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Foliar trait contrasts between African forest and savanna trees: genetic versus environmental effects

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Abstract. Variations in leaf mass per unit area (M_a) and foliar concentrations of N, P, C, K, Mg and Ca were determined for 365 trees growing in 23 plots along a West African precipitation gradient ranging from 0.29 to 1.62 m a^{-1}. Contrary to previous studies, no marked increase in M_a with declining precipitation was observed, but savanna tree foliar [N] tended to be higher at the drier sites (mass basis). Generally, M_a was slightly higher and [N] slightly lower for forest vs savanna trees with most of this difference attributable to differences in soil chemistry. No systematic variations in [P], [Mg] and [Ca] with precipitation or between trees of forest vs savanna stands were observed. We did, however, find a marked increase in foliar [K] of savanna trees as precipitation declined, with savanna trees also having a significantly lower [K] than those of nearby forest. These differences were not related to differences in soil nutrient status and were accompanied by systematic changes in [C] of opposite sign. We suggest an important but as yet unidentified role for K in the adaption of savanna species to periods of limited water availability; with foliar [K] being also an important factor differentiating tree species adapted to forest vs savanna soils within the 'zone of transition' of Western Africa.

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Additional keywords: foliar cations, leaf mass per unit area, leaf physiology, nitrogen, phenology, phosphorus, potassium, plant functional traits, tropical, West Africa, zone of transition.

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Introduction

Forest ($\mathbb{F}$) and savanna ($\mathbb{S}$) constitute the two major biomes of the tropics (Walter and Mueller-Dombois 1971). Typically differing in stand structure, a second major difference between $\mathbb{F}$ and $\mathbb{S}$ is their species composition, with little overlap in woody plant taxa even when growing in close proximity (Torello-Raventos *et al.* 2013). As well as $\mathbb{S}$ trees typically having trait characteristics associated with the tolerance and/or survival of the strongly pyrogenic regimes to which they are often exposed – for example, a greater bark thickness (Hoffmann and Franco 2003; Ratnam *et al.* 2011) numerous other $\mathbb{F}$ vs $\mathbb{S}$ trait differences typically exist (Ratnam *et al.* 2011). For example, as may be expected for trees growing in water-limited high-light environments, tropical savanna species have amongst the highest leaf mass per unit area (M_a) worldwide (Poorter *et al.* 2009). Indeed, even when growing in the same environment, the typically higher M_a of $\mathbb{S}$ species (Hoffmann *et al.* 2005*b*) has been cited as one characteristic of savanna trees that sets them apart from their proximally-growing forest-associated counterparts (Ratnam *et al.* 2011). Other systematic differences between forest and savanna trees have also been noted, for example, lower mass based nitrogen, $[N]_m$, and phosphorus, $[P]_m$, for savanna trees as well as differences in root-shoot ratios (Hoffmann and Franco 2003) and other structural/hydraulic properties (Gotsch *et al.* 2010).

Nevertheless, to date, our knowledge of non-fire associated trait differences is based almost entirely on phylogenetically controlled contrasts between gallery-forest and savanna species in the Cerrado Region of Brazil (Hoffmann and Franco 2003; Hoffmann *et al.* 2005*a*; Hao *et al.* 2008; Rossatto *et al.* 2009; Gotsch *et al.* 2010; Hoffmann *et al.* 2012). It is unclear to what extent the observed differences apply to other regions of the planet.

It is well established that for savanna trees physiological traits may vary with precipitation regime. For example, both Buckley *et al.* (2002) and Cernusak *et al.* (2011) reported an increase in area based foliar nitrogen concentrations, $[N]_a$, and M_a with increasing aridity for various *Eucalyptus* savanna species growing along a precipitation transect in the Australian Northern Territory. This is consistent with theory that suggests that $[N]_a$ will increase with declining water availability and increasing irradiance (Buckley *et al.* 2002), which is also seemingly the case in general (Wright *et al.* 2001, 2005). However, for M_a at least, such a precipitation effect may also depend on leaf habit with a decline in M_a with increasing rainfall not observed for deciduous species when examined on a global scale (Wright *et al.* 2005).

Evergreen vs deciduous species of the 'dry-tropics' (typically savanna regions) also typically show differences in leaf osmotic potential with evergreen 'dry-tropical' species typically having leaves operating at more negative osmotic potentials than those of their deciduous counterparts (Bartlett *et al.* 2012). A need for different concentrations of osmotically important ions such as potassium (Leigh and Wyn Jones 1984) might also reasonably be inferred, with higher [K] in the leaves of evergreen trees compared with their deciduous counterparts under identical edaphic and climatic growing conditions thus anticipated. Any such variations in foliar cation concentrations should also be related to variations in absolute carbon content (Poorter and Villar 1997; Patiño *et al.* 2012) potentially linking this trade-off to variations in M_a (Fyllas *et al.* 2012).

Here we report on variations in M_a as well as foliar nitrogen, phosphorus, carbon, potassium, magnesium and calcium concentrations along a precipitation transect in West Africa for which data on soil properties and leaf photosynthetic characteristics have already been presented (Domingues *et al.* 2010; Saiz *et al.* 2012). This data, collected within the ecotonal area of this transect where both savanna and forest exist in discrete patches – the so called 'zone of transition' (ZOT) – is also compared with a similar forest and savanna foliar trait dataset collected some 1000 km away in an independently replicated ZOT located in Cameroon. Specific questions addressed are as follows.

(1) Are the previously well documented differences between leaf traits of gallery forest vs savanna species for Central Brazil also observed within African ZOT?
(2) If (1) is true, to what extent are these contrasts due to inherent (genetic) differences as opposed to differing soil physical and chemical properties for forest *vs.* savanna vegetation formations types?
(3) Are West African savanna species characterised by increased M_a and N_a as precipitation declines?
(4) Given their likely role as osmotica, are there variations in foliar cation concentrations associated with variations in precipitation and/or the transition from savanna to forest within ZOT?

Materials and methods

This dataset derives from ten 1.00 (100 × 100 m) and four 0.25 ha (50 × 50 m) permanent sampling plots which were established as part of the Tropical Biomes in Transition (TROBIT) project in August to September 2006 (Ghana) and October–December 2007 (Cameroon). For further details on plot locations, climate and soil characteristics see Table S1.1 and Fig. S1.1, available as Supplementary Material to this paper.

All field campaigns were conducted towards the end of the wet season. Plot locations were selected according to the

following criteria: characteristic of the potential natural vegetation of the region; as low direct anthropogenic disturbance (in particular wood felling and arable agriculture) as possible; low altitudinal variation and no obvious hydrological differences. Further details are provided by Torello-Raventos *et al*. (2013).

The plots in Ghana, Boabeng-Fiema (BFI) Asukese (ASU) and Kogyae (KOG), were located in the transitional zone between dry semi-deciduous forest and the Guinea savanna. They experience an average dry season of 5 months duration (this being defined as the number of months with an average total precipitation of 100 mm or less) and an average annual rainfall of 1.21 m at ASU to 1.29 m at BFI (Hijmans *et al*. 2005).

The central African field sites (MDJ) are clustered in the Mbam Djerem National Park located in central Cameroon. This park covers 1765 km^2, and was established in 2000, forming part of the 4165 km^2 'Reserve de Faune de Pangar et Djerem'. Apart from local fishing and poaching, the park is relatively undisturbed due to low human population density in its surroundings. The dry season lasts for 4 months with an average annual rainfall around 1.65 m (Hijmans *et al*. 2005).

Species identification and classification

Leaf habit ($\mathbb{h}$)

Phenology was assembled using sources in the literature and expert knowledge. Trees were categorised as being deciduous (trees remain bare until leaf flush is induced by re-hydration), brevi-deciduous (short bare period in the dry season followed by leaf flush), semi-deciduous (trees losing old foliage as growth of new leaves starts) or evergreen (trees are never leafless but flush or shed leaves in regular periods or continuously throughout the year).

Vegetation formation type affiliation ($\mathbb{v}$)

Based on the ordination study by Torello-Raventos *et al*. (2013) sites were first grouped into one of either the forest or savanna vegetation domains on the basis of both floristics and structure, as detailed further in work by Veenendaal *et al*. (2014). Each species sampled was also classed as typically affiliated with either forest or savanna vegetation formation types using plot locality information for each species from Torello-Raventos *et al*. (2013), local expert knowledge and habitat information from local floras and the African Plant Database (http://www.ville-ge.ch/musinfo/bd/cjb/africa/recherche.php, accessed 23 July 2012).

Leaf sampling

Traits were assessed on an individual basis on at least 10 individuals with a diameter at breast height (1.3 m) greater than 0.1 m within each 1 ha plot. Trees were further selected on the basis that climbing the tree or cutting the branch from the ground could retrieve sun-exposed top-canopy branches. For each tree, a branch was harvested from the top canopy as described in work by Lloyd *et al*. (2010).

Sample preparation and analysis locations

Sample preparation was undertaken at the University of Leeds (UK). Leaf samples not used for the determination of M_a were dried as described above. About 20 g DW subsamples were then taken for the determination of foliar nutrients and isotopes. In order to prepare the leaf samples for these analyses, the main vein and petiole of each leaf were removed before drying and subsamples ground through a 1.0 mm (20 mesh) sieve.

Leaf mass per unit area (M_a)

The ratio of fresh, one-sided area of a leaf to its DW was obtained by separating at least 10 healthy adult leaves from the bulk leaf sampled from each branch. Each leaf was then scanned using a flatbed scanner attached to a laptop as fast as possible after harvesting in the field. Where scanning on the day of collection was impossible due to logistic reasons, leaves were stored in tightly sealed plastic bags under cool and dark conditions for a maximum of 2 days to avoid changes in the leaf area. The surface area of the leaf-scans was subsequently analysed on an individual basis using WinFOLIA (Regent Instruments Inc., Ottawa, Canada). The scanned leaves were then oven-dried to constant weight at 70°C for ~24 h to prevent enzymatic decomposition, and their dry mass determined after cooling in a desiccator. Where this was not possible due to logistical reasons, leaves were air-dried in the field and oven-dried as soon as possible.

Carbon and nitrogen determinations

Foliar nitrogen $[N]_m$ and carbon $[C]_m$ in the bulk leaf samples were determined on 15–30 mg of the ground plant material using elemental analysis (EURO EA CHNSO analyser, HEKAtech GbhB, Wegberg, Germany).

Cation and phosphorus determinations

Foliar cations (calcium, potassium and magnesium) and phosphorus in the ground samples were determined by inductively coupled plasma optical emissions spectrometry (ICP-OES) (Optima 5300DV, PerkinElmer, Shelton, CT, USA) following acid digestion (Lloyd *et al*. 2010).

Soil physical and chemical analyses

Soil sampling and laboratory analyses of the soil samples reported were performed by the University of Leeds, UK, using standard protocols (Quesada *et al*. 2010, 2011). In brief, across each plot, five soil cores up to 2 m depth (substrate permitting) were sampled in addition to one soil pit, which was dug to 2 m depth. Average values for 0–0.3 m depth are reported here (i.e. six samples per plot). Soil carbon and nitrogen were determined using an automated elemental analyser (Pella 1990), exchangeable cations by the silver thiourea method (Pleysier and Juo 1980) and particle size distribution using the Boyoucos method (Gee and Bauder 1986). Total soil exchangeable bases were calculated as the sum of Ca, Mg, K and Na. Phosphorus fractionations were as in work by Quesada *et al*. (2010).

Climatological data

Precipitation climatologies for all sites were obtained from the interpolated WorldClim dataset (Hijmans *et al*. 2005).

Statistical analyses

Exploratory analysis and precipitation effects

The effects of vegetation formation type ($\mathbb{V}$) and precipitation on the seven measured foliar traits were first examined using a generalised additive modelling approach using the gam mgcv. function (Wood 2006, 2011) as available within the R statistical package (R-Development-Core-Team 2012). The equation individually fitted for each trait (Θ) was

$$\Theta = \mu + \alpha\,\mathbb{S} + s(P_A) + U_S + \varepsilon, \quad (1)$$

where μ represents the dataset mean for trees located within the forest ($\mathbb{F}$) vegetation formation type, $\mathbb{S}$ is an indicator variable taking a value of one for all trees located within savanna formations and zero otherwise, s is a non-parametric smoother, P_A is the mean annual precipitation of the plot in which the tree was growing, U_S is a random effect assuming a different value for each species and ε is the residual error: In terms of fixed effects, the parameterisation of Eqn 1, allows an assessment of trait differences between forest and savanna stands after controlling for any precipitation effects (the trends in which could also be both visually inspected and statistically assessed). The variance in the dataset not accounted for by $\mathbb{V}$ or P_A is partitioned into that attributable to a trees' taxonomic identity (U_S) and then to other causes (ε, in this case, soils, other climatic effects, intra-specific variability and measurement error).

Differences in trait characteristics between forest and savanna species and between the four leaf pre-defined habit types ($\mathbb{h}$) for sites within the forest/savanna transition zone were then further assessed applying non-parametric multivariate ANOVA (Anderson 2001) and dispersion tests using similarity matrices (Anderson 2006) estimated using Gower's metric (Gower 1971) to help define our final three vegetation/habit groups ($\mathbb{G}$) using the adonis, betadisp and vegdist functions available in the R vegan package (Oksanen *et al*. 2012). These tests were conducted without reference to taxonomic identity. Here, in a multivariate sense, 'habit type' can be thought of as a measure of centrality (as in mean or median for the univariate case) with 'dispersion', by a similar analogy, being a measure of the multivariate scatter.

Forest/savanna differences in the zone of transition

Two approaches were used to assess the magnitude of trait differences between the three $\mathbb{G}$ obtained as described above. In the first a simple generalised linear model was fitted for each logarithmically transformed trait (Θ) according to

$$\log_2(\Theta) = \mathbb{G} + \varepsilon, \quad (2)$$

using the R function nlme(gls) (Pinheiro and Bates 2000; Pinheiro *et al*. 2011). The results of this *in loco* (in place, i.e. plot effects) analysis (effectively an unbalanced ANOVA) then being compared with a mixed-effect model with each tree's taxonomic affiliation and plot location incorporated as crossed random effects according to

$$\log_2(\Theta) = \mathbb{G} + U_S + U_P + \varepsilon, \quad (3)$$

where U_S is the species-level random effect and U_P the plot-level random effect. Comparison of Eqn 2 and Eqn 3 shows that the first analysis considers the estimate of the mean Θ for each $\mathbb{G}$ to be independent of the site conditions in which the tree was found as well as species identity. On the other hand, Eqn 3 allows for systematic variations in Θ to occur across plots, also probing the extent to which trait differences within the various $\mathbb{G}$ vary systematically with genotype. For each trait this allowed each species to be assigned a characteristic value ($\mathbb{G} + U_S$), with these 'species' estimates then representing the expected Θ at the dataset average site for that trait (i.e. with $U_P = 0$) with the U_P for each site giving a measure of the associated plot modulating effect on the trait values observed. We thus term the estimates of Θ so derived in this case as being *ab intra* ('from within', i.e. species effects); this being considered the trait value characteristic of each species before modulation by the environment (or more precisely, the mean value of the trait that would be expected for that species should all species have been uniformly distributed across all the plots sampled as part of this study).

Model diagnostics show that the log-transformation provided for a reasonable homoscedacity in the variances with unbiased estimates of the fixed effect $\mathbb{G}$ terms. There was, however, a tendency for the retrieved fitted values (involving both fixed and random terms) to overestimate at low Θ and *vice versa* to underestimate at high Θ. This is to be expected when random effects are quantified through the best linear unbiased predictor (BLUP) method, with shrunken estimates of the differences between terms and the overall means (Galwey 2006). Nevertheless, as shown in the supplementary information of work by Fyllas *et al*. (2009) this effect (which is greatest for those observations the farthest from the overall mean) affects bivariate slope estimates (see below) to only a trivial degree.

Bivariate relationships

Bivariate relationships were first assessed for each $\mathbb{G}$ and for the dataset as a whole using Pearson's correlation coefficient (r^2), and standardised major axis (SMA) line fits, which were subsequently investigated where r^2 was significantly different from zero. SMA regression lines represent the first axis of a principal component analysis (of a correlation matrix) and are often used in plant allometry studies. When the slopes of these lines were not statistically different we further tested for differences in elevation and shift across the SMA lines between high and low fertility plots, with all analyses being performed using the R smatr library (Warton *et al*. 2012). These analyses were undertaken for both the 'raw' dataset and for the species effects derived as outlined above (centred on the dataset mean) with the latter analyses also incorporating the inevitable error associated with the statistical retrieval of the *ab intra* 'species effects'.

Principal component analysis (PCA)

For the plot effects emerging from Eqn 3, a PCA was undertaken using the R prcomp(stats) function with all variances scaled to unity. Common principal components (CPC) analysis (Flury 1988) was undertaken using the program as outlined in work by Phillips and Arnold (1999) for which further details on the approach used may be found in work by Patiño *et al*. (2012).

Results

Trait variation in relation to precipitation

Individual tree values for each of the seven traits (Θ) examined *viz* leaf mass per unit area (M_a) and mass based concentrations of nitrogen $[N]_m$, phosphorus $[P]_m$, carbon $[C]_m$, potassium $[K]_m$, magnesium $[Mg]_m$, and calcium $[Ca]_m$ are plotted as a function of mean annual precipitation (P_A) in Figures 1–7. For each Θ there are three subpanels: (*a*) for trees growing in savanna vegetation formation types ($\mathbb{S}$); (*b*) for trees growing in forest vegetation formation types ($\mathbb{F}$) and (*c*) the Θ frequency distribution for $\mathbb{S}$ and $\mathbb{F}$ trees growing in the ZOT.

Leaf mass per unit area

For $\mathbb{S}$ trees there was a slight but insignificant trend towards an increase in M_a with P_A with the variability of M_a within plots (each of which has its own *x*-axis ordinate) typically much greater than between the overall plot means (Fig. 1*a*). With the more abundant species designated by a unique symbol/colour combination, clear systematic differences between species at the same site were also evident. For example, at the two (adjacent) lowest P_A sites of HOM-01 and HOM-02 (Sahel region) only three tree species were found, with *Combretum glutinosum* having M_a varying from 140 to 168 g m^{-2}, more than four times higher than for the two *Acacia* species also found in the same plots (*A. tortilis* and *A. senegal*) for which M_a varied from 27 to 43 g m^{-2}. It follows then with the gls model fit of Eqn 1 only accounting for 0.15 of the total dataset variance that an examination of the random terms shows that ~0.6 of the remaining 0.85 was attributable to the trees taxonomic affiliation with only 0.4 attributable to unmeasured factors such as edaphic effects and/or intra-species variability (see Table S2.1). In Fig. 1.1b, data is shown for trees growing in forest plots ($\mathbb{V}=\mathbb{F}$) but with the lines shown simply being a reproduction of the savanna fit of Eqn 1 (as in Fig. 1*b*). Comparing the points with the $\mathbb{S}$ line thus illustrates a clear tendency for $\mathbb{F}$ trees to have lower M_a than their $\mathbb{S}$ equivalents; as is also shown by the significant $\mathbb{S}$ term of 16 ± 5 g m^{-2} obtained from the Eqn 1 M_a model fit (Table S2.1).

Identified on Fig. 1*a*, *b* are several species that were sampled in both forest and savanna. Although the effect is by no means ubiquitous, a tendency for the same species to have a slightly lower M_a when growing in $\mathbb{F}$ as compared with $\mathbb{S}$ formations is suggested. With different symbol styles for different leaf habit characteristics, also note the virtual absence of evergreen species within $\mathbb{S}$ with no obvious differences between the three deciduous groupings in either $\mathbb{F}$ or $\mathbb{S}$. Nor are there any obvious differences in M_a between the forest evergreen and the various

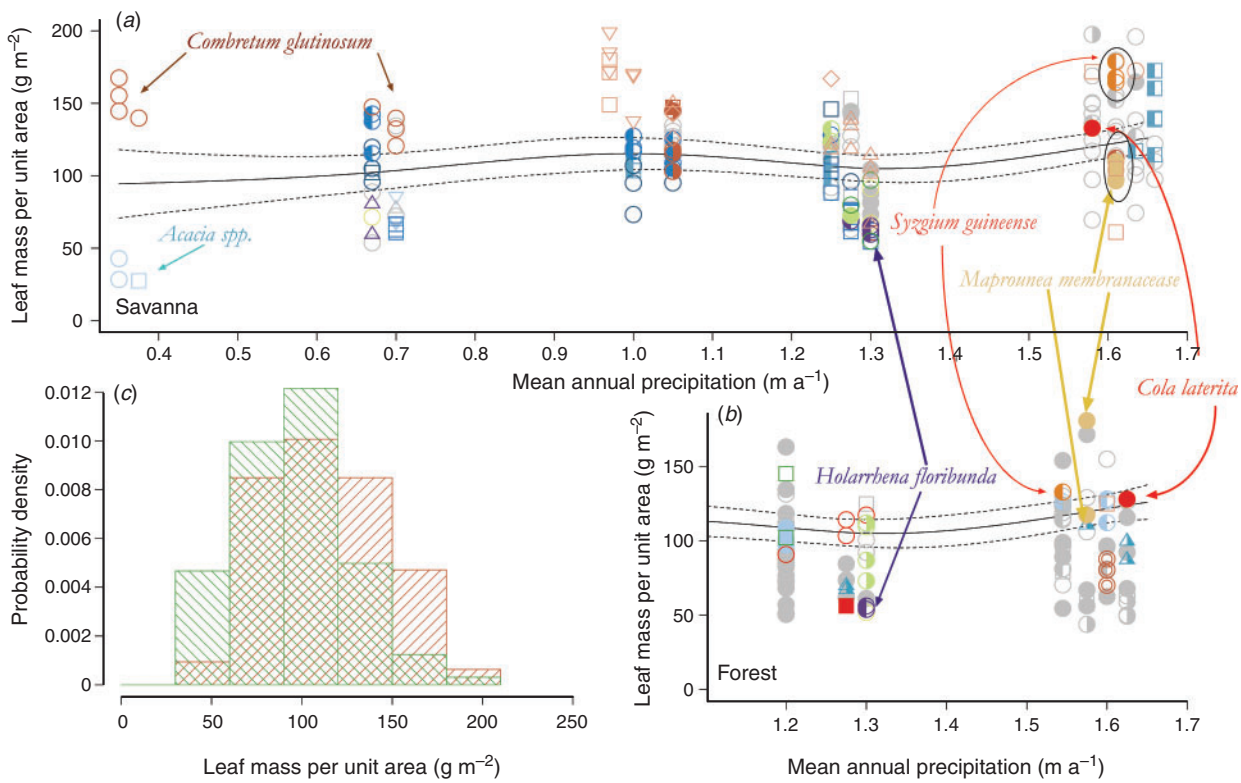

Fig. 1. Leaf mass per unit area of African forest and savanna trees in relation to rainfall. (*a*) plotted as a function of mean annual precipitation (P_A) for trees growing in savanna formation types ($\mathbb{S}$); (*b*) plotted as a function of P_A for trees growing in forest formation types ($\mathbb{F}$); (*c*) trait frequency distribution of trees in $\mathbb{F}$ vs $\mathbb{S}$ in the forest-savanna transition zone (overlapping region of (*a*) and (*b*)). The smoothed solid line represents the $s(P_A)$ function of Eqn 1 with $\mathbb{F}=0$ (i.e. the $\mathbb{S}$ fit) in both (*a*) and (*b*) with 0.95 confidence intervals also shown (dotted lines). To facilitate interpretation, the more frequent genera have been assigned a unique colour in (*a*) and (*b*) with different symbols of the same colour therefore representing different species within the one genus. Symbols have been assigned according to leaf habit: open symbols, deciduous; symbol with left half solid, brevideciduous; symbol with right half solid, semideciduous; solid symbols, evergreen. For (*c*) brown, $\mathbb{S}$; green, $\mathbb{F}$.

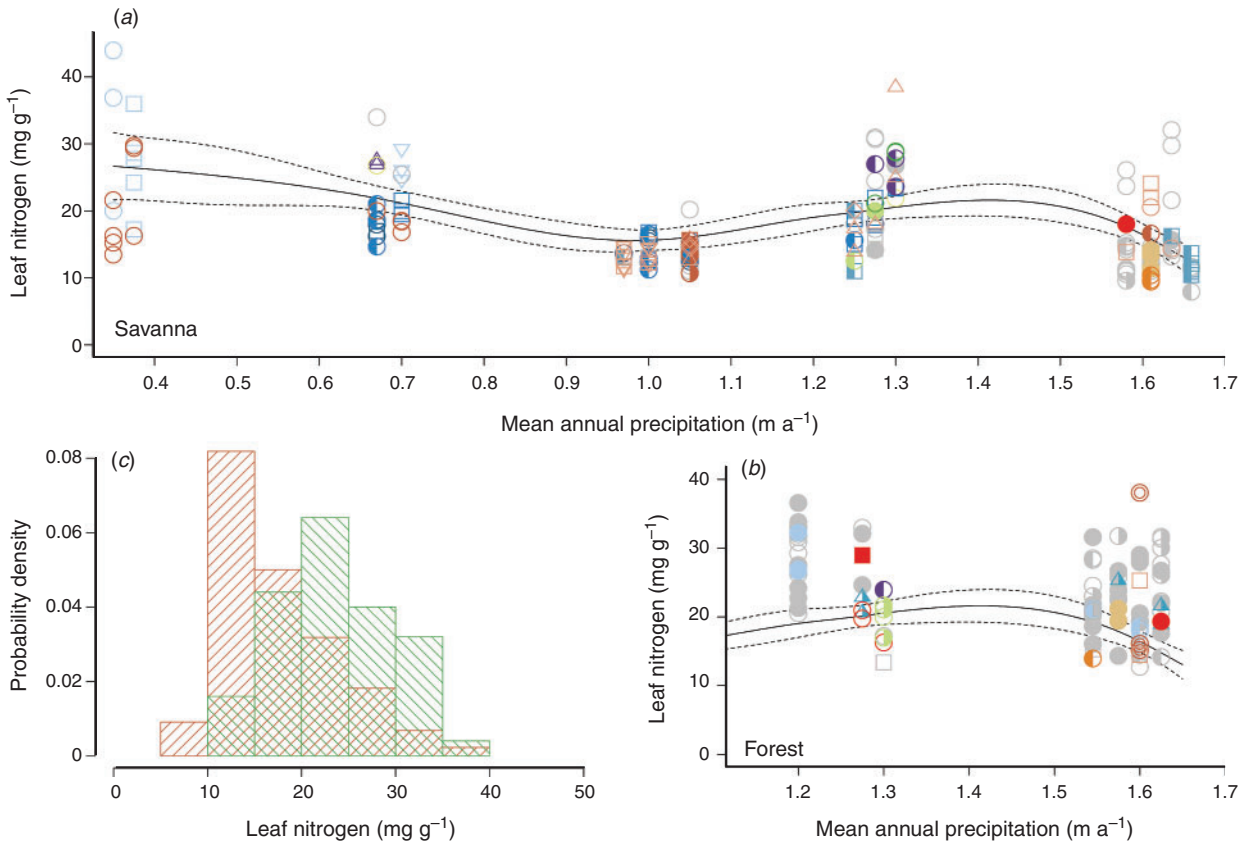

Fig. 2. Leaf nitrogen of Africa forest and savanna trees in relation to rainfall. See Fig. 1 caption for full explanation.

deciduous types. Fig. 1*c* shows the distribution of M_a for $\mathbb{F}$ and $\mathbb{S}$ trees within the ZOT. This shows little difference in the overall range observed, but with the M_a distribution of $\mathbb{S}$ trees skewed markedly towards the right.

Leaf nitrogen

For $\mathbb{S}$ trees, there was a decline in $[N]_m$ with increasing P_A from 0.35 to 1.1 m a^{-1} though with no systematic pattern within the higher rainfall ZOT (Fig. 2*a*). As for M_a there were large differences within sites, especially at the lowest P_A for which distinct species differences were similarly readily discernible (species identification accounting for ~0.6 of the variance not explained by the simple Eqn 3; see Table S2.2). Fig. 2*b* also shows a distinct tendency for $\mathbb{F}$ trees to have higher $[N]_m$ than their $\mathbb{S}$ counterparts within the ZOT (the fitted value for $\mathbb{S}$ in Eqn 1 as given in Table S2.2 being -6.2 ± 0.9 mg g^{-1}) and with the comparison of those few species found in both $\mathbb{F}$ and $\mathbb{S}$ suggesting lower $[N]_m$ in the latter. There are no obvious differences between the four leaf habit categories in the $[N]_m$ patterns observed. Very different frequency distributions in the variation of $[N]_m$ within the ZOT for the two $\mathbb{V}$ were observed with $\mathbb{F}$ tree $[N]_m$ being distributed more or less symmetrically around the mean, but with the $[N]_m$ distribution for $\mathbb{S}$ trees skewed markedly to the right (Fig. 2*c*). For example, $[N]_m$ ≤ 15 mg g^{-1} was five times more common for trees growing in savanna formation types than for forest.

Leaf phosphorus

Fig. 3*a* shows little systematic variation of $[P]_m$ with P_A for $\mathbb{S}$ and with no systematic difference in $[P]_m$ between the two $\mathbb{V}$ (the fitted value of $\mathbb{S}$ in Eqn 1 being 0.05 ± 0.07 mg g^{-1}; see Table S2.3). Table S2.3 also shows, through an analysis of the random terms, a slightly lower proportion of the variation in $[P]_m$ not explained by the simple precipitation model attributable to taxonomic identity (0.42) than was the case for $[N]_m$ or M_a. From Fig. 3*b* it can be see that there is some suggestion that the evergreen forest trees have lower $[P]_m$ than their deciduous counterparts.

For both $\mathbb{V}$, statistical distributions for leaves sampled within the ZOT were skewed to the right with the occasional very high value. As expected from Fig. 3*a*, *b*, there was no evidence of any systematic difference between $\mathbb{F}$ and $\mathbb{S}$ (Fig. 3*c*).

Leaf carbon

For $\mathbb{S}$, precipitation had a significant influence on $[C]_m$ (Fig. 4*a*) with the linear trend from the $s(P_A)$ model fit of 7.3 ± 1.9 mg being highly significant ($P < 0.001$). Within the ZOT, there was also a clear tendency for $\mathbb{F}$ trees to have lower $[C]_m$ (Fig. 4*b*), the fitted value for $\mathbb{S}$ from Eqn 1 being 13.6 ± 3.9 mg g^{-1} (Table S2.4). Again systematic species differences were evident (accounting for just less than 0.5 of the unexplained model variance) and with species found in both $\mathbb{F}$ and $\mathbb{S}$ tending to

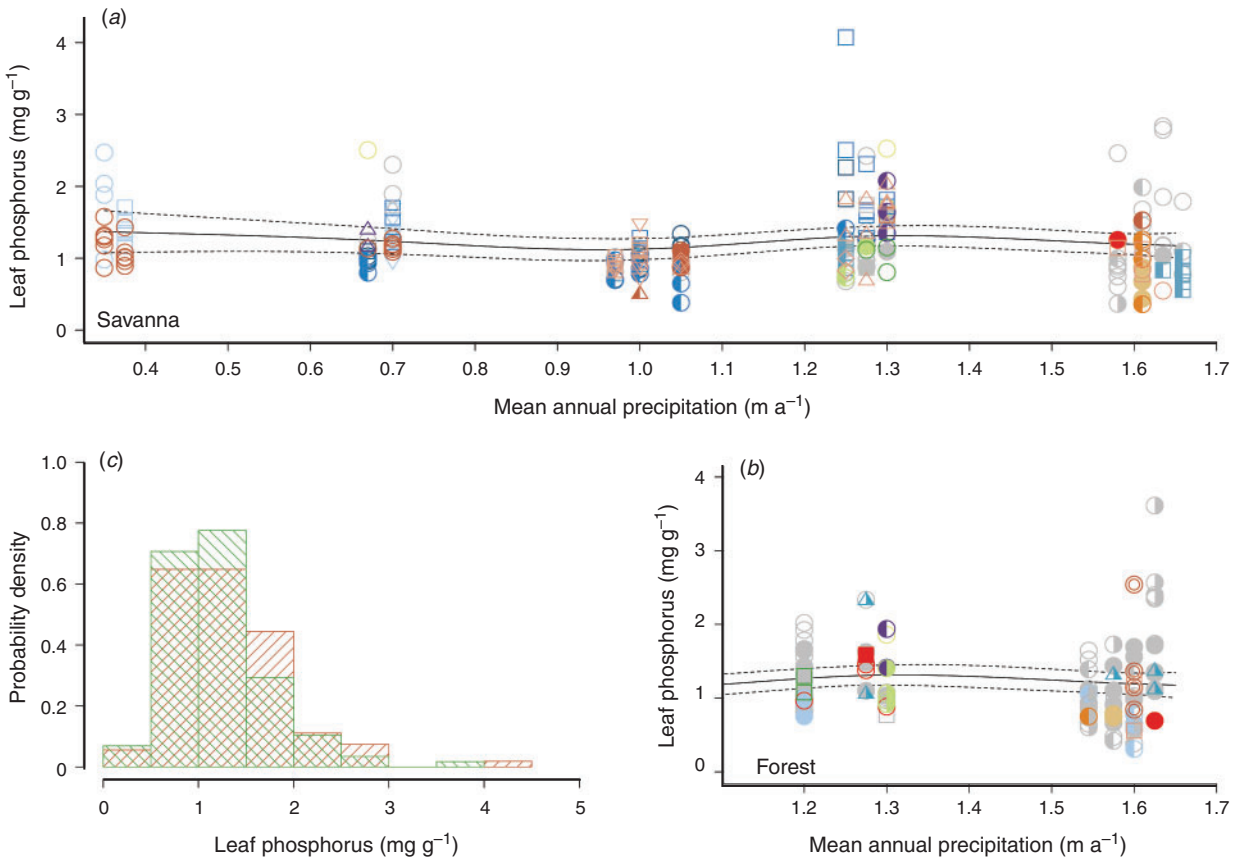

Fig. 3. Leaf phosphorus of Africa forest and savanna trees in relation to rainfall. See Fig. 1 caption for full explanation.

have slightly lower $[C]_m$ in the latter but with no obvious effect of leaf habit on $[C]_m$ for trees in $\mathbb{F}$ (Table S2.4). For $\mathbb{F}$ the $[C]_m$ distribution within the ZOT was skewed to the left with no values greater than 500 mg g^{-1} observed. In contrast, $\mathbb{S}$ trees had a distribution skewed towards the right and with less than 0.2 of observations $\leq$425 mg g^{-1} (Fig. 4*c*).

Leaf potassium

Fig. 5*a* reveals a marked increase in $[K]_m$ for $\mathbb{S}$ trees as precipitation decreases. Within the ZOT $\mathbb{F}$ tree values were clearly higher (Fig. 5*b*) with the fitted $\mathbb{S}$ term of Eqn 1 being -2.3 ± 0.5 mg g^{-1} (Table S2.5). There was, however, little evidence of a systematic difference between $\mathbb{F}$ and $\mathbb{S}$ for those species observed in both $\mathbb{V}$. Within $\mathbb{F}$ an effect of leaf habit was evident with the leaves of deciduous trees tending to have the highest $[K]_m$. As for most of the other traits examined, the proportion of variance not explained by the simple precipitation model but explained by taxonomic identity was just >0.5

For both $\mathbb{F}$ and $\mathbb{S}$ trees statistical distributions within the ZOT were skewed to the right and with the very highest $[K]_m$ ($\geq$15 mg g^{-1}) only observed for forest trees. Likewise, $[K]_m$ less than 6 mg g^{-1} formed the majority of the $\mathbb{S}$ tree population, but only about one-third of that of $\mathbb{F}$ trees (Fig. 5*c*).

Leaf magnesium

Across the precipitation gradient there was little evidence of an influence of a systematic effect of P_A on $[Mg]_m$ for $\mathbb{S}$ (Fig. 6*a*), although there was some suggestion of $\mathbb{F}$ trees having higher $[Mg]_m$ within the ZOT (Fig. 6*b*). Nevertheless the $\mathbb{S}$ term in the Eqn 1 model fit of -0.18 ± 0.24 mg g^{-1} was clearly non-significant (Table S2.6). Nor was there any evidence of a systematic effect of leaf habit on $[Mg]_m$. The proportion of variance not explained by the simple precipitation model but attributable to taxonomic identity was ~0.5 (Table S2.6). Fig. 6*c* shows little difference in the distributional properties of $[Mg]_m$ for $\mathbb{F}$ vs $\mathbb{S}$ trees within the ZOT, with both distributions skewed towards the right.

Leaf calcium

There was little evidence of any consistent effect of P_A on $[Ca]_m$ for $\mathbb{S}$ (Fig. 7*a*) with the slope of the linear gls fit found for Eqn 1 of -1.0 ± 0.5 only being significant at $P = 0.06$. The effect of $\mathbb{V}$ was also small with the $\mathbb{S}$ fitted term in Eqn 1 being -1.9 ± 1.1 mg g^{-1} and only significant at $P = 0.08$ (Table S2.7). Within the ZOT, there was little difference in the pattern of $[Ca]_m$ distributions between the two $\mathbb{V}$.

Trait variation within the forest/savanna transition zone

Multivariate leaf habit: vegetation formation affiliation differences

With a view to the possible amalgamation of the three 'deciduous' groups to simplify subsequent interpretations, applying the non-parametric ANOVA with $\mathbb{V}$ as a grouping

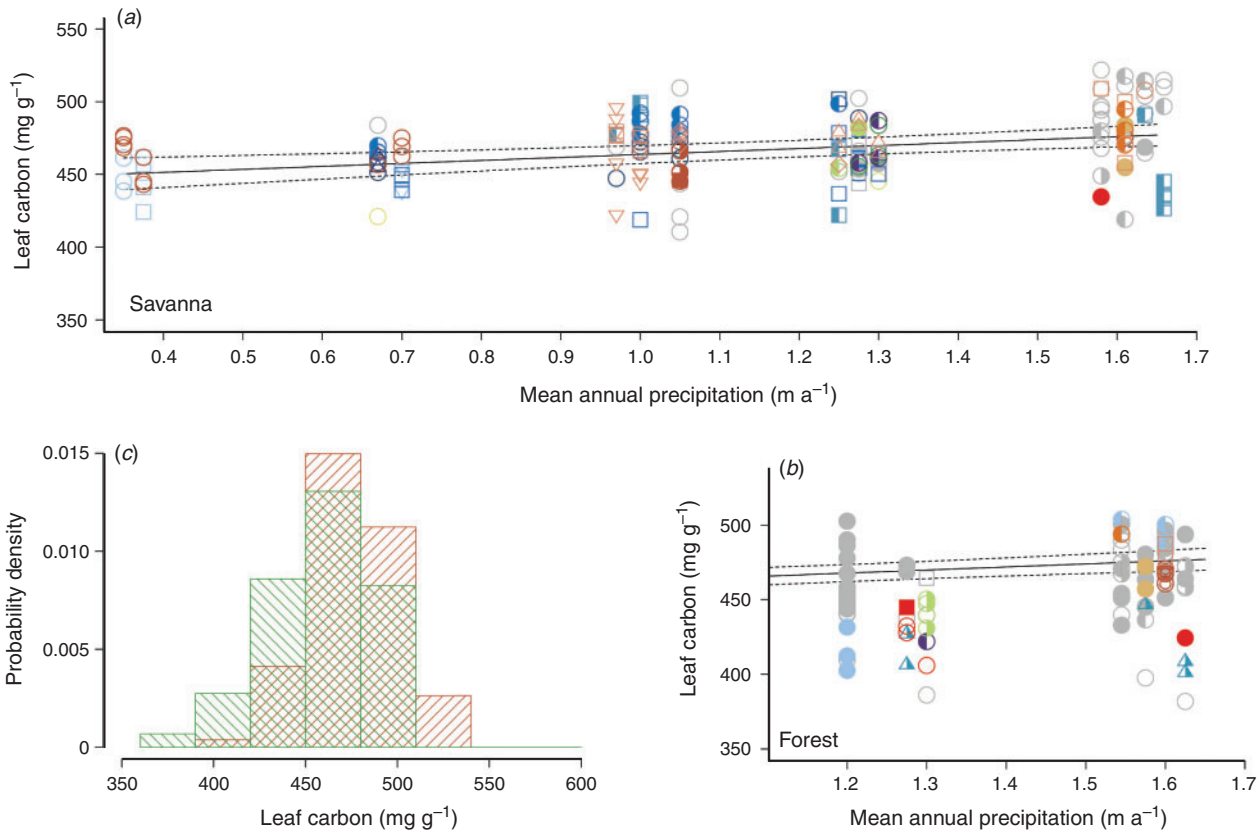

Fig. 4. Leaf carbon of Africa forest and savanna trees in relation to rainfall. See Fig. 1 caption for full explanation.

factor showed no indications of any major differences between the groups after adjustment for multiple testing (see Table S3.1). Also noting that the few evergreen trees sampled in savanna were all actually 'forest affiliated' (i.e. more commonly found in forests) we therefore amalgamated the seven original $\mathbb{V}$ into three 'vegetation formation: type-leaf habit' groups ($\mathbb{G}$) viz. 'Forest evergreen' (F_e), 'Forest deciduous' (F_d), and 'Savanna deciduous' (S_d). A MANOVA on these three groups then suggested significant trait differences, some of which may be attributable to differences in dispersion as well as location (Table 1) with the S_d grouping showing markedly greater multivariate trait variations than the F_e group (Tukey's honest significant difference = 0.035) and with the F_d leaves intermediate (data not shown). This categorisation of the three $\mathbb{G}$ was retained for the rest of the data analysis procedure.

Intrinsic versus environmental trait differences

Differences between the three $\mathbb{G}$ when considered both *in loco* and *ab intra* (Eqns 2 and 3) are summarised in Fig. 8. The *in loco* value assumes that all trait differences associated with the different $\mathbb{G}$ are intrinsic (i.e. not influenced by where the tree was growing) whereas the *ab intra* estimate allows for trees of the same species but located within different plots to assume a (systematically) different trait value (which is also dependent upon their species identity). For M_a there were only small differences between the three $\mathbb{G}$ in both cases. Specifically, S_d trees have slightly higher values than the two forest habit types, but with this small difference only being significant *in loco*. Irrespective of analysis approach evergreen and deciduous forest trees were statistically similar with regards to $[N]_m$, but with leaves of S_d trees significantly lower *in loco*. This difference was not, however, evident for the *ab intra* estimate; this then implying that most of the differences in $[N]_m$ between forest and savanna trees were more a consequence of plot location than a reflection of inherent physiognomic differences. Unlike $[N]_m$ there were no differences between the three $\mathbb{G}$ in their $[P]_m$.

Both trait quantification approaches gave similar results for $[C]_m$ for which there was a clear difference between the two deciduous $\mathbb{G}$ with S_d leaves having significantly higher carbon contents and with F_e leaves intermediate. Similarly large differences between the three $\mathbb{G}$ were found for potassium but reversed, with S_d leaves having significantly lower $[K]_m$ than their deciduous forest counterparts and with F_e lying between the two deciduous groups. Unlike $[K]_m$, but similar to $[P]_m$, for both $[Mg]_m$ and $[Ca]_m$, there was little suggestion of any systematic difference between trees of the three $\mathbb{G}$.

Bivariate relationships

Using $\log_2$ transformed data, Table 2 gives OLS Pearson correlation coefficients (r) for the *ab intra* (species effect) trait estimates (upper diagonal) with the associated plot effects estimates (both from Eqn 3) given in the lower diagonal. This shows *ab intra* trait statistically significant ($P < 0.05$) negative

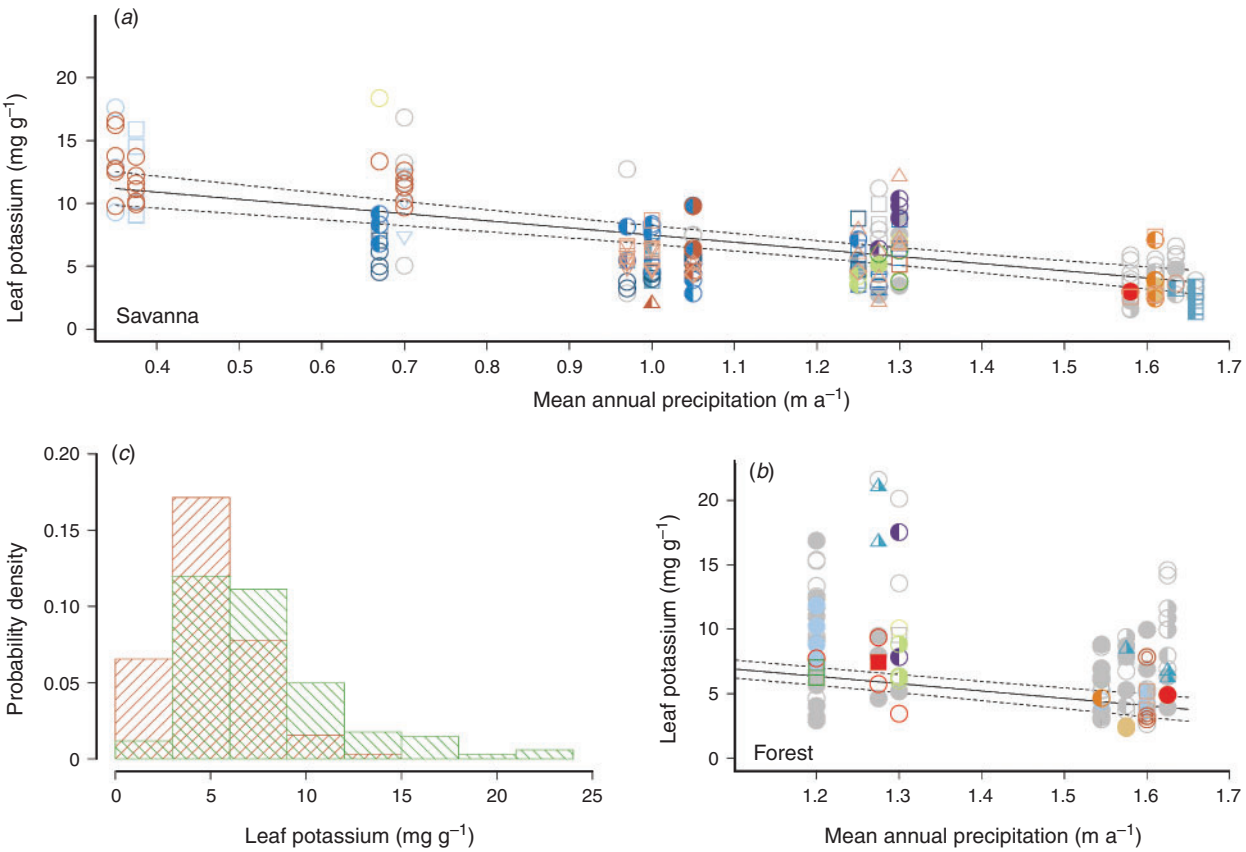

Fig. 5. Leaf potassium of Africa forest and savanna trees in relation to rainfall. See Fig. 1 caption for full explanation.

log-log relationships for M_a with $[N]_m$, $[P]_m$ and $[K]_m$ and with these three mass based chemical trait measures all positively correlating with each other. Also of note in the *ab intra* trait correlation matrix are significant negative correlations between all three of the studied cations and $[C]_m$. Although $[Mg]_m$ and $[Ca]_m$ were reasonably strongly correlated with each other ($r = 0.44$), neither of these divalent cations was significantly associated with $[K]_m$.

Broadly similar patterns were found for the plot effects but with the important difference that, in addition to the negative log-log correlations for M_a with $[N]_m$, $[P]_m$ and $[K]_m$ there was also a significant negative correlation between M_a and $[Mg]_m$ with M_a. and $[C]_m$ showing a significant positive correlation. No estimates of correlation coefficients could be made for $[Ca]_m$ due to our inability to retrieve meaningful plot estimate terms.

The *in loco* and *ab intra* bivariate relationships between M_a, $[N]_m$ and $[P]_m$ are shown in Fig. 9. Noting our use of the 'ℓ' superscript from here on where variables have been log-transformed (Lloyd *et al*. 2013), for the *in loco* ${}^{\ell}[N]_m$; ${}^{\ell}M_a$ relationship (Fig. 9*a*) we found significant negative correlations for all three $\mathbb{G}$ ($P < 0.001$) with no significant difference in SMA slope between the three $\mathbb{G}$ (estimated at –0.91). In contrast, S_d had a lower intercept than either the F_e and F_d leaves, which, in turn, did not differ from each other.

By contrast, for the *in loco* ${}^{\ell}[P]_m$; ${}^{\ell}M_a$ association - although again with significant negative correlations for all three $\mathbb{G}$ ($P < 0.05$) - differences in SMA slope were evident (Fig. 9*b*), with the gradient being shallower for F_e (–0.92) than for the forest and savanna deciduous types (–1.40) which themselves varied in intercept only. For the *in loco* ${}^{\ell}[P]_m$; ${}^{\ell}[N]_m$ association, significant positive associations were found for all three $\mathbb{G}$ ($P < 0.01$) but with differences in slope: with lower ${}^{\ell}[N]_m$ S_d tending to have a higher ${}^{\ell}[P]_m$ than their deciduous forest counterparts but with this difference becoming smaller (in a relative sense) at higher ${}^{\ell}[N]_m$ (Fig. 9*c*). The difference in *in loco* slope between F_d (1.80) and F_d (1.18) was also significant ($P = 0.002$) with the suggestion being that at high (but not low) $[N]_m$ evergreen forest tree leaves have a lower $[P]_m$ than their deciduous counterparts.

Performing the same three mass-based analyses, but this time with *ab intra* environmentally independent estimates, somewhat different patterns to the *in loco* case emerge, especially for the ${}^{\ell}[N]_m$; ${}^{\ell}M_a$ relationship (Fig. 9*d*: $P < 0.005$) with there being no significant difference in SMA slope between the three $\mathbb{G}$ (estimated at only –0.78) and also no difference in elevation ($P = 0.98$). Thus, the three SMA regression equations can be considered to be equivalent, although also with a significant difference in mean location along that common line (a shift) when contrasting F_d and S_d ($P < 0.05$). For the *ab intra* ${}^{\ell}[P]_m$; M_a association, significant regressions were again found for all three $\mathbb{G}$ ($P < 0.05$) and with similar differences as for the *in loco*. Contrasting with the *in loco* case, statistically significant relationships were not found for the *ab intra* relationship between ${}^{\ell}[N]_m$ and ${}^{\ell}[P]_m$ for F_e or S_d ($P > 0.10$) although a

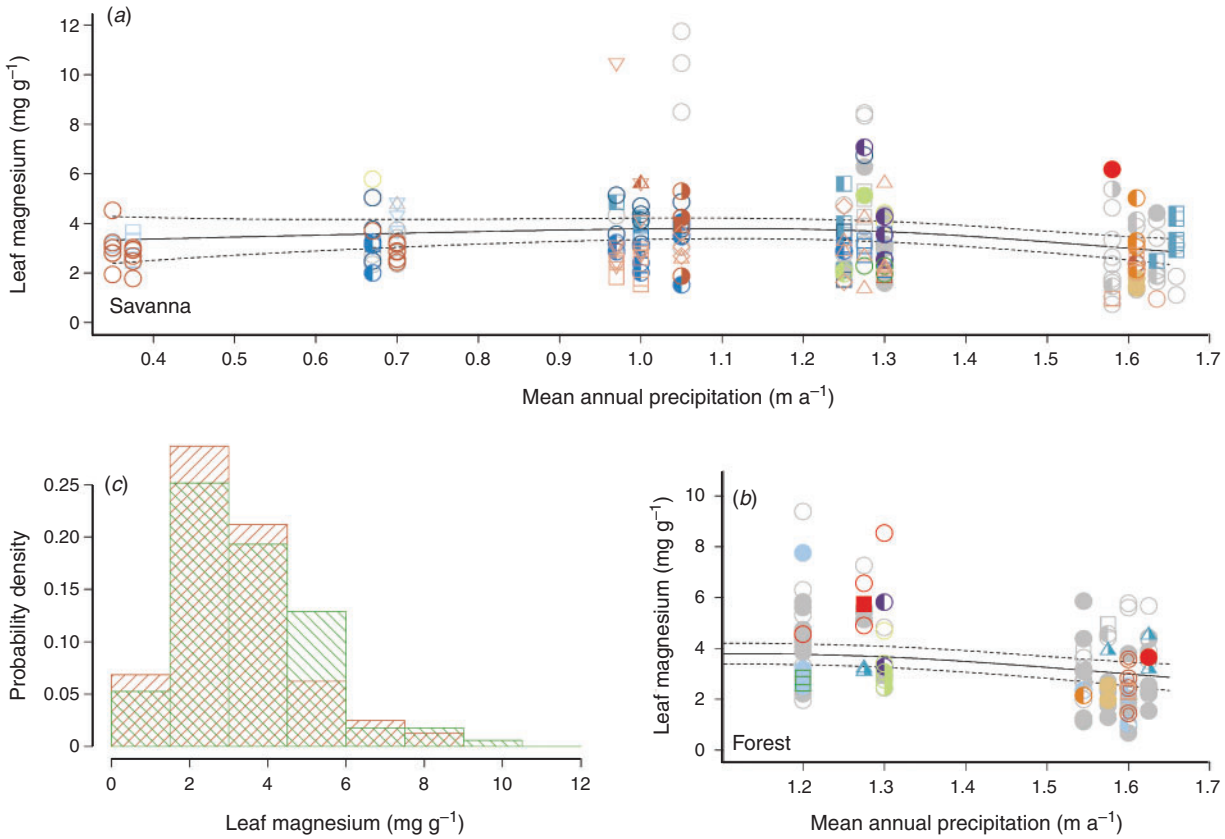

Fig. 6. Leaf magnesium of Africa forest and savanna trees in relation to rainfall. See Fig. 1 caption for full explanation.

reasonably strong relationship was still observed for F_d ($P<0.001$) with a slope of 1.05

Fig. 10 shows SMA scaling relations involving cations as selected with reference to Table 2. This shows negative *in loco* ${}^{\ell}[K]_m$; ${}^{\ell}M_a$ relationships (Fig. 10*a*) for which significant negative correlations were observed for all F_d ($P<0.001$) with a common slope estimated at –1.51. There were, however, significant differences in elevation ($P<0.05$) with the estimated elevation of F_d significantly greater than S_d but not significantly different to F_e ($P=0.05$).

With both ${}^{\ell}[Mg]_m$ and ${}^{\ell}[Ca]_m$ showing strong correlations with ${}^{\ell}[C]_m$ (Table 2) it was further found that the combined term ${}^{\ell}([Mg]_m+[Ca]_m)$ in molar units gave a stronger correlation with ${}^{\ell}[C]_m$ than when these cations were considered individually ($r=-0.47$), this also being marginally better than when potassium was included ($r=-0.45$). Fig. 10*b* thus shows the scaling relationships for ${}^{\ell}([Mg]_m+[Ca]_m)$ as a function of ${}^{\ell}[C]_m$ for which correlations were significant for F_d and S_d ($P<0.005$) but not for F_e ($P=0.40$). Comparing the two deciduous $\mathbb{G}$, significant differences in slope were found with greater (relative) differences between the two deciduous types at low ${}^{\ell}[C]_m$ (Fig. 10*b*).

Both ${}^{\ell}[N]_m$ and ${}^{\ell}[P]_m$ showed strong correlations with ${}^{\ell}[K]_m$ (Table 2) and with the *in loco* ${}^{\ell}[P]_m$; ${}^{\ell}[K]_m$ associations illustrated in Fig. 10*c*. Here the relationship was significant for all three $\mathbb{G}$ ($P<0.05$) with a common slope of 1.14. There were, however, differences in elevation with S_d being markedly lower than F_d and F_e which did not differ significantly from each other. Although not shown, a similar analysis was also undertaken for ${}^{\ell}[N]_m$ and ${}^{\ell}[K]_m$ but with slightly different results. Again all three $\mathbb{G}$ exhibited statistically significant relationships *in loco* ($P<0.001$), but in this case with a significant difference in slopes as a consequence of F_d being significantly higher than S_d. Here the slope for the F_e was intermediate between the two deciduous types (1.67) and not significantly different to either.

When these analyses were repeated using *ab intra* estimates, patterns remained essentially the same for ${}^{\ell}[K]_m$; ${}^{\ell}M_a$ and ${}^{\ell}([Mg]_m+[Ca]_m)$; ${}^{\ell}[\ C]_m$ associations (Fig. 10*d*, *e*) but with a greater divergence between ${}^{\ell}[K]_m$ and ${}^{\ell}[P]_m$ for the two deciduous $\mathbb{G}$ at high values as a consequence of differences in slope. Although the associations for the deciduous groupings were both clearly significant (Fig. 10*f*; $P<0.005$) this was not the case for F_e ($P=0.09$). Also observing the ${}^{\ell}[N]_m$; ${}^{\ell}[P]_m$ association *ab intra* (data not shown), moderately strong correlations were observed for all three $\mathbb{G}$ ($P<0.05$), although with no differences in slope ($P=0.07$) which was estimated for all three at 1.20 with large differences in elevation ($P<0.001$). Specifically, with $F_d>F_e=S_d$.

With photosynthesis primarily being an area based process (Lloyd *et al*. 2013; Osnas *et al*. 2013), we also examine the interrelationships of M_a and the area based nitrogen and phosphorus measures in Fig. 11. For the *in loco* M_a;$[N]_a$ association, a positive relationship was observed for all three $\mathbb{G}$ ($P<0.001$) with a common slope of 0.85 but with significant differences in

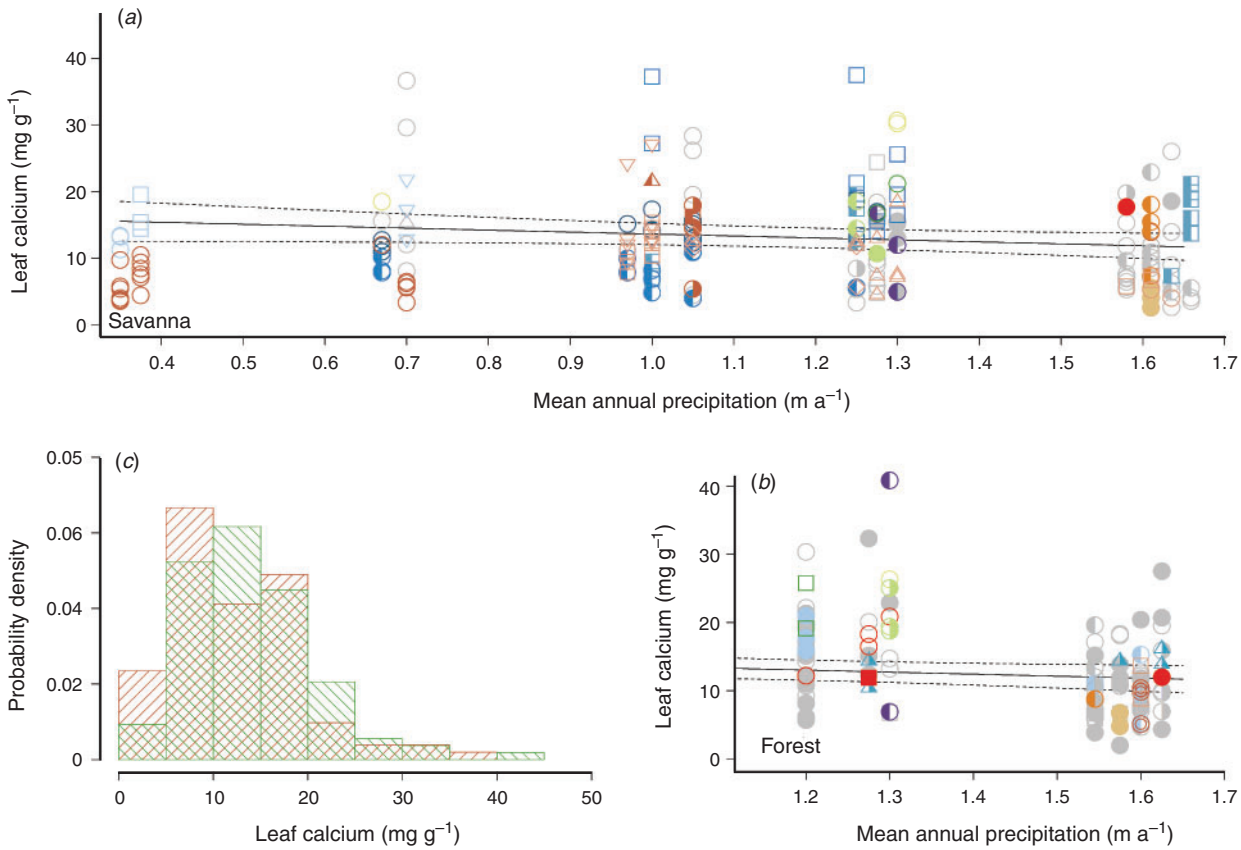

Fig. 7. Leaf calcium of Africa forest and savanna trees in relation to rainfall. See Fig. 1 caption for full explanation.

Table 1. Results of multivariate tests for differences in trait location vs dispersion between the three vegetation habit groups

Abbreviations: d.f., degrees of freedom; SS, sum of squares; MS, mean sum of squares. *, $P < 0.05$

	d.f.	SS	MS	F	p
Location					
Vegetation habit group (G)	2	0.2521	0.1261	4.35	<0.002
Residual	154	4.5209	0.0290*	–	–
Total	158	4.7730	–	–	–
Dispersion					
Vegetation habit group (G)	2	0.0365	0.01823	3.22	0.0437
Residual	154	0.0883	0.00566	–	–

elevation ($P < 0.001$) arising through the estimate for S_d being markedly lower than for either F_d or F_e. By contrast, the *in loco* M_a;$[P]_a$ association was significant for only F_e and S_d ($P < 0.001$) with no differences in slope or elevation, though with a significant shift along a common axis. For the F_d leaves there was clearly no evidence of any statistically meaningful relationship ($r^2 = 0.01$, $P = 0.61$). Examining the $[P]_a$;$[N]_a$ associations (Fig. 11*c*), a positive relationship was observed for all three $\mathcal{G}$ ($P < 0.05$) with a common slope of 1.46 but with different intercepts: That for the S_d leaves being greater than the two forest types between which any difference was marginal ($P = 0.052$).

Examining the same three bivariate relationships *ab intra*, for the M_a;$[N]_a$ association a positive relationship was observed for all three $\mathcal{G}$ ($P < 0.005$) with no significant differences between the three $\mathcal{G}$ in elevation and with a common slope estimated as 0.82. For phosphorus the situation was somewhat different with a common slope found (0.97) but, in contrast to the *in loco* case, with a significant difference in elevation associated with leaf habit; $F_d = S_d > F_e$. For the *ab intra* $[P]_a$;$[N]_a$ association the result was more or less akin to the *in loco* analysis with common slopes and differences in elevation: but in this case with the correlation for S_d not being significant.

Common principal components (CPC): species traits

Results of the CPC are shown in Table 3 for which results can be interpreted for an ordinary principal components analysis, the difference here being that the relative weightings (λ) have been allowed to differ between the three $\mathcal{G}$. Only the first two eigenvectors appeared meaningful with S_d and F_d weightings for the first component, Ψ_1 being similar but much greater than that observed for F_e. This first common principal component was characterised by strong positive weightings for $[N]_m$, $[P]_m$ and $[K]_m$ and a strong negative weighting for M_a. For Ψ_2, the λ for all three $\mathcal{G}$ were similar; although slightly higher for S_d, and involved substantial contributions from $[Mg]_m$ and $[Ca]_m$ and, of opposite sign, M_a and $[C]_m$.

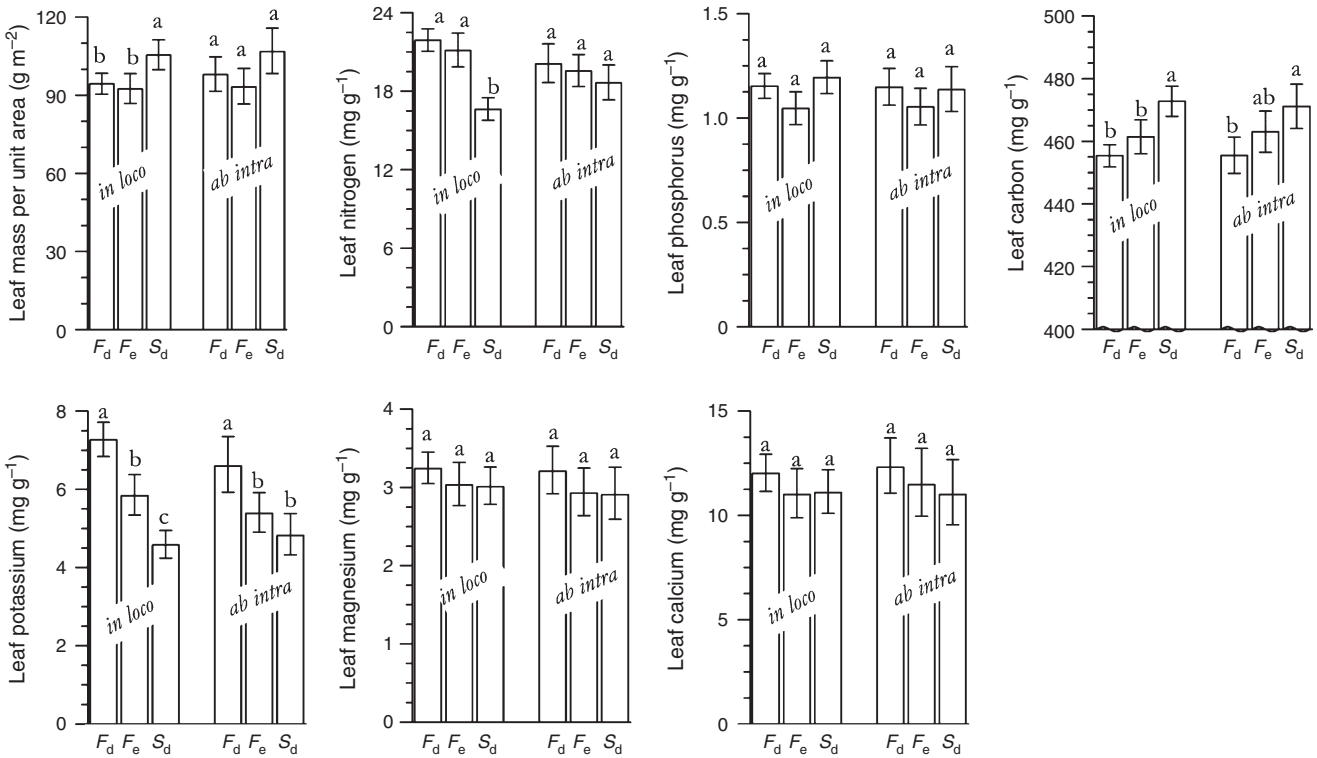

Fig. 8. Differences in foliar trait characteristics between the three vegetation formation types: leaf habit groups (*G*). Analyses have been done either ignoring (*in loco*) or including (*ab intra*) potential taxonomic and environmental effects. As defined in the text the three are 'forest deciduous' (F_d), 'forest evergreen' (F_e) and 'savanna deciduous' (S_d).

Table 2. Pearson correlation coefficients (*r*) between the studied trait pairs as separated into genetic and environmental components using Eqn 3

Values above the diagonal: 'species effects' (all data pooled); values below the diagonal: 'plot effects'. Significant correlations are indicated: *, $P < 0.05$

$^{\ell}M_a$	–0.57*	–0.02	–0.39*	–0.38*	–0.03	–0.19
–0.47*	$^{\ell}[N]_m$	–0.05	0.24*	0.45*	–0.05	–0.01
0.63*	–0.23	$^{\ell}[C]_m$	0.10	–0.24*	–0.37*	–0.42*
–0.47	0.34	–0.36	$^{\ell}[P]_m$	0.38*	0.08	0.17
–0.65*	0.60*	–0.54*	0.47*	$^{\ell}[K]_m$	–0.09	–0.04
–0.52*	0.30	–0.58*	0.38	0.52*	$^{\ell}[Mg]_m$	0.44*
–	–	–	–	–	–	$^{\ell}[Ca]_m$

Environmental (site) traits

Investigation of the random terms from the model fits for the various traits (Eqn 3) showed that, in addition to a plant taxonomic identity, a substantial portion of the variance not explained by the model was attributable to the site within which a tree was growing; the only exception being for $[Ca]_m$. Indeed in the case of $[N]_m$ and $[K]_m$ the random site effect term was the most important (data not shown). Retrieval of the trait specific terms for each site further showed strong correlations between these 'site effects' for the various Θ (Table 2). A principal components analysis (PCA) of these site effects was thus undertaken (see Table S5.1) the results of which showed over 0.6 of the variance being explained by a single axis (see biplot in Fig. 12). This shows strong anti-correlation between the site effects for M_a and both $[K]_m$ and $[P]_m$, these three Θ more or less aligning with the first PCA axis (denoted here as $Ч_1$). Negatively correlated effects of $[C]_m$ and $[Mg]_m$ were also evident, these two traits varying mostly along the first PCA axis and with a substantial contribution from $[N]_m$.

Of the soil variables investigated, two clearly emerged as being the most strongly correlated with the $Ч_1$ site scores, namely the soil C : N ratio and the total sum of (exchangeable) bases, Σ_B. Fig. 13 thus plots $Ч_1$ as a function of these two variables illustrating a strong, albeit non-linear relationship in both cases. Although there is some overlap, the different relative locations of the forest versus savanna sites can also be seen in terms of both, the response variate $Ч_1$, and the driving soil covariates C : N and Σ_B.

Discussion

One part of our work has focussed on examining foliar trait differences between distinct forest and savanna species, also taking advantage of the occasional 'casuals' that are found growing in the other habitat in order to help determine whether trait differences between the two species groupings within ZOT are a consequence of intrinsic (genetic) differences or a response to systematic differences between forest and savanna vegetation formations in soil chemical and/or physical conditions (Cochrane 1989; Ratter 1992; Thompson *et al.* 1992; Hoffmann *et al.* 2009; Lehmann *et al.* 2011; Saiz *et al.*

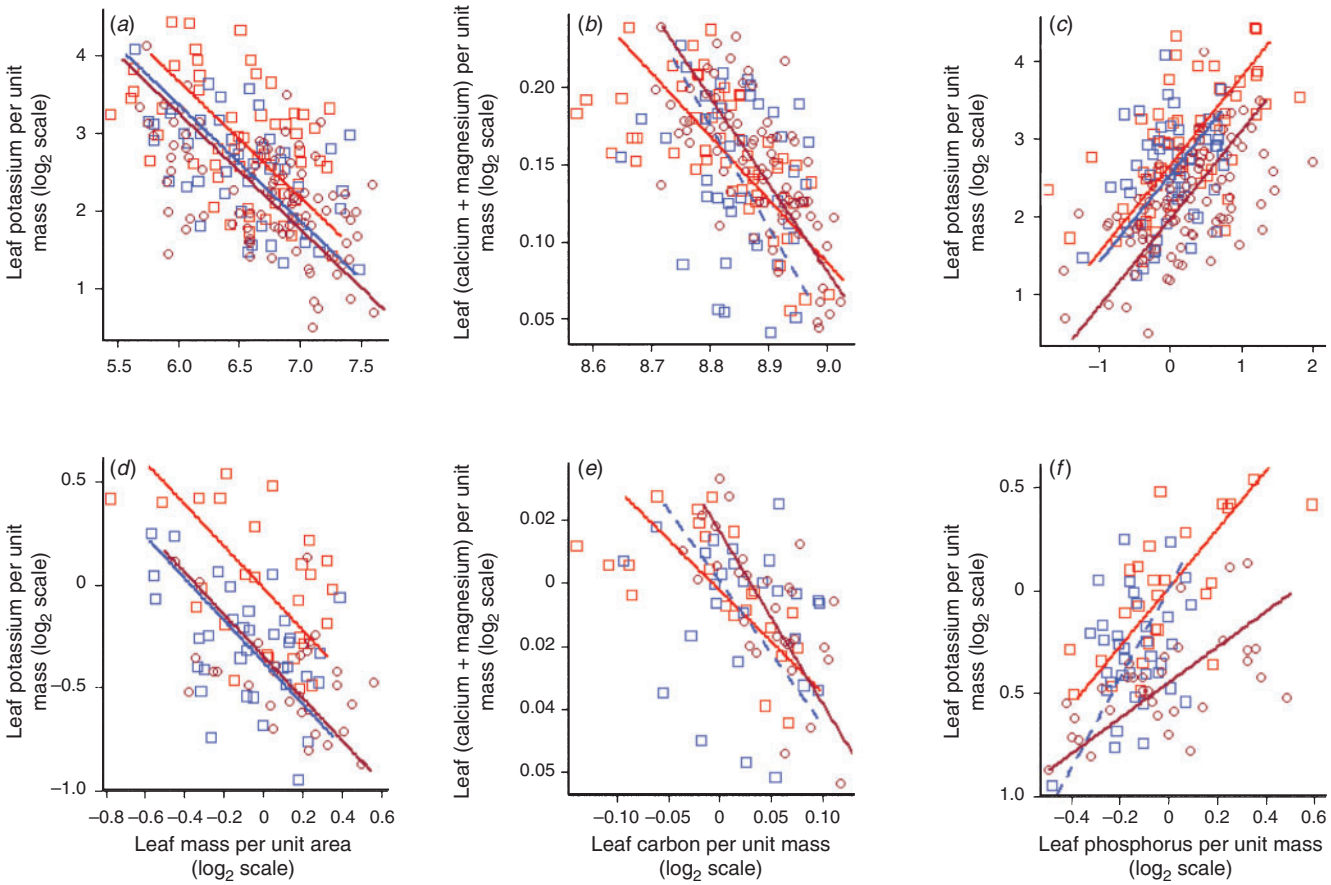

Fig. 9. Relationships between leaf mass per unit area, and mass based measures of leaf nitrogen and leaf phosphorus. Top row, *in loco* trait values (each point represents a different tree); bottom row, *ab intra* values (each point represents a separate species with the mean centred on zero). Open (red) circles: deciduous forest trees; open (blue) squares: evergreen forest trees; open brown circles: deciduous savanna trees. Note the $\log_2$ scale with underlying dimensions of mg g^{-1} (N and P) and g m^{-2} (M_a). Solid lines, ordinary least squares (OLS) regression significant at $P < 0.05$. Dashed lines, OLS significant only at $P > 0.05$.

2012). Here the *in loco* estimate simply takes the measured foliar value for each tree categorising it into either the 'forest' or 'savanna' group depending on where it was found growing (Eqn 2). Alternatively, the *ab intra* approach accounts for systematic trait variations occurring between plots (due, for example, to differences in soil fertility); also allowing each species to have its own unique trait value.

This second approach was only taken within the ZOT areas studied because of low species diversity and limited species overlap across sites in the drier areas. Nevertheless, as discussed below, the mixed model analysis for the precipitation component of the study (with the variance not explained by P_A partitioned into species and residual effects) does provide some insight into the importance of species traits.

Savanna foliar traits and precipitation/rainfall seasonality

It has frequently been reported that M_a tends to increase with declining precipitation (Schulze *et al*. 1998; Fonseca *et al*. 2000; Wright *et al*. 2002; Midgley *et al*. 2004; Cernusak *et al*. 2011) and this has now been suggested as representing a general trend (Poorter *et al*. 2009). Nevertheless, no such pattern was observed in our savanna tree dataset which showed, if anything, a slight tendency for a decline in M_a as P_A declined (Fig. 1*a*). Although this could be attributable to all our savanna species being of the deciduous habit (Wright *et al*. 2005), some of the above cited studies which report increases in M_a as P_A declines have also involved deciduous species (Schulze *et al*. 1998; Midgley *et al*. 2004). This suggests that leaf habit is not the sole explanation for the study differences.

With soil nutrient effects on ecosystem level M_a clearly being important (Cunningham *et al*. 1999; Fyllas *et al*. 2009) one possibility that could account for our different result is the relatively high cation/phosphorus status of the soils in this study (see Table S1.1) compared to the relatively highly weathered soils typical of both tropical Australia and southern Africa (Buckley *et al*. 1987), which is where the studies suggesting a high M_a–low P_A association have taken place. Alternatively, differences in the way rainfall seasonality changes as P_A declines may also be important in explaining differences between the continents as there is a tendency for both the seasonality and total amount of rainfall to decline as one approaches the desert regions of both Australia and Southern Africa but not West Africa. For example, the study by Cernusak

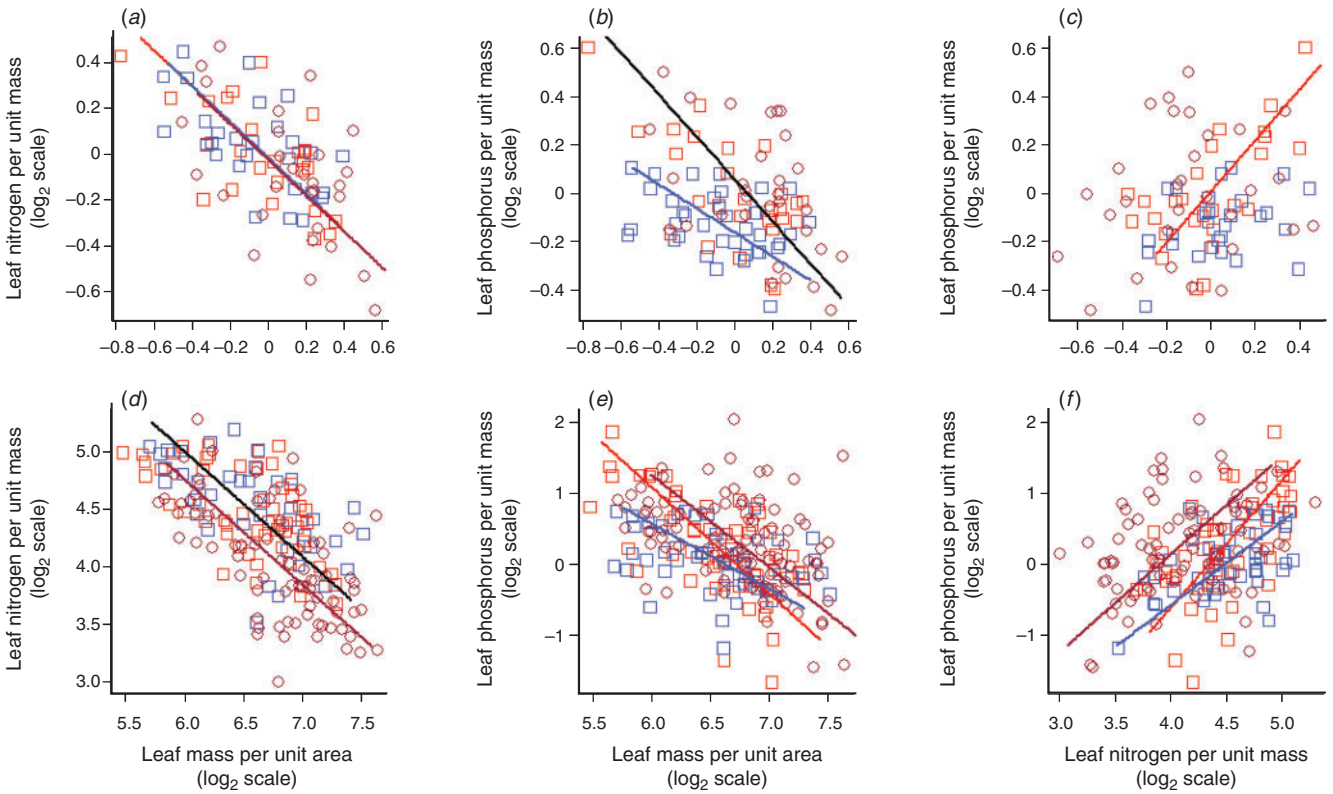

Fig. 10. Relationships between leaf mass per unit area, and leaf potassium (mass basis: *a*, *d*); leaf carbon per unit mass and the sum of leaf calcium and magnesium (mass basis: *b*, *e*); leaf phosphorus per unit mass and leaf potassium per unit mass (*c*, *f*). Top row, *in loco* trait values (each point represents a different tree); bottom row, *ab intra* values (each point represents and separate species with the mean centred on zero). Open (red) circles: deciduous forest trees; open (blue) squares: evergreen forest trees; open brown circles: deciduous savanna trees. Note the $\log_2$ scale with underlying dimensions of mg g^{-1} (K and P), mmol g^{-1} (Ca + Mg) and g m^{-2} (M_a). Dashed lines, ordinary least squares (OLS) significant only at $P > 0.05$.

et al. (2011) was conducted along a precipitation gradient in northern Australia extending from Howard Springs ($P_A = 1.71$ m a^{-1}) to Boulia ($P_A = 0.29$ m a^{-1}). In this study, a dimensionless seasonality index (υ), which potentially varies from 0 for uniform year-round rainfall to 1.0 where all rain occurs in 1 month (Chave *et al.* 2010), gives values of 0.71 and 0.49 respectively. Likewise, for the Kalahari precipitation transect study of Midgley *et al.* (2004), P_A varied from 0.59 m a^{-1} (Pandamatenga) to 0.30 m a^{-1} (Tshane) for which υ = 0.72 and 0.65 respectively. In contrast, for our West Africa study, the opposite is observed, with the driest HOM-01 and HOM-02 sites along our precipitation transect (for which $P_A = 0.29$ m a^{-1}) having υ = 0.85 (see Table S1.1) compared with much lower values in the higher precipitation regions further south. This strong negative υ–P_A association, arising through the West African monsoon providing a highly seasonal but very stable rainfall pattern across our study region (Hall and Peyrillé 2006), means that for the northern areas close to the Sahara desert, the probability of any rain at all for over nearly 8 months of the year is effectively zero. This is in contrast with the more commonly studied low P_A, low υ situation (rainfall becoming 'less predictable' in more arid regions) for which the structural resilience associated with a high M_a would be expected to contribute to leaf survival during extended periods between rainfall events, themselves of an intrinsically more stochastic nature.

Although we detected a slight tendency for M_a to increase with declining P_A, variability within a site was as large as or larger than variability across sites: the most striking examples of which were the two driest sites (HOM-01 and HOM-02). There, despite only three woody species being present (*Acacia senegal*, *Acacia tortilis* subsp. *raddiana* and *Combretum glutinosum*) – all of which are considered to be of exceptional drought tolerance (Arbonnier 2004) – a variation in M_a more or less spanning the range of the entire dataset was observed. This was clearly genetically controlled (Fig. 1*a*) with these large inter-species differences in M_a possibly reflecting different leaf phenological strategies. For example, the *Combretum* species with the higher M_a have been shown to typically exhibit a much longer leafing period compared with the low M_a *Acacia* species, even when in the same environment (Poupon 1979; Devineau 1999). This is consistent with the now generally accepted M_a↔leaf lifetime trade-off (Chabot and Hicks 1982). We therefore postulate that the successful *Acacia* and *Combretum* spp. surviving in this extreme Sahelian environment represent two end points of possible leaf construction cost vs potential carbon return strategies: on the one hand, the *Acacia* spp. produce a very low cost leaf that nevertheless survives long enough to make

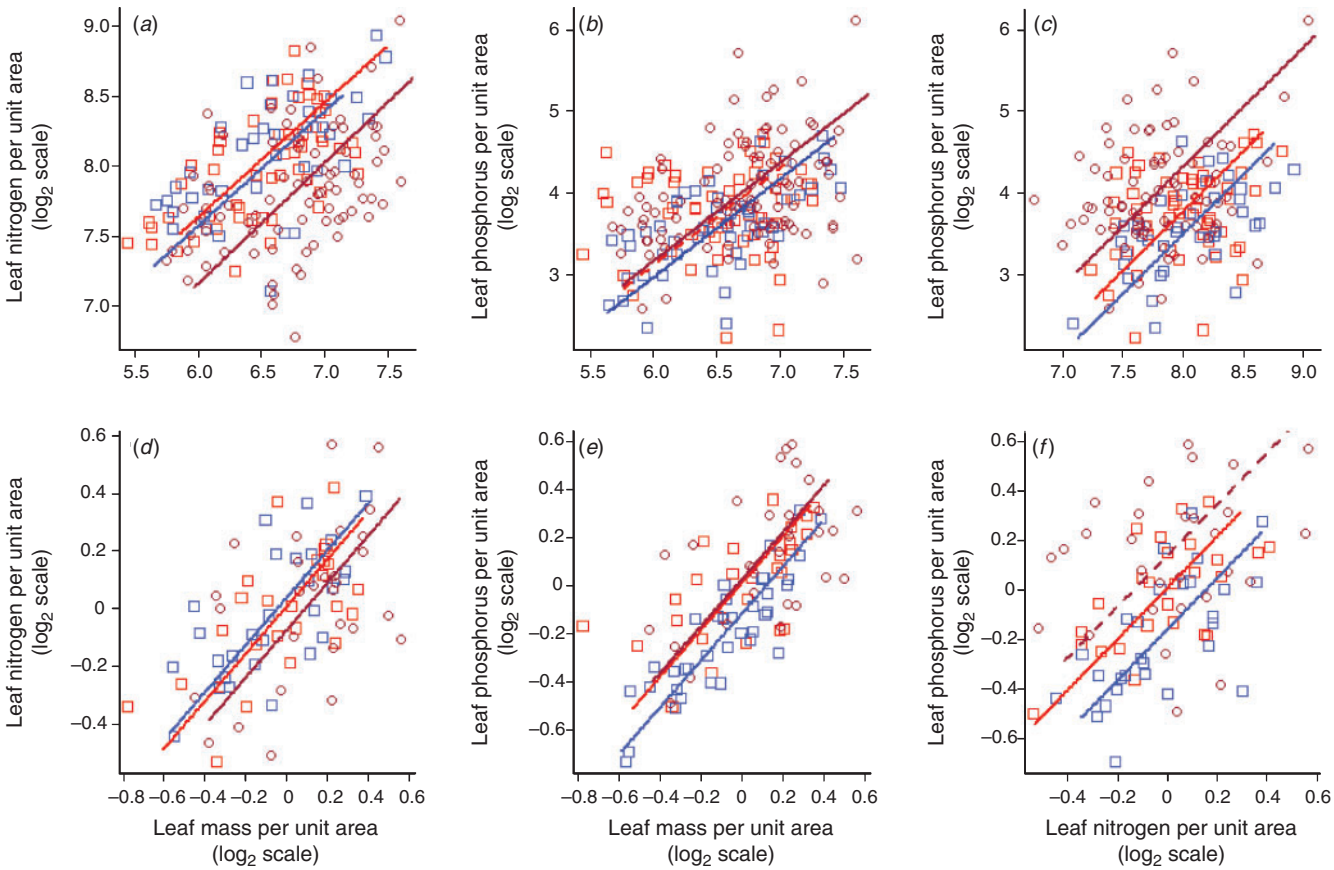

Fig. 11. Relationships between leaf mass per unit area, and area based measures of leaf nitrogen and leaf phosphorus. Top row, *in loco* trait values (each point represents a different tree); bottom row, *ab intra* values (each point represents a separate species with the mean centred on zero). Open (red) circles: deciduous forest trees; open (blue) squares: evergreen forest trees; open brown circles: deciduous savanna trees. Note the $\log_2$ scale with underlying dimensions of mg g^{-1} (N and P) and g m^{-2} (M_a). Dashed lines, ordinary least squares (OLS) significant only at $P > 0.05$.

use of the soil water associated with the rains occurring in the most intense phases of the short wet season. *Combretum* spp. on the other hand, make a more durable leaf, capable of surviving more extended drought periods and hence capable of accessing any later season rains, the value of which would be lost on the already leafless *Acacia* spp. Consistent with this conjecture is the observation by De Bie *et al*. (1998) that West African *Combretum* typically prolong their leafing period when water availability is extended, with *Acacia* spp. apparently lacking the same degree of phenological plasticity.

The clear tendency for different species to systematically differ in M_a is also evident in the mixed-model variance partitioning (see Table S2.1) for which 0.57 of the total dataset variance not accounted for by the fixed effect terms (viz. biome or precipitation) was attributed to species differences (with the rest reflecting within-species variation and experimental error). Some systematic variations at the genus level are also apparent. For example, strongly negative random effect terms (U_S), such as those found for the two *Acacia* species mentioned above, were also found for all other sampled members of this genus. In contrast, for all *Combretum* species, positive random effect terms were found, although some other members of this genus were not characterised by an appreciably higher M_a than average (e.g. *C. adenogonium*, also sampled at MLE-01 for which U_S is only slightly positive). A second savanna genus characterised by consistently higher than average M_a was *Terminalia* (pink coloured symbols in Figs 1–7) for which all five species sampled had a positive U_S.

For $[N]_m$ an even high proportion of the random model component was explained by species identity with $\tau_0^2/(\sigma_0^2+\tau_0^2) = 0.70$ (see Table S2.2), and with the positive (fixed effect) slope for $P_A < 1.0$ m a^{-1} (Fig. 2) also indicating that the higher $[N]_m$ under low precipitation regimes have an additional climate induced component. Especially as there was proportionally a less marked decline in M_a (Fig. 1*a*), this increase in $[N]_m$ was also associated with an increase in $[N]_a$ and the high area based photosynthetic capacities as water availability declines (Domingues *et al*. 2010). This is consistent with theory (Buckley *et al*. 2002) and has also been observed for transect studies in Australia (Cernusak *et al*. 2011) and China (Wei *et al*. 2011) with a general increase in $[N]_a$ with decreasing precipitation also having been demonstrated with the global dataset presented by Wright *et al*. (2005).

However, our dataset also showed an increase in foliar [N] for savanna species for P_A above 1.0 m a^{-1} suggesting that factors

Table 3. First two common principal component axes for the measured foliar properties of the three vegetation formation: habit groups

Values in brackets indicate s.e. Coefficients marked with * are those whose absolute values (Ч) are larger than 0.30 and where the s.e. is <0.5Ч. Abbreviations: M_a, leaf mass per unit area; elemental concentrations are on a dry weight basis; F_d, deciduous forest trees; F_e, evergreen forest trees; S_d, deciduous savanna trees

Variable	Component	
	Ч$_1$ 1	Ч$_2$ 2
log(M_a)	0.51 (0.15)*	–0.40 (0.19)*
log[N]	–0.43 (0.08)*	0.16 (0.16)
log[C]	–0.28 (0.15)	–0.38 (0.12)*
log[P]	–0.43 (0.11)*	0.26 (0.16)
log[K]	–0.39 (0.06)*	–0.06 (0.16)
log[Mg]	0.31 (0.20)	0.54 (0.13)*
log[Ca]	0.24 (0.21)	0.56 (0.10)*
Characteristic roots ($\times 10^3$)		
$\lambda_{j(Fd)}$	187 (58)	112 (35)
$\lambda_{j(Fe)}$	95 (26)	120 (33)
$\lambda_{j(Sd)}$	151 (40)	166 (44)

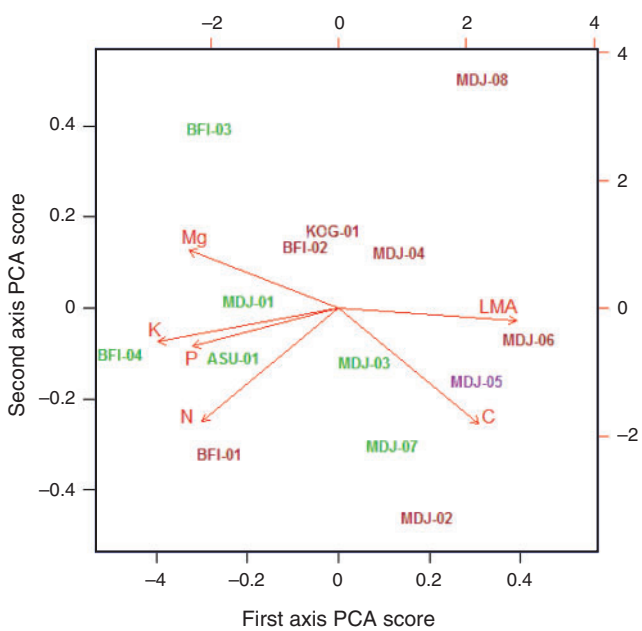

Fig. 12. Principal coordinates analysis biplot of the 'site effects' retrieved from Eqn 2.2. Brown symbols, savanna; green symbols, forest; purple symbol, transitional forest.

other than climate and species identity may have been influencing $[N]_m$ and $[N]_a$ at the moister end of the savanna spectrum. One potential candidate is the declining fire frequency associated with reductions in herbaceous cover as P_A increases (Saiz *et al*. 2012). Nevertheless, although rates of nutrient loss through biomass combustion may be considerable, net fire-induced nutrient losses are usually minimal at a regional scale due to the subsequent return of emitted nutrients through wet- and dry-atmospheric deposition. Thus, other factors may be involved with a general increase in savanna soil N cycling rates with higher P_A having been suggested by Aranibar *et al*. (2004).

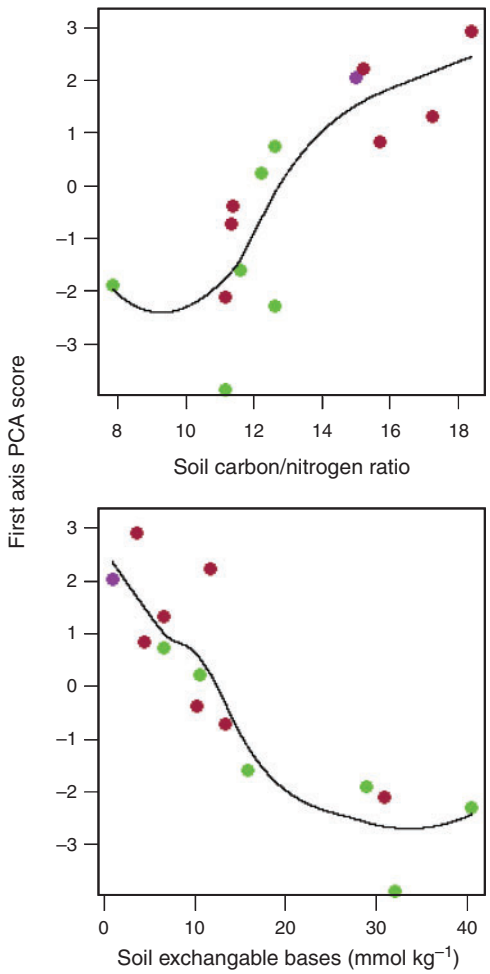

Fig. 13. Relationship between first axis scores of the site effects biplot (Fig. 6) and soil C : N ratio (top) and total soil exchangeable bases. Brown symbols, savanna; green symbols, forest; purple symbol, transitional forest. The fitted line is a non-parametric localised regression (LOESS) smoother.

The pattern of variation in $[P]_m$ in relation to precipitation (Fig. 3) was similar to that observed for $[N]_m$ but with a much less marked increase at lower P_A. Working along this same transect, Domingues *et al*. (2010) suggested that, in addition to N, P may also be important in modulating the photosynthetic capacity of tropical tree species. Although much of the analysis by Domingues *et al*. (2010) was centred on mass-based expressions which we now know can potentially lead to spurious conclusions as to causation where the entities involved are primarily area based (Lloyd *et al.* 2013), we also note that the general conclusions made in that study were demonstrated to hold when area-based expressions were employed (see table 4 in Lloyd *et al.* 2013). That study also found that - irrespective of model – savanna trees growing at lower P_A tend to have higher photosynthetic nutrient use efficiencies than their higher precipitation regime counterparts; also with some suggestion that savanna species typically have a higher photosynthetic capacity at any given level of N and/or P than their forest counterparts. This suggests that any direct translation of simple measurements of foliar N and/or P values into area-based

photosynthetic capacities may not be straight forward for analyses such as ours where significant climatic gradients and/or different vegetation formation types constitute a fundamental part of the analysis.

Although the overall effect was small, the observed linear increase in $[C]_m$ with P_A (7.6 mg g^{-1} m^{-1}) was highly significant (see Table S2.4) and this can only partly have been due to concomitant changes in cation content. For example, the fixed model precipitation term for $[K]_m$ was lower than for $[Ca]_m$ which, along with the observation that $[Mg]_m$ tended to decrease with P_A at the lower rainfall savanna sites suggests that at least half of the increase in $[C]_m$ with P_A must have been due to changes in foliar carbohydrate chemistry. For example, this might be associated with a tendency for increased production of strongly reduced constitutive defence compounds, such as lignin, as potential leaf longevity increases with increasing wet season length, as the phenomenon has been well documented for tropical forest species (Fyllas *et al*. 2009), and it is also the case that at any one site $[C]_m$ can vary by as much as 80 mg g^{-1}. This variability also clearly has a strong genetic component (see Table S2.4) and implies variations in foliar construction costs per unit area due to variations in carbon content to be of a similar order of magnitude to that attributable to variations in M_a.

Of the cations, it was only potassium which showed strongly significant variations with P_A, with average $[K]_m$ for trees in savanna stands increasing markedly as precipitation declined (Fig. 5*a*). Such a trend, which as far as we know has not been reported before, cannot be readily attributed to variations in soil cation availability (see Table S1.1). This therefore suggests an increasingly important role of potassium in S_d leaf water relations as P_A declines. Given its prime role as an osmotically active cation (Leigh and Wyn Jones 1984) one possibility may relate to the leaves of lower P_A areas needing to have more negative osmotic potentials in order to survive more extended rain-free periods during the dry season; this notion being consistent with results of a recent literature survey where it was found that the leaves of 'tropical-dry' woody species tend to have more negative osmotic potentials than their 'tropical-wet' counterparts (Bartlett *et al*. 2012).

Forest/savanna differences

Within the ZOT, the extent to which *in loco* trait differences were accountable in terms of intrinsic (*ab intra*) differences between species typically associated with forest vs savanna as opposed to effects mediated by differences in growing environment depended upon the trait examined. For example, although all trait differences between forest and savanna were inevitably less when considered *ab intra* (as opposed to *in loco*) this difference was much more marked for $[N]_m$ as opposed to $[C]_m$ or $[K]_m$ and with M_a being intermediate (Fig. 8). These contrasts were also reflected in differences in scaling relationships. For example, although S_d leaves showed a lower intercept than their forest counterparts when the $[N]_m \leftrightarrow M_a$ relationship was examined *in loco* (Fig. 9*a*), no such difference was evident *ab intra* (Fig. 9*d*). Likewise, the contrast in slope between F_e and the two deciduous $\mathcal{G}$ was greater when examined *ab intra* (Fig. 9*e*) than *in loco* (Fig. 9*b*). In some cases relationships were significant when examined *in loco* but not *ab intra* (e.g. $[N]_m \leftrightarrow [P]_m$ for F_e and S_d in Fig. 9). Although this was no doubt in some part due to the multiple coordinated trait effects mediated by site as evidenced by the PCA analysis shown in Fig. 12 and Table S4.1, it must also be borne in mind that the *in loco* regressions inevitably have more degrees of freedom (as they are often replicates of the same species) and hence have a higher likelihood of a statistically significant association being obtained.

As noted by Lloyd *et al*. (2013), differences in the nature of bivariate relationships when expressed on a mass basis are readily explainable in terms of the relative variances of the traits being examined on an area basis and the strength of the area-based association (and *vice versa*). As might be expected from that analysis then, the area based relationships of both nitrogen and phosphorus with M_a are of an opposite sign, and for $[N]_m \leftrightarrow M_a$ with the relative intercept rankings being retained (though with a small difference in intercept between the S_d leaves and the forest types). Nevertheless, for phosphorus a second more subtle difference in mass- vs area-based relationships was observed with a difference in mass-based slope between F_e leaves and the two deciduous types (Fig. 9*b*, *e*) which was reflected only in a difference in intercept when examined on an area basis (Fig. 11*b*, *e*). At first sight this might be surprising, but when one examines the mathematics of area- to mass- transformations it is clear that any differences between the three $\mathcal{G}$ in their (in our case log-transformed) variances will be reflected in differences in mass-based SMA slopes, even if the area-based SMA slopes are identical. This is because, with the SMA slope simply being the ratio of the variances for the *y* vs *x* variable and given the slope has the same sign as the regression coefficient (Legendre and Legendre 2012) using eqns A2.1 and A4.6 in work by Lloyd *et al*. (2013) the mass-based SMA slope expressed in terms of the area-based variances becomes:

$$\operatorname{sign}\frac{\sigma_{\ell_{\Theta_m}}}{\sigma_{\ell_{M_a}}} = \operatorname{sign}\sqrt{\frac{\sigma^2_{\ell_{\Theta_a}} + \sigma^2_{\ell_{M_a}} - 2r\sigma_{\ell_{\Theta_a}}\sigma_{\ell_{M_a}}}{\sigma^2_{\ell_{M_a}}}}, \qquad (4)$$

where ${}^{\ell}\Theta_m$ is the log-transformed trait value on a mass basis, ${}^{\ell}\Theta_a$ is the equivalent trait value on an area basis, ${}^{\ell}M_a$ is the log-transformed mass-per unit area and *r* is the ${}^{\ell}\Theta_a \leftrightarrow {}^{\ell}M_a$ regression coefficient. From Eqn 4 it follows that identical area-based bivariate slopes must differ when converted to a mass basis should either the strength of the area-based association and/or the magnitude of the areas trait variance differ between treatments. This then presumably being the reason that the $[N]_m \leftrightarrow [P]_m$ relationships show different slopes (Fig. 9*c*, *f*) but with a common slope (although with different intercepts) being observed for $[N]_a \leftrightarrow [P]_a$ (Fig. 11*c*, *f*).

Although there is a clear rationale for examining foliar traits such as nitrogen and phosphorus which are related to photosynthetic carbon acquisition on an area-basis (Lloyd *et al*. 2013; Osnas *et al*. 2013) for traits such as foliar carbon content and cation concentrations the situation is less clear and we have therefore limited our analysis here to these traits being expressed on a mass basis as is usually the case. As is illustrated in Fig. 10, the strongest correlations for $[K]_m$ were with M_a and $[P]_m$ with $[C]_m$ showing its strongest correlation with

the term $[Mg]_m + [Ca]_m$; this also being stronger for deciduous leaves. Taken with the association of M_a, $[N]_m$ and $[P]_m$ discussed above, these relationships are also consistent with the results of the CPC analysis in Table 3 where (with a greater weighting towards the F_d and S_d groups) the first axis links $[K]_m$ with M_a, $[N]_m$ and $[P]_m$ and with the second axis having $[C]_m$ negatively associated with both $[Mg]_m$ and $[Ca]_m$.

As for the different patterns observed for $[K]_m$ vs $[Ca]_m$ and $[Mg]_m$ along the savanna precipitation transect as noted above, this may reflect not only the well documented role of the former in stomatal function, but also through a potential role in chloroplast metabolism itself (Battie-Laclau *et al*. 2014). This possible role for higher potassium concentrations in helping to maintain the typically higher photosynthetic rates of the low P_A trees (area basis) would be in addition to its contribution to a significant cation–carbon trade-off, which has also been observed for tropical tree species by Patiño *et al*. (2012). In brief, this is considered to reflect different plant strategies in terms of leaf construction costs, with the tendency for low M_a in leaves of high mineral content presumably attributable to a low tissue density associated with thinner, less lignified cell walls with the higher cation content presumably also balanced by higher levels of organic acids which are, themselves, of a relatively low C content. Such leaves also have lower overall construction costs and less investment of phenols and other carbon rich compounds in defence (Poorter and Villar 1997).

Although evergreen species are often thought of as being associated with a higher investment in expensive defence and structural compounds than deciduous or semi-deciduous species an increasing number of studies support the results obtained here. That is, at least for co-occurring tropical forest trees, there seems to be surprisingly little difference in the foliar chemistry and hydrology-related traits of these functional groups (Brodribb *et al*. 2002; Villar *et al*. 2006; Williams *et al*. 2008). Studies which have reported significant variations (e.g. an unpublished study cited in work by Poorter *et al*. (2009) and Ishida *et al*. (2008)) also found that differences due to phylogeny were as large or larger than differences due to phenology.

Environmental versus genetic effects

Finally we comment on the inferred importance of site conditions in influencing the foliar traits observed, especially as implied by the difference between the *in loca* and *ab intra* $[N]_m$ (and to a lesser extent M_a) estimates for savanna species (Fig. 8). Here, the clear implication is that the higher M_a and lower $[N]_m$ typically observed for trees growing in savanna formations as compared with their forest counterparts (Hoffmann *et al*. 2005*b*; Ratnam *et al*. 2011) are, at least in our case, attributable more to differences in the edaphic characteristics underlying savanna versus forest vegetation than inherent differences between 'forest' vs 'savanna' species *per se*. We noted that the first (and only significant) axis of the 'environmental effects' PCA (Table S4.1) contained many substantial weightings which more or less also replicated those implied by the first two axes of the CPC on the species effects (see also Fig. 13). This suggests similar patterns of co-ordinated trait variation not only between different species, but also when the foliar trait gamut of any given species is modified by its growing conditions. Both, soil cation availability and soil C : N ratio, emerged as good predictors of the magnitude of the co-ordinated 'environmental response', which is not surprising given their generally close associations to tropical soil types (Quesada *et al*. 2010). As pointed out by Lloyd *et al*. (2009), immobilisation of nitrogen through litter chemistry feedbacks may also be an important characteristic of savanna ecosystems which – generally speaking – also show more widespread indications of limitations to their productivity by nitrogen availability than is the case for tropical forest systems as suggested by the high soil C : N ratio.

Conclusions

Supplementing our knowledge of already well documented differences between forest and savanna trees in terms of trait adaption to pyrogenic environments, we found significant contrasts in leaf mass per unit area and mass-based foliar N concentrations attributable to both genotype and differing site conditions for naturally growing dry land forest and savanna trees in West and Central Africa. No difference was found for foliar phosphorus concentrations. The most marked contrast between the two groupings was, however, in foliar potassium concentrations which were appreciably lower in savanna species as compared with their forest counterparts. Savanna tree foliar potassium concentrations were also markedly higher at lower precipitation sites. Considered in conjunction with previous studies from moister tropical forests (Quesada *et al*. 2012), this suggests an important yet unidentified role for this cation in the functioning of the tropical woody species.

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Appendix 1. List of Symbols and abbreviations

Symbol	Definition
$[\ ]_a$	leaf area-based nutrients
$[\ ]_m$	leaf mass-based nutrients
ab intra	from within analysis ("species effects")
ASU	Asukese field site (Ghana)
BFI	Boabeng-Fiema field site (Ghana)
C	carbon
Ca	calcium
CPC	common principal component analysis
d.f.	degrees of freedom
F_d	deciduous forest
F_e	evergreen forest
HOM	Hombori field site (Mali)
in loco	in place analysis ("plot effects")
K	potassium
KOG	Kogyae field site (Ghana)
ℓ	log-transformation
M_a	leaf mass per unit area
MDI	Central African field sites (Cameroon)
Mg	magnesium
MS	mean sum of squares
N	nitrogen
P	phosphorus
P_A	mean annual precipitation
PCA	principal component analysis
s	non-parametric smoother
SS	sum of squares
r^2	Pearson's correlation coefficient
S_d	deciduous savanna
SMA	standardised major axis
T_A	mean annual temperature
U_P	plot-level random effect (mixed model analysis)
U_S	species-level random effect (mixed model analysis)
ZOT	zone of transition
ε	residual error
λ	eigenvector weighting (common principal components analysis)
Θ	trait
σ, σ^2	standard deviation and variance (general)
σ_0^2	residual variance (mixed model analysis)
$\sum_B$	sum of exchangeable bases (soil)
τ_0^2	variance associated with species identity (mixed model analysis)
υ	seasonality index
Ч	(common)n principal components axis
$\mathbb{F}$	forest vegetation formation type
$\mathbb{G}$	vegetation formation/leaf habit group affiliation
$\mathbb{h}$	leaf habit
$\mathbb{S}$	savanna vegetation formation type
$\mathbb{V}$	vegetation formation type