-based model fits (Domingues et al. 2010), this then raises the obvious question to what extent are some previous conclusions of Domingues et al. (2010) regarding the relative roles on N and P still valid? In answer, with only minor modifications they still hold. For example, in that paper we also showed that area-based fits of the min-min model implied a role for both N and P as alternate limiting factors for photosynthesis (in addition to the mass based models) and with area-based comparisons with simple linear models also showing the min-min model to have the lowest $AIC$. Indeed, the analysis here should be best considered a refinement of the work of Domingues et al. (2010), probing further into the nature of the apparent different nutrient/photosynthesis relationships identified for species associated with the different rainfall environments first identified there. Within Domingues et al. (2010) we also discussed at length possible reasons for forest trees having an apparent less efficient use of N; with these focussing on including increased allocation of N to non-photosynthetic compounds when conditions favouring a longer leaf longevity are also combined with a more variable light environment.

That when growing under the same climatic conditions we have now found forest trees photosynthesis to be less sensitive to variations in $P_a$ than for savanna species (at least at the foliar concentrations apparent in this study which were relatively high: see Table 2) requires, however, further explanation and biome history may be important here, For example any forest refugia in Africa at the LGM (Anhuf et al. 2006) would have been most likely to have occurred where both precipitation regime and soil conditions remained most favourable for forest tree function. So, with deeper tropical soils of a high water holding capacity also typically being of a low phosphorus status due to their long history of extreme weathering (Quesada et al. 2010), specific adoptions to a chronically low $P_a$ for forest trees seem likely, for example in the
replacement of phospholipids by galactolipids and sulfolipids under condition of low P-supply (Lambers et al. 2012; Tjellström et al. 2008; Zhang et al. 2014). The question remains, however, as to the extent to which the original parameterisation of Domingues et al. (2010), or the new forest parameterisation developed here, validly apply to tropical forest trees growing on low-P availability soils, such as those which cover much of the eastern Amazon Basin (Quesada et al. 2011), especially as already investigated as part of the modelling studies of Mercado et al. (2011) and Fyllas et al. (2014). Trees on such soils do, nevertheless, typically have a foliage of a much lower \( P_a \) than encountered here (Table 2) and so it will only be with further dedicated measurements under the full spectrum of \( P_a;N_a \) variability and across a range of different growth forms that we will be able to ascertain the generality (or most likely otherwise) of any photosynthesis-nutrient relationships developed.

Most likely the dual-limitation model applies because for some specific locations and/or for some particular times of the year, phosphorus is rate-limiting whilst for other times/places it is nitrogen which constrains photosynthetic productivity. As for the Amazon Basin forest case discussed above, these regional variations, arising mostly from soil variations – but also clearly depending on vegetation formation type - will give rise to variations in the rates of photosynthesis, and these are presumably important when parameterizing global vegetation models - for example Sitch et al. (2008) and Piao et al. (2013) – placing great importance on the development of realistic models of ecosystem N and P cycling that include soil biogeochemical processes in a realistic manner (Fisher et al. 2010; Goll et al. 2012; Ostle et al. 2009; Thomas et al. 2013; Xu et al. 2012; Yang et al. 2013).
Acknowledgments

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Table 1. Soil and vegetation properties of the study plots. ECEC = Effective cation exchange capacity; $M_a$ = leaf mass per unit area, $V_{25}$ = estimated maximum rate of Rubisco limited carboxylation at 25 °C; $J_{25}$ = estimated maximum rate of electron transport at 25 °C; $N_a$ = leaf nitrogen per unit area; $P_a$ = leaf phosphorus per unit area.

<table>
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<tr>
<th>Location</th>
<th>MDJ-01</th>
<th>MDJ-03</th>
<th>MDJ-07</th>
<th>MDJ-05</th>
<th>MDJ-02</th>
<th>MDJ-04</th>
<th>MDJ-08</th>
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<td>25.6</td>
<td>25.6</td>
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<td>4.70</td>
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<td>5.32</td>
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<td>11.5</td>
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<td>307</td>
<td>738</td>
<td>576</td>
<td>997</td>
<td>316</td>
<td>364</td>
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<td>ECEC, mmol eq kg⁻¹</td>
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<td>10.5</td>
<td>7.3</td>
<td>1.6</td>
<td>9.3</td>
<td>5.2</td>
<td>16.4</td>
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<td>Leaf traits (mean ± standard deviation)</td>
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<td>$M_a$, g m⁻²</td>
<td>80±31</td>
<td>97±26</td>
<td>109±21</td>
<td>113±41</td>
<td>136±23</td>
<td>127±26</td>
<td>135±32</td>
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<td>$V_{25}$, μmol m⁻² s⁻¹</td>
<td>39.2±13.6</td>
<td>42.3±12.2</td>
<td>45.7±12.1</td>
<td>44.8±8.3</td>
<td>54.6±11.8</td>
<td>39.2±9.2</td>
<td>27.8±0.1</td>
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<td>$J_{25}$, μmol m⁻² s⁻¹</td>
<td>76.8±20.5</td>
<td>79.3±19.5</td>
<td>88.2±17.8</td>
<td>81.0±18.6</td>
<td>87.6±17.5</td>
<td>67.9±12.3</td>
<td>47.5±10.2</td>
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<td>$N_a$, g m⁻²</td>
<td>1.97±0.55</td>
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<td>2.12±0.56</td>
<td>2.67±1.39</td>
<td>1.48±0.27</td>
<td>1.44±0.06</td>
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<td>$P_a$, g m⁻²</td>
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<td>0.10±0.03</td>
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<td>-30.5±0.3</td>
<td>-30.3±0.3</td>
<td>-29.3±0.1</td>
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Table 2. Comparisons of the main leaf traits (shown as medians) between (1) forest and savanna in Cameroon (this study); (2) and (3) forest and savanna in this study as compared to a previous study in West Africa (Domingues et al. 2010); (4) forest in this study as compared to a previous study in Amazon Basin for which soils have been classified into two fertility groups (Fyllas et al. 2009). Asterisks denote the result of a Kruskal-Wallis test of significance (*p* < 0.05, **p** < 0.01, ***p** < 0.001).

<table>
<thead>
<tr>
<th>Location</th>
<th>Vegetation Type</th>
<th>M (g m⁻²)</th>
<th>N (g m⁻²)</th>
<th>P (g m⁻²)</th>
<th>A_{sat,a} (μmol m⁻² s⁻¹)</th>
<th>A_{sat,m} (μmol g⁻¹ s⁻¹)</th>
<th>Nm (mg g⁻¹)</th>
<th>Pm (mg g⁻¹)</th>
<th>N:P (g g⁻¹)</th>
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<tbody>
<tr>
<td>Cameroon</td>
<td>Forest</td>
<td>95</td>
<td>2.12</td>
<td>0.10</td>
<td>10.3</td>
<td>23.5</td>
<td>1.11</td>
<td>20.9</td>
<td>0.105</td>
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<tr>
<td>Cameroon</td>
<td>Savanna</td>
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<td>12.8</td>
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<tr>
<td>West Africa</td>
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<td>1.76</td>
<td>0.11</td>
<td>8.6</td>
<td>24.7</td>
<td>1.11</td>
<td>18.6</td>
<td>0.105</td>
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<tr>
<td>West Africa</td>
<td>Savanna</td>
<td>92</td>
<td>1.73</td>
<td>0.14</td>
<td>9.8</td>
<td>18.5</td>
<td>1.68</td>
<td>12.7</td>
<td>0.107</td>
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<tr>
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<td>Forest</td>
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<td>1.90</td>
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<td>ND</td>
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<td>ND</td>
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<td>0.11</td>
<td>ND</td>
<td>21.6</td>
<td>1.11</td>
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<tr>
<td>Amazon</td>
<td>&quot;high nutrient soil&quot; Forest</td>
<td>95</td>
<td>2.09</td>
<td>0.11</td>
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<td>21.6</td>
<td>1.11</td>
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<td>Equation</td>
<td>$\hat{r}$</td>
<td>AIC</td>
<td>BIC</td>
<td>$p$</td>
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<tr>
<td><strong>Forest Evergreen</strong></td>
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<tr>
<td>$V_{\text{EV}} = 16.43 + 11.72 N_i$</td>
<td>0.17</td>
<td>371.35</td>
<td>377.14</td>
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<td>381.21</td>
<td>387.01</td>
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<td>380.77</td>
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<tr>
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<td>830.27</td>
<td>838.23</td>
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<td>854.61</td>
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<td>842.36</td>
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<tr>
<td>$V_{\text{F+D}} = 18.61 + 12.03 N_i$</td>
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<td>1208.24</td>
<td>1217.39</td>
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<td>&lt;0.001</td>
<td></td>
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<tr>
<td>$V_{\text{S}} = 22.20 + 5.27 N_i + 66.68 P_a$</td>
<td>0.23</td>
<td>775.52</td>
<td>785.98</td>
<td>&lt;0.001</td>
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<tr>
<td>$V_{\text{S}} = 1.57 + 17.67 N_i + 167.62 P_a - 57.46 N_i P_a$</td>
<td>0.27</td>
<td>770.21</td>
<td>783.29</td>
<td>&lt;0.001</td>
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<tr>
<td>$V_{\text{S}} = \min(34.87 + 5.83 N_i; 9.79 + 251.86 P_a)$</td>
<td><strong>0.30</strong></td>
<td>765.86</td>
<td>779.94</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>Forest and Savanna (Deciduous and Evergreen)</strong></td>
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<tr>
<td>$V_{\text{F&amp;S}} = 22.13 + 10.46 N_i$</td>
<td>0.26</td>
<td>2151.39</td>
<td>2162.29</td>
<td>&lt;0.001</td>
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<tr>
<td>$V_{\text{F&amp;S}} = 36.03 + 57.33 P_a$</td>
<td>0.04</td>
<td>2223.43</td>
<td>2234.33</td>
<td>0.001</td>
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<tr>
<td>$V_{\text{F&amp;S}} = 21.53 + 10.24 N_i + 8.83 P_a$</td>
<td>0.25</td>
<td>2153.07</td>
<td>2167.60</td>
<td>&lt;0.001</td>
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<tr>
<td>$V_{\text{F&amp;S}} = 6.44 + 17.70 N_i + 118.70 P_a - 51.98 N_i P_a$</td>
<td><strong>0.29</strong></td>
<td>2141.92</td>
<td>2160.10</td>
<td>&lt;0.001</td>
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<tr>
<td>$V_{\text{F&amp;S}} = \min(22.13 + 10.46 N_i; 75.85 + 19.52 P_a)$</td>
<td>0.25</td>
<td>2154.39</td>
<td>2173.56</td>
<td>&lt;0.001</td>
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Table 3. Comparisons of predictive models of area based maximum carboxylation capacity ($V_{\text{max,area}}$ area 25 °C, μmol m$^{-2}$ s$^{-1}$) based on leaf nitrogen and/or phosphorus content. Coefficients in black are significantly different from zero ($p < 0.01$); coefficients in red are not significantly different from zero ($p > 0.05$) and; coefficients in green are marginally different from zero (0.01 > $p > 0.05$): $N_i$ = nitrogen per unit leaf area (g m$^{-2}$); $P_a$ = phosphorus per unit leaf area(g m$^{-2}$).
\[
\begin{array}{l}
\text{Forest Evergreen} \\
J_{25a} = 32.69 + 21.14N_s, \quad 0.18 \quad 426.88 \quad 432.68 \quad 0.001 \\
J_{25a} = 64.15 + 64.28P_s, \quad 0.02 \quad 436.24 \quad 442.03 \quad 0.181 \\
J_{25a} = 32.66 + 20.68N_s + 8.58P_s, \quad 0.16 \quad 428.85 \quad 436.58 \quad 0.005 \\
J_{25a} = 28.78 + 22.89N_s + 48.23P_s - 21.78N_sP_s, \quad 0.15 \quad 430.81 \quad 440.47 \quad 0.015 \\
J_{25a} = \min(32.69 + 21.14N_s; 78.99 + 64.40P_s), \quad 0.16 \quad 429.88 \quad 440.54 \quad 0.028 \\
\text{Forest Deciduous} \\
J_{25a} = 40.42 + 19.60N_s, \quad 0.24 \quad 946.15 \quad 954.12 \quad <0.001 \\
J_{25a} = 83.76 - 9.89P_s, \quad -0.01 \quad 975.38 \quad 983.34 \quad 0.888 \\
J_{25a} = 49.03 + 21.89N_s - 121.73P_s, \quad 0.25 \quad 944.48 \quad 955.10 \quad <0.001 \\
J_{25a} = 25.94 + 32.89N_s + 99.60P_s - 101.93N_sP_s, \quad 0.26 \quad 945.34 \quad 958.61 \quad <0.001 \\
J_{25a} = \min(30.05 + 24.93N_s; 105.64 - 55.54P_s), \quad 0.26 \quad 945.12 \quad 959.39 \quad <0.001 \\
\text{Forest (Evergreen & Deciduous)} \\
J_{25a} = 35.21 + 21.30N_s, \quad 0.29 \quad 1376.58 \quad 1385.73 \quad <0.001 \\
J_{25a} = 73.34 + 62.94P_s, \quad 0.01 \quad 1428.19 \quad 1437.34 \quad 0.172 \\
J_{25a} = 38.25 + 22.58N_s - 52.86P_s, \quad 0.29 \quad 1376.94 \quad 1389.14 \quad <0.001 \\
J_{25a} = 15.48 + 34.54N_s + 164.81P_s - 109.80N_sP_s, \quad 0.29 \quad 1376.58 \quad 1391.83 \quad <0.001 \\
J_{25a} = \min(26.97 + 25.76N_s; 105.46 - 59.78P_s), \quad 0.30 \quad 1374.66 \quad 1390.91 \quad <0.001 \\
\text{Savanna (Deciduous)} \\
J_{25a} = 45.60 + 14.01N_s, \quad 0.23 \quad 861.59 \quad 869.44 \quad <0.001 \\
J_{25a} = 46.59 + 170.38P_s, \quad 0.18 \quad 868.26 \quad 876.11 \quad <0.001 \\
J_{25a} = 40.56 + 10.27N_s + 83.73P_s, \quad 0.25 \quad 859.78 \quad 870.24 \quad <0.001 \\
J_{25a} = 21.18 + 21.91N_s + 178.51P_s - 53.96N_sP_s, \quad 0.26 \quad 859.05 \quad 872.12 \quad <0.001 \\
J_{25a} = \min(51.28 + 13.06N_s; 29.55 + 337.17P_s), \quad 0.29 \quad 855.17 \quad 869.25 \quad <0.001 \\
\text{Forest and Savanna (Deciduous and Evergreen)} \\
J_{25a} = 38.23 + 19.42N_s, \quad 0.30 \quad 2431.46 \quad 2442.36 \quad <0.001 \\
J_{25a} = 68.53 + 68.45P_s, \quad 0.02 \quad 2528.29 \quad 2539.19 \quad 0.017 \\
J_{25a} = 40.06 + 20.09N_s - 26.70P_s, \quad 0.30 \quad 2432.37 \quad 2446.90 \quad <0.001 \\
J_{25a} = 15.87 + 32.06N_s + 149.52P_s - 83.38N_sP_s, \quad 0.33 \quad 2421.91 \quad 2440.09 \quad <0.001 \\
J_{25a} = \min(29.42 + 24.36N_s; 97.57 + 6.46P_s), \quad 0.33 \quad 2422.10 \quad 2441.28 \quad <0.001 \\
\end{array}
\]

Table 4. Comparisons of predictive models of area based maximum electron transport rate \(J_{\text{max}}\) area 25 \(^\circ\)C, \(\mu\text{mol m}^{-2} \text{s}^{-1}\) based on leaf nitrogen and/or phosphorus content. Coefficients in black are significantly different from zero \((p < 0.01)\); coefficients in red are not significantly different from zero \((p > 0.05)\) and; coefficients in green are marginally different from zero \((0.01 > p > 0.05)\); \(N_s\) = nitrogen per unit leaf area (g m\(^{-2}\)); \(P_s\) = phosphorus per unit leaf area(g m\(^{-2}\)).
Fig. 1 A section of a vegetation map of Africa taken from Mayaux et al. (2004), showing the location of the study area within Cameroon (left panel) with field plot locations within the forest/savanna mosaic shown (centre and right hand panels). The latter come from the satellite imagery analysis of Mitchard et al. (2009) for which forest areas are typically green with savanna areas blue.
Fig. 2 Venn-Euler diagram showing the abundance of tree species across sample plots considered as “forest”, “savanna” and “transitional forest” according to the classification of Torello-Raventos et al. (2013). Numbers refer to number of individual species.
Fig. 3 Partition of the total variance between plots, species, individual trees. The residual term includes between-leaf variation and experimental error: $M_a = \text{leaf mass per unit area}$; $N_a = \text{nitrogen per unit leaf area}$; $P_a = \text{phosphorus per unit leaf area}$; $A_{\text{max},a} = \text{Net CO}_2 \text{ assimilation rate per unit leaf area at saturating light and } [\text{CO}_2]$. 
Fig. 4. Statistical comparison of leaf attributes of forest (white bars), savanna (light grey) and transitional forest (dark grey) in sampled in central Cameroon. Also shown (right of vertical line) are data from a previous study in West Africa (Domingues et al. 2010) designated as "WA-F" and "WA_S" respectively. 

Mₐ = leaf mass per unit area; Nₐ = nitrogen per unit leaf area; Pₐ = phosphorus per unit leaf area; Aₐsat(a) = Net CO₂ assimilation rate per unit leaf area at saturating light and ambient [CO₂]; Aₐsat(m) = Net CO₂ assimilation rate per unit leaf mass at saturating light and ambient [CO₂]; Nₐm = nitrogen per unit leaf mass; Pₐm = phosphorus per unit leaf mass; N:P = leaf nitrogen/phosphorus ratio (g g⁻¹).
Fig. 5 Bivariate plots of observed relationships between area based measures of light saturated photosynthetic rate ($A_{sat}$), nitrogen and phosphorus. Symbols are deciduous forest (red), evergreen forest (green) and savanna (black) with filled symbols indicating taken the transitional forest (MDJ-05). Lines shown are for standard major axis (SMA) regression fits. Solid lines: ordinary least squares regression (OLS) fit significant at $p < 0.05$; dashed lines (OLS) regression significant at $p > 0.05$.
Fig. 6 Model predictions of estimated Rubisco activity standardised to 25 °C ($V_{25}$) and (b) estimated electron transport capacity @ 25 °C according to Eq. 2.
Fig. 7 Area-based relationships between (a) estimated Rubisco activity standardised to 25 °C ($V_{25}$) and leaf nitrogen; (b) $V_{25}$ and leaf phosphorus; (c) estimated electron transport capacity @ 25 °C ($J_{25}$) and leaf nitrogen; ($J_{25}$) and leaf phosphorus. Symbols are: deciduous forest (red), evergreen forest (green) and savanna (brown). Filled savanna symbols show points modelled to be limited by phosphorus as per the model fit of Eq. 2 as detailed in Supporting Information Table 1. Also shown from this study are the model fits of Eq. 2 for forest (purple) and savanna (brown), along with a previous fit of the same model to a mixture of forest and savanna species sampled along a precipitation transect in West Africa (grey) as detailed in Domingues et al. (2010).