Leaf traits of African woody savanna species across climate and soil fertility gradients: evidence for conservative versus acquisitive resource-use strategies

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Summary

1. Establishing trade-offs among traits and the degree to which they covary along environmental gradients has become a key focal point in the effort to develop community ecology into a predictive science. While there is evidence for these relationships across global data sets, they are often too broad in scale and do not consider the particularities of local to regional species pools. This decreases their usefulness for developing predictive models at scales relevant for conservation and management.
2. We tested for trade-offs between traits and relationships with environmental gradients in trees and shrubs sampled across southern African savannas and explored evidence for acquisitive versus conservative resource-use strategies using a phylogenetically explicit approach.
3. We found a distinct trade-off between two major poles of specialization indicative of acquisitive (high leaf nitrogen concentration, leaf phosphorus concentration, leaf N:P, specific leaf area and average leaf area) and conservative resource-use strategies (high leaf carbon to nitrogen ratios (C:N), tensile strength and leaf dry matter content). Although we found that trait variance and species occurrence were constrained by phylogeny, phylogenetically informed analyses did not contradict non-phylogenetic analyses, strengthening relationships in most cases.
4. The high intrasite trait variability and weak relationships with soils and climate may in part be explained by the high levels of deciduousness and disturbance (i.e. fire and herbivory) inherent in African savannas.
5. *Synthesis*. The relationships between traits and between traits and environmental gradients were far weaker than, and often contradictory to, broad-scale studies that compare these relationships across biomes and growth forms, cautioning against making generalizations about relationships at specific sites based on broad-scale analyses.

Key-words: communities, determinants of plant community diversity and structure, evolutionary history, leaf economic spectrum, plant functional traits, rainfall, soil nutrients, southern Africa

Introduction

The effort to develop community ecology into a predictive science over the last decade has seen a strong focus on

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exploring the role of functional traits in determining species distributions and their ability to co-occur (McGill *et al.* 2006; Shipley, Vile & Garnier 2006; Westoby & Wright 2006). This has involved establishing trade-offs and relationships among traits, and the degree to which they covary along environmental gradients (see Cornwell & Ackerly 2009; Ordoñez *et al.* 2009). Earlier efforts explored large numbers of species from large areas, spanning many biomes and including multiple growth forms, establishing relationships between traits and trait–environment correlations indicative of a key trade-off between rapid acquisition and the conservation of resources (Reich *et al.* 1999; Díaz *et al.* 2004; Wright *et al.* 2004). While these studies were ground-breaking in drawing attention to the topic, they are too broad in scope to be useful for developing predictive models at the intraregion or community scale (Shipley, Vile & Garnier 2006; Díaz *et al.* 2007). Indeed, subsequent studies have found that relationships evident at these broad scales do not necessarily hold when explored at finer scales (Wright *et al.* 2007), where disturbance history and the necessarily restricted species pools may play an important role.

The leaf economics spectrum identified axes of trait variation that define a continuum of functional strategies spanning two extremes (Wright *et al.* 2004). At one end of the spectrum are species that adopt resource acquisition strategies that allow for rapid resource capture and growth rates, typically characterized by thin, short-lived leaves of high quality, and dominant in highly productive environments. At the other end of the spectrum are species with a conservative resource-use strategy, typically with long-lived, high-density leaves with low nitrogen concentrations, and common in low productivity environments or nutrient-limited soils (Coley 1988; Reich *et al.* 1991, 1998; Poorter & Garnier 1999; Wright, Reich & Westoby 2001; Díaz *et al.* 2004; Ruíz-Robleto & Villar 2005; Villar *et al.* 2006; Cornwell *et al.* 2014).

The availability of soil resources is known to be a strong environmental filter, limiting plant community composition to those species from a regionally available pool that have the traits required to persist at a site (Tilman 1982; Woodward & Diament 1991; Díaz, Cabido & Casanoves 1998; Grime 2006; García-Palacios *et al.* 2012). In low-nutrient ecosystems, slow growth rates and higher nutrient-use efficiencies are typical (Hobbie 1992). This minimizes the demand for nutrients, and losses thereof, resulting in plant traits that promote slow nutrient cycling. In high-nutrient ecosystems, plants are able to achieve rapid growth rates with low nutrient-use efficiency, thereby promoting rapid nutrient cycling (Hobbie 1992). While the availability of soil nutrients is thought to be one of the main factors determining plant community composition (Ordoñez *et al.* 2009), plant species and communities have been shown to play an important role in determining soil fertility through feedbacks to nutrient cycling (Hobbie 1992; Jobbágy & Jackson 2001; Wigley *et al.* 2013), reinforcing patterns of nutrient availability in natural systems through their uptake and use of nutrients.

The relationships between traits and environment are complicated by phylogenetic relatedness among plant species.

Plant communities can represent non-random samples of the phylogenetic lineages that occur in the regional species pool (Cavender-Bares *et al.* 2009). This occurs because, through their descent from a common ancestor, close relatives commonly share characteristics that may be important in determining their habitat requirements (Felsenstein 1985). Sharing similar requirements may lead to filtering of close relatives into the same community, creating a signal of phylogenetic clustering, or competition for the same resources and competitive exclusion, leading to a pattern of phylogenetic evenness (Cavender-Bares *et al.* 2009). Yessoufou *et al.* (2013) found evidence of phylogenetic clustering from sites within our study area, and many of the 81 dominant woody species found at the 16 study sites in this study are concentrated in few, distantly related lineages. For example, our data include 13 species from the Combretaceae, which typically dominate dry broad-leaved savannas on poor soils, while 15 species from the Mimosoideae (i.e. acacias) typically dominate fine-leaved savannas on soils with higher nutrient status (Scholes 1997).

Because relationships among species influence their trait values and probability of occurrence at any particular site, they often cannot be considered as independent observations in statistical analyses comparing traits with environmental conditions (i.e. there is phylogenetic autocorrelation; Felsenstein 1985). For example, an observed relationship between leaf traits and soil properties in African savannas could be entirely driven by an unobserved trait that is similarly structured by phylogenetic history (for instance, nitrogen fixation in acacia species). To account for these issues, there are a number of statistical approaches that allow one to account for phylogenetic history and derive more accurate inference of adaptive trends in cross-species data sets (Felsenstein 1985; Pignata & Diniz-Filho 1996), and which can be applied to plant trait data sets (Swenson & Enquist 2007).

Despite a recent increase in studies investigating the relationships between plant traits and climatic and/or edaphic factors (e.g. Díaz *et al.* 2004; Wright *et al.* 2004; Ordoñez *et al.* 2009; Reich 2014), there have been very few studies in savanna ecosystems that have attempted to quantify the effects of soil fertility and climate on plant traits. This study explores relationships among eight leaf traits (Table 1) and between these traits and soil fertility and climate in southern African savannas. We expect to find 'conservative' or 'retentive' species with associated leaf traits that result in 'slow and tight' nutrient dynamics at resource-limited sites. At resource-rich sites, we expect to find 'acquisitive' species with associated leaf traits that result in 'fast and leaky' nutrient dynamics. Intermediate trait values are expected in-between, in accordance with the findings and predictions of Díaz *et al.* (2004), Wright *et al.* (2004) and Grime (2006). We perform both traditional (non-phylogenetic) and phylogenetically explicit statistical analyses and explore how phylogeny affects relationships and trade-offs among traits and between traits, soils and climate (Price 1997; Blackburn & Gaston 1998; Schluter 2000; Freckleton, Harvey & Pagel 2002). Specifically we ask (i) Are there relationships among the eight leaf

Table 1. List of measured traits, abbreviations and units used

Trait	Abbreviation	Unit
Leaf nitrogen concentration	LNC	mg g^{-1}
Leaf carbon to nitrogen ratio	Leaf C:N	NA
Leaf phosphorus concentration	LPC	mg g^{-1}
Leaf nitrogen to phosphorus ratio	Leaf N:P	NA
Specific leaf area	SLA	cm^2 g^{-1}
Average leaf area	ALA	cm^2
Leaf dry matter content	LDMC	mg g^{-1}
Leaf tensile strength	TS	N mm^{-1}

traits [at both the species and community levels, that is species values versus community-weighted means (CWMs)] that reflect a trade-off between acquisitive versus conservative resource-use strategies? (ii) Are there significant relationships between leaf traits and environmental variables (soil fertility and climate), and do these suggest that particular habitats favour acquisitive versus conservative resource-use strategies? (iii) Do any of these relationships depend on the phylogenetic affinities of the species under study?

Materials and methods

SITE SELECTION, SOILS AND CLIMATE

Sixteen sites (Table S1 in Supporting Information) spanning from semi-arid (< 300 mm mean annual precipitation, MAP) to mesic savannas (> 1200 mm MAP) were selected across conservation areas in South Africa and Zimbabwe. Sites were chosen to include a wide range of underlying geologies and soil fertility status. The sites were not of a fixed size but were large enough to be representative of the savanna woody communities growing in that area. MAP, potential evapotranspiration (PET), mean annual temperature (MAT) and solar radiation (SR) were determined for each site using nearby long-term weather stations when available, alternatively these were acquired from the South African Atlas of Agrohydrology and Climatology (Schulze *et al.* 1997). Evapotranspiration rates at the sites were calculated from the global MODIS evapotranspiration data set described by Mu, Zhao & Running (2011) available at ftp://ftp.ntsg.umt.edu/pub/MODIS/Mirror/MOD16/. The sites also span strong gradients of herbivory and fire frequencies.

For each site, five sets of soil samples were taken at least 5 m apart at depths of 0–10, 10–20 and at 50–60 cm according to the methods described by Wigley *et al.* (2013). A soil pit was also excavated in order to determine bulk density (BD). Four replicate BD measurements were taken at the same depth intervals as the soil samples. BD samples were collected by vertically knocking a sharpened 48-mm-diameter steel pipe 10 cm into the soil on each of the four sides of the soil pit. A spade was then used to dig out the side of the pit until the pipe was exposed, and the spade was placed underneath the rim of the pipe to ensure no soil was lost while the core was retrieved. The soil cores were then carefully emptied into a labelled brown paper bag which was dried to constant weight in a 60 °C drying oven. The BD was calculated as $\rho = M_s/V_s$ (Boone *et al.* 1999), where ρ is BD (g cm^{-3}), M_s is mass of oven-dried soil (g) and V_s is the field-moist soil volume (cm^3).

Soil samples were analysed for carbon (C), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K), as well

as pH at the Elsenburg Laboratory, Institute for Plant Production, Stellenbosch, South Africa. Carbon was analysed by a rapid dichromate oxidation method using the Walkley–Black procedure (Walkley 1947). Samples were not pre-treated for carbonates as the pH was well below 7.4, which suggests that carbonates are not significant in our samples (Nelson & Sommers 1996). Extractable P, Ca, Mg, Na and K were extracted with 1% citric acid and analysed by using a Thermo ICP iCAP 6000 Series Spectrometer (Thermo Fisher Scientific, Surrey, UK). Duplicate samples analysed for C (%), Ca, Mg (both cmol kg^{-1}), Na, K and P (all three mg kg^{-1}) had CVs of 0.08 (C), 0.10 (Ca), 0.07 (Mg), 0.20 (Na), 0.05 (K) and 0.13 (P), respectively. The pH was determined in a 1:10 ratio of 0.01 M KCl (McLean 1982). Sum of bases (SB) was calculated as the sum of Ca, Na, Mg and K and is reported as cmol$^+$ (kg oven dry soil)$^{-1}$ (Da Silva & Batalha 2008). Total soil nitrogen (N) was analysed by total combustion at Bemlab (Pty) Ltd., Somerset West, South Africa, using a Leco Nitrogen Analyser FP 528 (LECO Corporation, St. Joseph, MI, USA). BD measurements allowed for soil nutrients to be expressed as total nutrient stocks for each site, taking the depth of the soil into account. Total stocks of N, P and C are expressed as g m^{-2}, while SB is expressed as cmol m^{-2}. Soil nutrient stocks were calculated to a depth of 1 m for all sites except Makhohlolo, where the soil profile was 50 cm in depth.

Given the large number of soil and climate variables, we used a principal component analysis (PCA) to reduce the number of soil and climate predictors for all subsequent analyses. All measures of soil fertility were approximately log-normally distributed and were therefore log-transformed to attain approximate normality and homogeneity of residuals before input into the PCA. The first two components of the soil PCA (Table S2) explained 81% of the variance in the soil data and loaded highest for soil C:N and soil SB (component 1), and soil N and soil P (component 2). The first two components of the climate PCA (Table S3) explained 90% of the variance in the climate data and loaded highest for PET and SR (component 1), and for MAP and MAT (component 2). All statistical analyses in this study were performed using R 3.2.2 (R Development Core Team 2012).

LEAF TRAIT MEASUREMENTS

At each site, the species that formed the dominant woody community (> 80%) of standing biomass (see Table S4) were identified and sampled for eight leaf traits (Table 1) which are thought to correlate with productivity. The specific leaf area (SLA; inverse of leaf mass per area) of a species is usually a good correlate of its potential growth rate, leaf life span, and should also closely correlate with many of the other measured leaf traits such as leaf nitrogen concentration (LNC) and leaf phosphorus concentration (LPC) (Reich *et al.* 1999; Wright *et al.* 2004). Lower SLA values tend to correspond with relatively high investment in defences, both structural and chemical, and long leaf life span. Species growing in resource-rich environments usually have higher SLA values than nutrient-poor environments (Pérez-Harguindeguy *et al.* 2013). Average leaf area (ALA) has important consequences for leaf energy and water balances. ALA is known to be connected to climatic variation, geology and altitude, whereby heat stress and cold stress select for small leaves (Pérez-Harguindeguy *et al.* 2013). Leaf size variation can also be linked to allometric factors such as plant size, anatomy and architecture. Environmental nutrient stress, disturbance and phylogeny contribute to variation in leaf size (Cornelissen *et al.* 2003; Pérez-Harguindeguy *et al.* 2013). Leaf size and SLA were considered to change in parallel (e.g. Mooney & Dunn 1970; Parsons 1976) but more recent studies

suggest that leaf size and SLA may not be closely linked (Fonseca *et al.* 2000; Ackerly *et al.* 2002).

Leaf nitrogen concentration and LPC are the total amounts of N and P per unit dry mass, expressed as mg g^{-1}. LNC tends to be closely correlated with maximum photosynthetic rate as it is an integral component of the protein Rubisco (Field, Merino & Mooney 1983).

Specific leaf area, ALA, LNC and LPC are generally positively related to plant relative growth rates, leaf carbon assimilation rates and energy supply (e.g. Lambers & Poorter 1992; Niklas *et al.* 2005). Leaf C:N can be used as a proxy of nutrient limitation (e.g. Norby & Cotrufo 1998; Oren *et al.* 2001). Leaf N:P is a common proxy for whether phosphorus or nitrogen is the limiting nutrient (e.g. Koerselman & Meuleman 1996; Güsewell 2004). N-fixing species such as legumes tend to have higher N:P ratios than other plants growing at a site (Pérez-Harguindeguy *et al.* 2013).

Leaf dry matter content (LDMC) has been shown to be negatively correlated with potential growth rates and positively related to leaf life span. Species with low LDMC tend to be associated with productive environments (Cornelissen *et al.* 2003; Hodgson *et al.* 2011). Leaf tensile strength (TS) is strongly related to leaf life span, litter quality and rates of decomposition (Onoda *et al.* 2011; Pérez-Harguindeguy *et al.* 2013), thereby influencing nutrient cycling and availability.

A total of 81 species (of which several were found at more than one site) were sampled across all 16 sites. All analyses were performed on mature, sun-exposed leaves, sampled from the edge of the canopy. Four leaves were removed from five individuals of each species for SLA and ALA measurements while a further two leaves were sampled for TS. All leaf material was put on ice after harvesting and leaf area scanned and wet mass weighed as soon as possible. Leaves were then oven-dried at 65 °C for 72 h before dry mass was determined. An additional 100 g of wet leaf material was sampled from the same five individuals for nutrient analyses. Leaf N and C were analysed using a Leco TruSpec CN Analyser (LECO Corporation). Leaf P was analysed using inductively coupled plasma-optical emission spectrometry (ICP-OES; Varian Vista MPX, Palo Alto, CA, USA). SLA, ALA, TS and LDMC were measured according to the methods described by Cornelissen *et al.* (2003). Leaf area measurements included all parts of the leaf including the petiole and as well as the rachis in compound leaves. All leaf traits were approximately log-normally distributed and were therefore log-transformed to attain approximate normality and homogeneity of residuals prior to all analyses.

Community-weighted means were calculated for each leaf trait at each site as $\Sigma\ P_i \times \text{Trait}_i$, where P_i is the relative abundance of species 'i' in the community, and Trait_i is the average trait value obtained for species 'i' (Violle *et al.* 2007; Domínguez *et al.* 2012). The relative abundance of each species at each site was determined by walking four (100 m × 4 m) randomly assigned transects at each site and recording the species identity and total number of occurrences for all encountered plants that were > 0.5 m in height.

PHYLOGENETIC TREE

A phylogenetic hypothesis was reconstructed using PHYLOMATIC (Pearse & Purvis 2013) based on molecular sequence data for the rbcL and matK chloroplast gene regions downloaded from www.ncbi.nlm.nih.gov (see Table S4 for Genbank accessions). Sequences were aligned using MUSCLE (Edgar 2004), trimmed using trimAL (Capella-Gutiérrez, Silla-Martínez & Gabaldón 2009) and concatenated for analysis. Phylogenetic reconstruction was performed

in BEAST (Drummond & Rambaut 2007) using a backbone tree derived from Yessoufou *et al.* (2013) as a prior. Where our species did not occur in the backbone tree, we either chose its closest available relative as a surrogate or inserted the species into the tree based on current taxonomy (14 surrogates, six inserts of 81 species, see Table S5). Six chains were run for 20 000 000 iterations each using GTR-GAMMA. Posterior distributions were checked for stationarity using TRACER version 1.5.4 (Rambaut & Drummond 2007a), the burn-in period discarded (the first 2 000 000 iterations from each chain in this case), and the runs concatenated and processed to produce a maximum clade credibility tree (MCC) in TREEANNOTATOR version 1.5.4 (Rambaut & Drummond 2007b).

TRAIT EVOLUTION AND SPECIES, FUNCTIONAL AND PHYLOGENETIC TURNOVER

To explore whether we should be concerned about the phylogenetic relationships among species influencing trait–trait and trait–environment relationships in our study system, we investigated the extent to which variability in the eight leaf traits under study depends on phylogeny, and whether there are strong associations among measures of turnover in species, functional and phylogenetic diversity between sites. We explored the influence on traits by plotting trait values on the phylogenetic tree for the 81 species in the study and tested for significant phylogenetic signal in the traits based on the method of Blomberg, Garland & Ives (2003) using 999 iterations of the null model, as implemented in the R package 'picante'. We then calculated measures of species, functional and phylogenetic turnover between sites based on the dissimilarity of Sorenson's coefficient, where turnover = $1 - 2C/(A + B)$, A and B are the number of species in samples A and B, respectively, and C is the number of species shared by the two samples. Phylogenetic and functional turnover were calculated using an adaptation of the coefficient following Bryant *et al.* (2008), substituting the number of species with branch length from the phylogeny and a functional dendrogram, respectively. The functional dendrogram for all traits (Fig. 1) was derived following the method of Petchey & Gaston (2002), whereby the Euclidean distance between all species was calculated based on the eight traits, standardized to a mean of zero and unit variance, and clustered using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). We report the distributions of species, functional and phylogenetic turnover values between sites and test the relationship between the three measures using Mantel tests (Mantel 1967).

TRAIT–TRAIT RELATIONSHIPS AND THEIR DEPENDENCE ON PHYLOGENETIC HISTORY

To test for relationships among the eight leaf traits, we tested for correlations among traits and trait CWMs. We performed phylogenetic least squares (PGLS) regression on species means to account for phylogenetic dependence (Duncan, Forsyth & Hone 2007), using the 'caper' package (version 0.5.2; Orme 2013) in R. We fitted both ordinary least squares (OLS) models, which ignored phylogenetic relatedness, and PGLS models on species trait means and tested which was the most appropriate model for each comparison using both the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). We ran two sets of PGLS analyses, one estimating only λ (the extent to which covariance in traits depends on phylogenetic branch length – the Brownian motion model of evolution), while the other explored more complex evolutionary models, estimating lambda (λ), kappa (κ) and delta (δ) phylogenetic branch length transformations
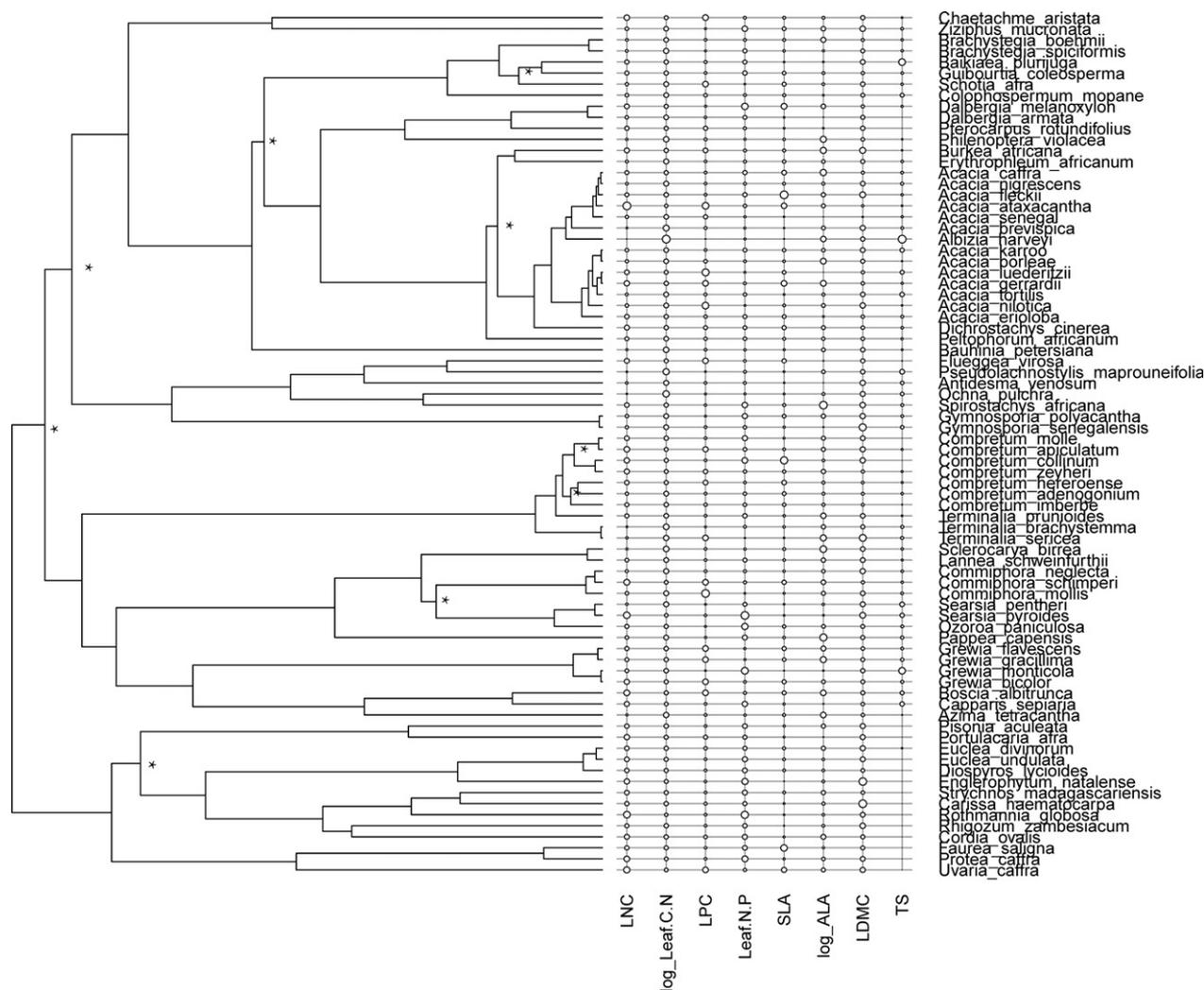
Fig. 1. Maximum clade credibility phylogenetic tree used in this analysis with bubble plots showing variation in values of the eight leaf traits. Branch lengths are proportional to time, but the phylogeny was not calibrated to actual dates so no axis is shown. The tree topology is consistent with published accounts (Bremer *et al.* 2009; Yessoufou *et al.* 2013), and nodes that are not supported with posterior probabilities > 0.9 are marked with *.

simultaneously (Pagel 1999; see Supporting Information for more detail).

TRAIT–ENVIRONMENT RELATIONSHIPS

We performed several different analyses to explore trait–environment relationships. This allowed us to cover a range of approaches commonly used in the literature and to account for the influence of phylogeny. First, we took a community aggregate approach, testing for correlations among trait CWMs and site environmental conditions. We then tested for relationships between species trait means and environmental conditions using the same PGLS versus OLS approach used to explore the trait–trait relationships. Lastly, to account for the influence of spatial autocorrelation among species co-occurring within sites, we explored multivariate relationships between each of the eight leaf traits and the PC axes for soil and climate using Markov chain Monte Carlo generalized linear mixed models (MCMCglmm) in the 'MCMCglmm' package in R (version 2.19; Hadfield 2010). For each leaf trait, models were fit with uninformative priors with soil and climate PC axes set as fixed effects and, to account for phylogenetic

and spatial autocorrelation, phylogenetic relatedness among species and site set as random effects. Models were run for 13 000 iterations, all estimates inspected to ensure that stationarity had been reached, and summaries were based on the last 10 000 iterations. The D^2 regression coefficient (Guisan & Zimmermann 2000) was used to calculate the proportion of deviance explained by the fixed effects (over and above the random effects).

Results

TRAIT EVOLUTION AND TURNOVER AMONG SITES

There was almost no overlap in species composition among sites, with turnover values of 1 for the majority of site pairs (Fig. 2), that is no species in common between the members of a pair. Conversely, there was high overlap in functional and phylogenetic composition, with turnover values centring on 0.4 (i.e. more than 50% overlap). The strong correlation between functional and phylogenetic turnover (Fig. S1)
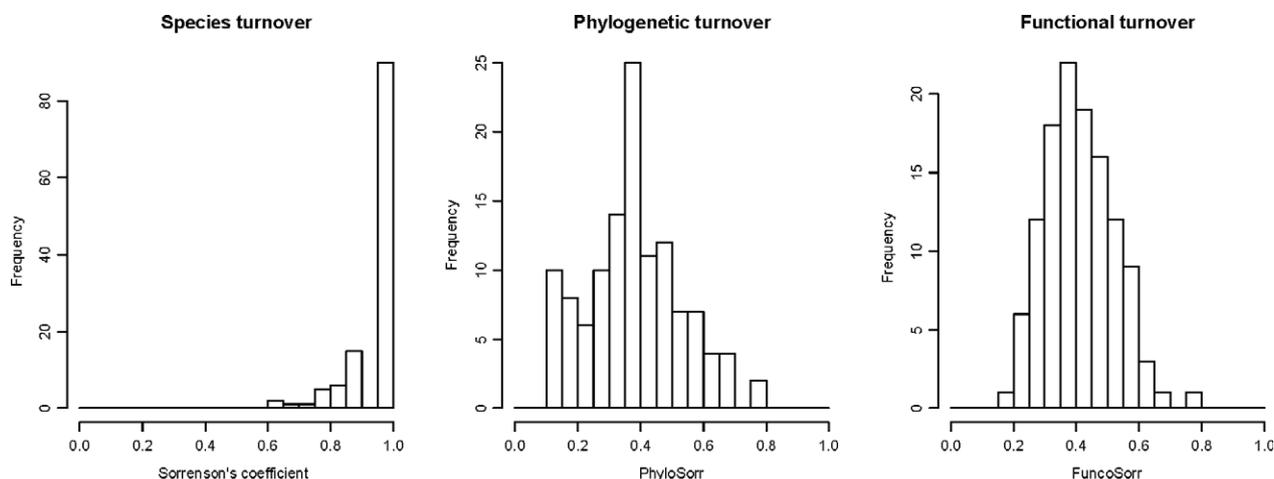
Fig. 2. Frequency histograms of turnover in species composition, phylogenetic relatedness and functional similarity among the 16 study sites. All measures are based on the equation for dissimilarity in Sorenson's coefficient (see Materials and methods for details) and range from 0 (sites are identical) to 1 (no overlap in composition).

Table 2. Tests for phylogenetic signal in the eight measured leaf traits

Trait	K	Observed variance	Mean random variance	P	z-score
LNC	0.093	5391	22 714	**0.001**	−2.123
Leaf C:N	0.122	22 691	118 546	0.066	−0.728
LPC	0.038	60	103	0.112	−1.103
Leaf N:P	0.073	6764	22 141	**0.001**	−1.952
SLA	0.027	684 925	835 937	0.409	−0.410
ALA	0.170	109 757	842 167	**0.002**	−1.131
LDMC	0.023	4 567 159	4 360 532	0.633	0.113
TS	0.148	45	265	**0.001**	−2.129

ALA, average leaf area; LDMC, leaf dry matter content; LNC, leaf nitrogen concentration; LPC, leaf phosphorus concentration; SLA, specific leaf area; TS, tensile strength.
P values for traits with significant phylogenetic signal are highlighted in bold. Tests are based on the method of Blomberg, Garland & Ives (2003) using 999 iterations of the null model as implemented in the R package 'picante'.

indicates that major changes in the functional composition of sites mostly only occur when there is loss or gain of major lineages. This is not surprising given that many of the traits studied show significant phylogenetic signal (Table 2). These results suggest that phylogenetic autocorrelation needs to be accounted for in this data set.

TRAIT–TRAIT RELATIONSHIPS AND THE INFLUENCE OF PHYLOGENY

The PGLS model provided a better fit than the OLS model for all comparisons (ΔAIC and ΔBIC ≥ 2; Table S6), implying that evolutionary history (phylogeny) has a strong impact on the observed patterns. The λ parameter estimates were > 0.5 for all but one comparison (LNC versus leaf C:N; Table 3), suggesting that trait divergence between species is highly dependent on the phylogenetic branch length that links

them (i.e. a Brownian motion model), and were largely unchanged between PGLS models when all three parameters were estimated simultaneously versus λ only (see Results section in Appendix S1 and Table S6).

Irrespective of the history of trait evolution, the PGLS and OLS results were largely consistent and provide evidence for a distinct trade-off along the acquisitive–conservative axis (i.e. high nutrient/fast growth-related traits, for example high LNC, LPC, leaf N:P, SLA and ALA versus low nutrient/slow growth/high retention-related traits, for example high leaf C:N, TS, LDMC). For example, LNC was negatively correlated with leaf C:N and leaf TS ($P < 0.001$, see Table 3 for all correlations) and positively correlated with LPC, leaf N:P, SLA and ALA ($P < 0.05$). LPC was negatively correlated with leaf N:P ($P < 0.001$) and positively correlated with SLA ($P < 0.001$). Leaf N:P was negatively correlated with leaf TS ($P < 0.01$), while SLA was positively correlated with ALA and negatively correlated with LDMC ($P < 0.01$). ALA was positively correlated with leaf TS ($P < 0.001$). Leaf C:N was negatively correlated with LPC, leaf N:P and SLA ($P < 0.001$) and positively correlated with leaf TS ($P < 0.01$). Non-phylogenetic correlations based on CWMs of the eight leaf traits were largely consistent with the OLS and PGLS results, but revealed fewer significant relationships on the whole (Table S7). Interestingly, the CWM analysis revealed significant relationships between LDMC and LNC and LDMC and LPC that were not evident in the OLS and PGLS analyses.

RELATIONSHIPS BETWEEN LEAF TRAITS AND ENVIRONMENTAL VARIABLES

There were several significant correlations between leaf traits and measures of soil fertility and climate (summarized by the first two axes of the soil and climate PCAs, Fig. 3 and Table S8). The PGLS analysis revealed that ALA was positively correlated ($P < 0.05$) with soil PC1 (mostly influenced by soil C:N and soil SB, see Table S2) and climate PC1

Table 3. Relationships among species-level leaf traits [PGLS model coefficients, significance, phylogenetic correlation (λ)]

	LNC	Leaf C:N	LPC	Leaf N:P	SLA	ALA	LDMC	TS
LNC	–	λ = 0.10	λ = 0.86	λ = 0.90	λ = 0.86	λ = 0.92	λ = 0.90	λ = 0.89
Leaf C:N	−0.86***	–	λ = 0.76	λ = 0.83	λ = 0.66	λ = 0.89	λ = 0.86	λ = 0.85
LPC	0.5***	−0.52***	–	λ = 0.81	λ = 0.56	λ = 0.54	λ = 0.54	λ = 0.57
Leaf N:P	0.49***	−0.57***	−0.56***	–	λ = 0.80	λ = 0.85	λ = 0.81	λ = 0.88
SLA	0.31***	−0.33***	0.19*	0.08	–	λ = 0.56	λ = 0.80	λ = 0.66
ALA	−0.07*	0.09*	−0.03	−0.03	0.09*	–	λ = 0.87	λ = 0.78
LDMC	−0.19	0.33	−0.11	−0.04	−0.61**	0.02	–	λ = 0.62
TS	−0.03	0.04	0.01	−0.03	0.02	0.33***	−0.01	–

ALA, average leaf area; LDMC, leaf dry matter content; LNC, leaf nitrogen concentration; LPC, leaf phosphorus concentration; SLA, specific leaf area; TS, tensile strength.
The coefficients of the phylogenetic generalized least squares (PGLS) models are given in the lower left section of the matrix. The phylogenetic correlation (λ) is shown in the upper right section of the matrix. All trait data were log-transformed.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. All P values indicated by *** ($P < 0.001$) remain significant after applying the Bonferroni correction. See Table S6 for kappa (κ) and delta (δ) phylogenetic branch length transformations, and differences in Akaike Information Criterion and Bayesian Information Criterion for PGLS versus ordinary least squares models.

(mostly influenced by PET and SR, see Table S3) and negatively correlated with soil PC2 (mostly influenced by N and P, see Table S2) and strongly dependent ($λ > 0.5$) on phylogenetic history for all comparisons. LNC and leaf C:N were both significantly correlated ($P < 0.05$) with climate PC2 (mostly influenced by MAP and MAT, see Table S3) and strongly dependent on phylogenetic history. SLA was significantly correlated with climate PC1 and climate PC2. The CWMs of the leaf traits mostly showed no significant relationships with the measures of soil fertility and climate (Fig. S2).

The MCMCglmm analyses (Table 4), which controlled for the effects of phylogenetic history and spatial autocorrelation among species co-occurring within sites, showed that soils and climate were significant but poor predictors of half the leaf traits investigated. LNC was negatively related to climate PC2 (posterior mean = −0.25, $P_{MCMC} < 0.001$) and soil PC1 (posterior mean = −0.1, $P_{MCMC} = 0.008$), with the combined fixed effects accounting for 18% of the variance of LNC. LPC was negatively related to climate PC2 (posterior mean = −0.23, $P_{MCMC} < 0.001$), but this only accounted for 2% of the variance in LPC. SLA was positively related to climate PC1 (posterior mean = 0.12, $P_{MCMC} = 0.016$) and climate PC2 (posterior mean = 0.13, $P_{MCMC} = 0.044$), accounting for 3% of the variance of SLA. Leaf C:N was positively related to climate PC2 (posterior mean = 0.28, $P_{MCMC} < 0.001$) and soil PC1 (posterior mean = 0.13, $P_{MCMC} < 0.001$) with the combined fixed effects accounting for 14% of the variance of leaf C:N. None of the measures of soil fertility or climate were strongly related to leaf N:P, ALA, LDMC and TS, with all of these models accounting for < 1% of the variance in each of these leaf traits.

Discussion

Identifying and understanding the major dimensions of trait variation among the vegetation of the world have become a central focus in ecological studies over the past few decades (e.g. Reich *et al.* 1999; Díaz *et al.* 2004; Wright *et al.* 2004,

2005; Ordoñez *et al.* 2009; Liu *et al.* 2012; Jager *et al.* 2015). Studies undertaken at the regional to global scale often include many different taxonomic groups of contrasting plant functional types (PFTs) from several biomes; therefore, they are considerably less prone than more local studies to biases due to species pool limitations. Here, we set out to determine whether the expected trade-offs along the conservative–acquisitive axis of trait variation are evident when tested on a smaller subset of PFTs (trees and shrubs) within a single biome, by determining how resource gradients (soil nutrient stocks and climate) affect savanna woody plant leaf traits. Furthermore, we investigated the degree to which these trade-offs and relationships depend on the phylogenetic relationships among the species under study.

THE INFLUENCE OF PHYLOGENY ON TRAIT ANALYSES

We explored whether phylogeny had a strong influence on the relationships among traits and trait–environment relationships, following the suggestion by Wright *et al.* (2007) that taxonomic biases could significantly influence the results of cross-species analyses of leaf traits, particularly in the face of species pool limitations. Our results showed that the phylogenetically informed model (PGLS) always provided a better fit than the ahistorical (OLS) model (Tables S6 and S8). There was a strong dependence on phylogeny for most trait comparisons ($λ > 0.5$), and stronger relationships were evident once phylogenetic dependence had been accounted for. While these analyses, tests of the degree of phylogenetic signal in traits, and exploration of species, functional and phylogenetic turnover all suggested that it was likely that phylogenetic autocorrelation would hinder analysis in this study, the phylogenetically informed and ahistorical analyses were largely consistent.

RELATIONSHIPS AMONG TRAITS

We found relationships among leaf traits in the sampled species that support the existence of two opposing strategies
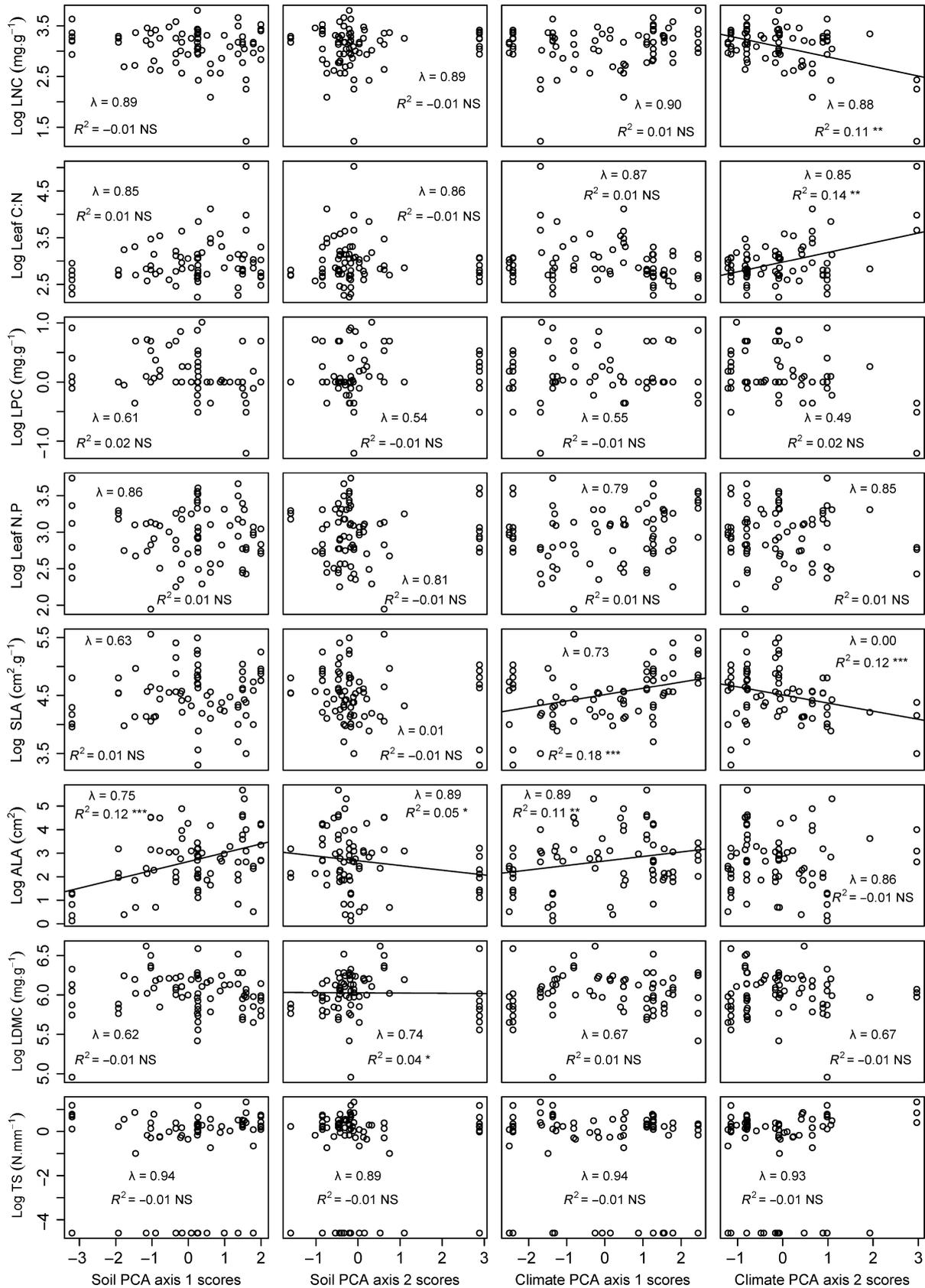
Fig. 3. Relationships between the eight leaf traits and the first two axes of the PCAs based on the measures of soil fertility and climate for all species. Lines were plotted for relationships with $P < 0.05$. The phylogenetic correlation (λ) and R^2 value are shown for all correlations. All trait data were log-transformed. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, $n = 104$, sites = 16. All P values indicated by *** ($P < 0.001$) remain significant after applying the Bonferroni correction.

Table 4. Summary of the Markov chain Monte Carlo generalized linear mixed models (MCMCglmm) used to predict the leaf traits

	Log LNC		Log Leaf C:N		Log LPC		Log N:P	
	pm	P_{MCMC}	pm	P_{MCMC}	pm	P_{MCMC}	pm	P_{MCMC}
Soil								
PC1	−0.10	0.008**	0.13	< 0.001***	−0.09	0.010*	−0.01	0.860
PC2	−0.01	0.946	0.01	0.914	−0.10	0.160	0.09	0.208
Climate								
PC1	0.06	0.144	−0.07	0.090	−0.03	0.478	0.08	0.088
PC2	−0.25	< 0.001***	0.28	< 0.001***	−0.23	< 0.001***	−0.01	0.880
Comparison								
D^2_{MCMC}	0.18		0.14		0.02		0.01	
DIC_{model}	49.7		59.7		64.2		67	
DIC_{null}	61.8		71.5		67.6		64.3	

	Log SLA		Log ALA		LDMC		TS	
	pm	P_{MCMC}	pm	P_{MCMC}	pm	P_{MCMC}	pm	P_{MCMC}
Soil								
PC1	−0.01	0.918	0.17	0.244	−6.0	0.580	0.03	0.658
PC2	0.04	0.562	0.02	0.894	13.4	0.464	0.12	0.424
Climate								
PC1	0.12	0.016*	0.11	0.496	10.2	0.430	−0.04	0.742
PC2	0.13	0.044*	−0.14	0.558	−2.6	0.890	0.29	0.056
Comparison								
D^2_{MCMC}	0.03		0.01		0.002		0.01	
DIC_{model}	83.7		208.5		956		186.5	
DIC_{null}	87		203.3		950		186.4	

ALA, average leaf area; LDMC, leaf dry matter content; LNC, leaf nitrogen concentration; LPC, leaf phosphorus concentration; SLA, specific leaf area; TS, tensile strength.

Fixed effects included the first two components (PC1 and PC2) of both the soil and climate PCAs; site was included as a random effect to account for the non-independence of trait measurements at each site. D^2_{MCMC} is the proportion of deviance explained by the fixed effects (Guisan & Zimmermann 2000); pm is the posterior mean while P_{MCMC} is the P value for each variable included in the model. Deviance Information Criterion (DIC) values are also shown for each full model and the accompanying null model.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

(conservative versus acquisitive). For example, plants with higher LNC values also had higher LPC, N:P and SLA and TS values and *vice versa*. That LNC, which is positively correlated with SLA, was negatively correlated with leaf size was surprising, as other studies have found that leaf size is positively correlated with SLA and leaf nutrients. For example, Díaz *et al.* (2004) found that plants adopting an 'acquisitive' strategy tended to have larger leaves and higher SLA values. Another surprising finding was that leaf TS was found to increase with leaf size. Díaz *et al.* (2004) found that species adopting a 'conservative' strategy tended to have small, thick, tough leaves with low SLA values. In our study, savanna woody plant species with 'acquisitive' strategies tended to have higher quality (high LNC, LPC, N:P and SLA but low C:N and TS), smaller leaves, while species with 'conservative' strategies tended to have lower quality, larger leaves. Many of these differences may be due to the differences in vegetation types and growth forms studied, given that Díaz *et al.* (2004) included xeric shrublands, which often include sclerophyllous species, and covered all lineages and growth forms, including grasses and other monocotyledons. Furthermore, our study sites are typically exposed to high

temperatures and irradiance with seasonal droughts, and Pérez-Harguindeguy *et al.* (2013) note that heat stress, drought stress and high-radiation stress tend to select for smaller leaves.

Analyses of CWMs have become increasingly common in ecology and often reveal stronger relationships than do direct analyses of species means (e.g. Ackerly *et al.* 2002; Garnier *et al.* 2004; Cingolani, Noy-Meir & Díaz 2005; Vile, Shipley & Garnier 2006). We therefore analysed the data from the same eight leaf traits, using the CWM for each trait at each site, instead of species means, to determine whether it improved the relationships between the leaf traits. In general, the results from these analyses were in accordance with the species-wise comparisons, but did not tend to improve the strength of the observed relationships as might have been expected. We also found significant relationships between LDMC and LNC and LDMC and LPC that were not evident in the OLS and PGLS analyses. These differences in results and statistical power are likely the result of high levels of variation in leaf traits among species at the sites (Fig. 2) or the effect of weighting the community means by abundance.

TRAIT–ENVIRONMENT RELATIONSHIPS

Surprisingly, several of the leaf traits (LPC, leaf N:P and TS) showed no significant correlations with any of the measures of soil fertility or climate. The widespread occurrence of legumes with higher N:P ratios across sites could partially explain the weak relationships between leaf N:P, soil fertility and climate. SLA was only found to correlate with climate but not soil fertility. For the most part, savanna woody leaf traits tended to be poorly predicted by both soil fertility and climate [< 4 % of variance for all traits except LNC (18%) and leaf C:N (14%)]. Furthermore, intrasite variability in all of the leaf traits tended to be high (see Fig. 3).

Previous studies have shown strong trait–environment relationships; for example, higher SLA and lower LDMC have been shown to correspond with higher relative growth rates associated with higher soil nutrient availability (Pérez-Harguindeguy *et al.* 2013). The weaker than expected relationships in our study can possibly be explained by the fact that nutrient-rich savanna sites are often dominated by fine-leaved species such as acacias (Scholes 1997), which often have compound leaves. Levels of herbivory in African savannas are typically highest at these more fertile sites; thus, plants that grow large leaves with high LNC and SLA would be easily removed by herbivores and therefore selected against. This could partly explain why leaves on more fertile soils in this study tended to be smaller and denser than might be expected from other studies (e.g. Rusch, Skarpe & Halley 2009). Surprisingly, leaf TS, which is known to be tightly coupled with leaf life span and therefore nutrient cycling (Cornelissen *et al.* 2003), showed no clear pattern across sites. TS was expected to be lower at nutrient-rich sites as a result of higher quality leaf material, which in turn would improve the litter quality and nutrient availability. The leaf N:P ratios found in this study suggest that the vegetation at the study sites is limited by P as species from many of the sites had N:P ratios above 20, while very few species showed N:P of below 10, which would suggest N to be more limiting (Güsewell 2004).

High levels of variability in relationships between leaf traits, soils and rainfall have been found in a number of previous studies (e.g. Poorter & De Jong 1999; Fonseca *et al.* 2000; Wright *et al.* 2004, 2005; Ordoñez *et al.* 2009). Causes for such variability have been attributed to disturbance (Grime 2006), microsite variability and alternative evolutionary solutions to similar environmental challenges and frequency-dependent processes (Westoby & Wright 2006).

Deciduousness in savannas provides an example of an alternative evolutionary solution to seasonal drought. The trade-off is that deciduous species need to invest more nitrogen in their leaves to support higher assimilation rates during the growing season when soil water is available (Eamus 1999). These short-lived leaves are better at fixing carbon but fixation is limited to the growing season. Evergreen species on the other hand have lower leaf nitrogen and consequent lower assimilation rates throughout the year (Eamus 1999). These trade-offs in leaf physiology between deciduous and evergreen species are likely to account for some of the variability in leaf traits and relatively weak relationships found between leaf traits and resources in this study.

Furthermore, the exceptionally high levels of disturbance in African savannas, which still contain a diverse and abundant array of mammalian herbivores and are frequently burnt, have probably been a long-term key driving factor in shaping leaf trait syndromes. For example, plant species may evolve smaller leaves (Brown & Lawton 1991) and invest more resources in structural defences such as spines (Hean & Ward 2012), or invest in higher concentrations of structural and secondary compounds in leaf tissue, in order to reduce both mammal and insect herbivory (Herms & Mattson 1992). This could partially account for the high levels of within-site variability of the leaf traits and the poor predictive power of soils and climate at predicting leaf trait values in southern African savannas.

Concluding remarks

We explored relationships between savanna leaf traits and soil fertility and climate for multiple sites across southern African savannas, finding some evidence for a fundamental trade-off in plant functioning as defined by the acquisitive versus conservative syndromes described by Díaz *et al.* (2004), or the leaf economic spectrum of Wright *et al.* (2004). In general, stronger relationships were evident when we accounted for phylogenetic non-independence, highlighting the value of accounting for phylogeny in analyses of this nature. Nevertheless, relationships between traits and between traits and environmental gradients in this study were far weaker, less consistent, or contradictory to, many broader scale studies that compare across multiple growth forms or sites across biomes and continents (e.g. Díaz *et al.* 2004; Wright *et al.* 2004; Ordoñez *et al.* 2009). This suggests that one should be cautious when making generalizations about relationships at specific sites based on broad-scale analyses, as some of the broader scale pattern may be the result of Simpson's paradox (Simpson 1951), whereby the pattern across sites or growth forms differs from the patterns within them. The high levels of intrasite variability and low levels of variance of each trait explained by soils and climate in our study likely reflect that factors other than these are important in explaining trait variability. Such factors might include the high levels of disturbance and prevalence of deciduousness inherent in many African savannas. Further analyses of plant traits in savanna systems should control for deciduousness and include measures of disturbance to improve our understanding of the relationships between savanna traits, abiotic conditions and disturbance regime.

Acknowledgements

Data accessibility

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Calculation and interpretation of lambda (λ), kappa (κ) and delta (δ) estimates.

Figure S1. Biplot of functional turnover versus phylogenetic turnover indicating the 1:1 line and the trendline.

Figure S2. Plotted correlations between the CWMs of the eight leaf traits and the first two axes of the PCAs based on the measures of soil fertility and climate.

Table S1. Site names, mean annual precipitation (MAP, mm), and mean annual temperature (MAT, °C) and GPS co-ordinates.

Table S2. Eigenvector scores for the PCA based on the four measures of soil fertility.

Table S3. Eigenvector scores for the PCA based on the four climate variables.

Table S4. Woody species identity, family and site names, nomenclature is from Palgrave (2012).

Table S5. Species which did not occur in Yessoufou *et al.* (2013) for which surrogate taxa were used or tips were inserted based on current taxonomy.

Table S6. Relationships among species-level leaf traits [PGLS and OLS model coefficients, significance, lambda (λ), kappa (κ) and delta (δ) branch length transformation estimates and difference in AIC and BIC values (ΔAIC and (ΔBIC) for PGLS versus OLS models].

Table S7. Relationships between the eight plant traits from the 16 sites using the community weighted means for each trait at each site, d.f.$_{1,14}$.

Table S8. Results from the PGLS and OLS models used to predict the eight leaf traits using the first two axes of PCAs based on soil and climate variables at the 16 study sites.