

Leaf adaptations of evergreen and deciduous trees of semi-arid and humid savannas on three continents

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Summary

1. Drought stress selects for a suite of plant traits at root, stem and leaf level. Two strategies are proposed for trees growing in seasonally water-stressed environments: drought tolerance and drought avoidance. These are respectively associated with evergreen phenology, where plants retain their leaves throughout the year, and deciduous phenology, where plants drop their leaves during dry seasons. Evergreen species are thought to have leaf traits supporting lower photosynthesis and transpiration rates, in order to conserve water during dry periods.

2. We evaluated 18 morphological, chemical and physiological leaf traits of 51 abundant savanna tree species that differed in leaf habit (deciduous and evergreen), selected from two climate types (semi-arid and humid) in three continents (Australia, Africa and South America) (annual rainfall range: 500–1550 mm), and grown in a common garden experiment. We hypothesised that evergreen species have more conservative water use and differ more across climate types than deciduous species because evergreen species are forced to endure extended water deficits during dry seasons.

3. Trait shifts between semi-arid and humid savannas did not differ between evergreen and deciduous species.

4. Evergreen species had similar assimilation rates but lower photosynthetic water-use efficiency (PWUE) than deciduous species, possibly to extend their leaf lifespans by protecting their photosynthetic machinery from overheating through evaporative cooling.

5. Species of humid and semi-arid environments did not differ with respect to assimilation rate or PWUE, but semi-arid species did have smaller leaf sizes and greater leaf potassium and phosphorus concentrations. These traits may enable semi-arid species to maximize growth during episodes of favourable moisture availability.

6. Species from the three continents differed in their leaf traits. These probably reflect the greater proportion of evergreen species in Australia as compared to the other continents and generally infertile soils in the South American sampling sites compared to the wider fertility range in the African sites.

7. Synthesis: Water stress in savannas does not select for more conservative water use, but may select for rapid adjustment to prevailing water conditions and for heat avoidance mechanisms.

Key-words: Africa, Australia, deciduous, evergreen, leaf physiology, leaf traits, plant–climate interactions, seedlings, South America, water stress

Introduction

Water is one of the main factors that limits net primary production and affects species' distributions globally (Wright, Reich & Westoby 2001; Ordoñez *et al.* 2009). Traits which are thought to be selected in response to water-limiting conditions include root traits that increase water uptake from deeper, more reliable water sources (Schenk & Jackson 2002; Tomlinson *et al.* 2012), stem traits that minimise risk of vessel cavitation and impairment of water transport (Ackerly 2004; Kursar *et al.*

2009; Markesteijn *et al.* 2011) and leaf traits that reduce water loss (Westoby *et al.* 2002; Wright, Westoby & Reich 2002; Sterck *et al.* 2011) or improve water-use efficiency (Wright, Reich & Westoby 2001). However, much of the evidence for these trait shifts comes from *in situ* comparisons across environmental gradients (e.g. Wright, Reich & Westoby 2001; Markesteijn & Poorter 2009), and some observed patterns may simply reflect plant plastic responses to the local environment. One way to disentangle adaptation from acclimation is to grow species in common garden experiments.

A substantial portion of the world's terrestrial ecosystems are dominated by seasonal climates composed of wet and dry

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seasons (Kottek *et al.* 2006). Two distinct strategies have been proposed for coping with seasonal drought, drought tolerance and drought avoidance. These are thought to be associated with leaf habit (Givnish 2002; Nelson, Barnes & Archer 2002; Ackerly 2004; Markesteijn & Poorter 2009). Drought tolerance has been associated with evergreen species because they retain their leaves throughout the dry season which allows them to be active at the transition between the wet and dry season, or proceed at lower activity during the dry season. Consequently, they are thought to possess traits that allow them to be active at low soil water potentials, including adaptations that reduce xylem cavitation (such as small vessels, high stem density) and minimize water loss [small leaf size (LS), low specific leaf area (SLA) and low transpiration rates] (Ackerly 2004; Kursar *et al.* 2009; Markesteijn *et al.* 2011). Drought avoidance has been associated with deciduous species, which are able to drop their leaves when soil water potentials decline during dry seasons (Reich & Borchert 1984). Because deciduous species avoid dry periods, they may be able to sustain high photosynthetic rates during wet seasons when conditions are favourable for growth (Cornelissen, Castro Diez & Hunt 1996; Givnish 2002; Markesteijn & Poorter 2009). One aspect of these patterns that has not been explored is how leaf traits of evergreen and deciduous species change across gradients of moisture deficit severity. Seasonally dry environments range from very dry savannas (200 mm year⁻¹, > 8 months dry season) to dry forests (up to 2000 mm year⁻¹, > 3 months dry season) (Sánchez-Azofeifa *et al.* 2005; Bond 2008; Hirota *et al.* 2011). Drier environments are also characterised by greater rainfall variability and more frequent drought events during the growing season than wetter environments (Ananthakrishnan & Soman 1989; Nicholls & Wong 1990), which should lead to differences in traits between semi-arid and humid species. In addition, the difference in dry season length could select for greater trait differences among evergreen species than among deciduous species: evergreen species have to endure the severe and extended water deficits during dry seasons and may be forced to even more conservative water use than deciduous species towards drier environments.

We compared leaf traits of seedlings of tree species from humid and semi-arid savannas from three different continents. Studies of functional trait diversity across moisture gradients have previously considered temperate herbaceous (Silvertown *et al.* 2006) and woody species (Wright, Reich & Westoby 2001; Wright, Westoby & Reich 2002; Sánchez-Gómez, Valladares & Zavala 2006) and tropical forest species (Markesteijn & Poorter 2009; Sterck *et al.* 2011). By contrast, patterns of functional trait diversity across savannas remain poorly understood despite the fact that they represent a major biome (Staver, Archibald & Levin 2011). Sub-tropical and tropical savannas extend down to much lower mean annual precipitations (MAP) (range: 200–4000 mm year⁻¹) than tropical forests (> 1000 mm year⁻¹) (Bond 2008; Hirota *et al.* 2011), occurring in arid to humid climate types (*sensu* Kottek *et al.* 2006). Therefore, research into the trait variation among savanna species will also extend our understanding of

the traits and syndromes selected by water stress. In addition, there are clear biogeographic patterns in the distribution of leaf habits in savannas such that Australian savannas are dominated by evergreen tree species whereas African and the American savannas are dominated by deciduous species (Bowman & Prior 2005; Orians & Milewski 2007). If evergreen and deciduous species possess different suites of traits, this may mean that savannas in Australia diverge from savannas in Africa and the America in functional leaf trait adaptations.

We hypothesized that leaf traits of evergreen species are more conservative with respect to water-use and differ more between humid and semi-arid savanna environments than deciduous species, because they are subject to severe water stress during the dry season, which deciduous species avoid. Based on this hypothesis we had the following predictions: (i) Evergreen species have lower water-use efficiency and consequently lower photosynthetic rates than deciduous species, to survive dry seasons. (ii) Species found in semi-arid climates have leaf traits that reduce water loss and enhance water-use efficiency relative to species found in humid climates. (iii) Evergreen species of humid and semi-arid savannas show greater leaf trait differences than deciduous species of each environment.

We evaluated 18 morphological, chemical and physiological leaf traits of 51 abundant savanna tree species that differed in leaf habit (deciduous and evergreen). Species were selected from two climate types (semi-arid and humid) in three continents (Australia, Africa and South America), and grown in a common garden experiment (see Tomlinson *et al.* 2012). We deliberately grew all species in a similar, favourable environment for a fair comparison of presumably genetically based (inherent) variation in the relevant traits. We concentrated on seedlings because this is the stage at which plants are most sensitive to soil water stress (Bond 2008; Van Langevelde *et al.* 2011). At this stage they lack deep root structures possessed by adults that allow them to access more reliable water sources (Ogle & Reynolds 2004; Burgess 2006), and therefore are most likely to express differences selected by water stress.

Materials and methods

SPECIES SELECTION

We sampled dominant or very abundant tree species from humid and semi-arid savannas in Africa, Australia and South America (previously described in Tomlinson *et al.* 2012). We identified semi-arid and humid environments using the Köppen-Geiger climate classification (Kottek *et al.* 2006). This system estimates the relative water deficits of particular locations by comparing a dryness threshold based on the mean annual temperature (T_{ann} , °C) with MAP (mm) at those locations, adjusted for whether the region has predominantly winter or summer rainfall. Given a dryness threshold $\text{Pth} = 28 + 2(T_{\text{ann}})$, then semi-arid environments are defined in the range: $5 \text{ Pth} < \text{MAP} < 10 \text{ Pth}$, and humid environments are defined as areas where $\text{MAP} > 10 \text{ Pth}$. We sampled dominant or very abundant tree species from humid and semi-arid summer-rainfall savannas in

southern Africa (coastal and inland savannas in South Africa and Zimbabwe) (Frost 1996; Mucina & Rutherford 2006), in north-eastern Australia (coastal and inland woodlands in Queensland) (Cole 1986), and eastern South America (Cerrado and Caatinga biomes in Brazil) (Cole 1986). Table S1 (Supporting Information) provides descriptive information for the sampled environments. The South American species were all sampled over low-fertility soils whereas the African and Australian species were sampled over a wider range of soil fertilities.

A total of 51 species were grown, including 18 species from Australia (8 humid, 10 semi-arid), 21 species from Africa (10 humid, 11 semi-arid) and 12 species from South America (eight humid, four semi-arid). The species belonged to a wide variety of clades, representing nine plant orders, 13 families and 28 genera. A full species list is provided in Table S2 (see Supporting Information), including their family, order, continent of origin, climate type of origin and leaf habit traits. The sampled species reflected continental biases in dominant plant families (Cole 1986): Australian savannas are dominated by Myrtaceae and Fabaceae, African savannas are dominated by Combrataceae and Fabaceae, whereas humid South American savannas are composed of a large number of co-dominant families and semi-arid savannas are dominated by Fabaceae. Consequently, two plant orders (Fabales and Myrtales) formed the bulk of the species sample.

GREENHOUSE EXPERIMENT

We conducted a greenhouse experiment to evaluate the effect of leaf habit and climate type on traits of seedlings of the 51 savanna tree species selected from the three continents and grown in one common growth environment. We deliberately grew all species in a similar, favourable environment for a fair comparison of presumably genetically based (inherent) variation in the relevant traits. Plants were grown in a greenhouse chamber at Radix Research Farm, Wageningen University, The Netherlands (51°59'17"N, 5°39'45"E) between September 2008 and October 2010. Temperature in the greenhouse was set at 28 °C for 12 h (daytime) and 23 °C for 12 h (night time). These temperatures were chosen to lie within the range of mean minimum temperatures (17.4–24.2 °C) and mean daily temperatures (24.8–30.3 °C) during the warmest month of the locations where seed was collected for the experiments. Daily variation of light in the greenhouse was from 150 to 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$, supplied from sunshine and supplementary light. Supplementary light (150 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was provided for 12–16 h (seasonally adjusted) to ensure that the daily supply of photon flux density exceeded 10 $\text{mol m}^{-2} \text{day}^{-1}$. Other studies have shown that the total daily photon flux density, rather than peak irradiance, is most important for plant growth (Poorter & Van der Werf 1998). Although the total amount of light is low by comparison with full sun environments in the tropics, plant growth was substantial in some species and varied substantially among species (Table S3 in Supporting Information; Tomlinson *et al.* 2012), indicating that available light was sufficient to distinguish growth abilities of different species.

Tree seedlings were grown in plastic tubes of 10 cm diameter and 100 cm length, to reduce pot limitation of root growth. Pots were filled with river sand mixed with slow-release fertiliser [Osmocote 18-6-12 (N-P-K) fertilizer (8–9 month mixture)] at a concentration of 5 kg fertiliser m^{-3} river sand. We used a high level of fertility to facilitate maximum growth and differentiation among the species during the period of the experiment. Water was supplied through irrigation three times per day at a rate of 40 mL per pot per day, equivalent to 800 mm of rainfall over 20 weeks of growth. This was a much more even water supply than most of these species would receive in their natural envi-

ronment, but the intention was to ensure that all the plants were well-watered while they grew, so that water limitation did not contribute to any trait variation between species.

Seeds were germinated for the experiment in the following manner: seed coats of hard-coated species were cut to allow water imbibition, and seeds of all species were soaked in distilled water for at least 12 h to allow imbibition. Subsequently, seeds were placed on petri dishes with agar to germinate and put into the greenhouse. Germination varied from 1 day to more than 4 weeks. Most species were planted into experimental pots within a week after root emergence from the seed, but a few species were kept on agar for longer because their root growth was very slow.

Seedlings were grown for 20 weeks and then harvested. A maximum of 10 individuals per species were grown (individuals per species: range = 5–10; average = 8.8; mode = 10) (actual replications provided in Table S3). Due to space limitations it was necessary to grow plants in three batches. Initially, we had intended only to run two batches of the experiment with even planting for each species in each batch. However, for numerous reasons [species' sample size, seed availability and viability, species distribution across the associated phylogenetic tree (see Tomlinson *et al.* 2012)] we decided an additional batch would be necessary. This meant that species were grown in one batch (10 individuals) or two batches (five individuals per batch) of the three.

DATA COLLECTION

Eighteen leaf traits were measured for each individual. This data set (of up to 457 individuals) is hereafter termed 'individual data'. Mean values of each trait were also calculated per species, hereafter termed 'species data' (provided in Table S3).

Leaf morphological traits were measured or estimated that are thought to affect water loss by tree crowns (Westoby *et al.* 2002; Cornelissen *et al.* 2003a,b; Poorter *et al.* 2009). Morphological traits were estimated per individual using the following procedure. At harvest time, up to six fully expanded fresh leaves were sampled from each plant. These were immediately scanned with a flatbed scanner (CanoScan LIDE 100; Canon, Tokyo, Japan) to obtain their surface area, weighed for their fresh weight, dried at 70 °C for at least 48 h, and weighed for their dry weight. The remaining plant biomass was separated into organ fractions (cotyledon, leaf, stem, root), dried and weighed to obtain dry masses of cotyledon, leaf, stem, root and total (M_T). Leaf size (cm^2) was estimated as the mean area of the sampled fresh leaves. Petioles, and rachides of compound-leaved species, were retained on the leaves for these measurements. Phyllodes of evergreen *Acacia* species were similarly treated as individual leaves of these species. Specific leaf area ($\text{cm}^2 \text{g}^{-1}$) was calculated as leaf area of the sampled leaves divided by their dry mass; leaf dry matter content (LDMC, g g^{-1}) as leaf dry mass divided by leaf fresh mass; leaf water content per unit area (LWC_a , $\text{g H}_2\text{O cm}^{-2}$) as leaf fresh mass minus dry mass, divided by leaf area; leaf mass fraction (LMF, g g^{-1}) as plant leaf mass divided by plant total mass (M_T); and leaf area ratio (LAR, leaf area per unit total plant mass, $\text{cm}^2 \text{g}^{-1}$) as the product of SLA and LMF. Low LS, LMF, SLA and LAR are all thought to reduce water loss. LWC_a reflects leaf surface area to volume; low leaf surface to volume may lead to lower evaporative demand per unit volume.

Leaf nutrient contents and photosynthetic parameters were measured that indicate leaf productivity and photosynthetic resource-use efficiency (Wright, Reich & Westoby 2001). Leaf chemical and physiological traits were estimated per individual using the following

procedures. Dry mass-based estimates of leaf nutrient contents (N_m , P_m , K_m , mg nutrient g^{-1}) were estimated using a Skalar San-plus autoanalyzer after digestion with a mixture of H_2SO_4 , Se and salicylic acid (Novozamsky *et al.* 1983). Area-based measurements of leaf nutrient contents (N_a , P_a , K_a , mg nutrient cm^{-2}) were calculated by dividing mass-based estimates by associated SLA (e.g. $N_a = N_m \times SLA$). Area-based assimilation rates (A_a , $\mu mol CO_2 m^{-2} s^{-1}$) and stomatal conductance (g_s , $\mu mol H_2O m^{-2} s^{-1}$) were measured with an infra-red gas-exchange system (CIRAS-1; PP-System, Hitchin, UK) on the youngest fully developed leaves on plants 1–2 weeks prior to harvesting. Mass-based assimilation rate (A_m , $nmol CO_2 g^{-1} s^{-1}$) was calculated as $A_m = A_a \times SLA$. Photosynthetic resource-use efficiency traits were evaluated by calculating water-use efficiency ($PWUE = A_a/g_s$, $\mu mol CO_2 \mu mol^{-1} H_2O$) and nitrogen-use efficiency ($PNUE = A_a/N_a$, $\mu mol CO_2 s^{-1} g^{-1} N$). Nitrogen and phosphorus content were measured because they are required in key compounds of photosynthesis, namely rubisco and ATP. Potassium content was measured because it is involved in osmotic regulation in cells and is considered to be important for regulating stomatal guard cells (Benlloch-González *et al.* 2008). Photosynthetic measurements were only taken for the first two batches and thus cover 40 species.

Leaf assimilation rates were measured at an irradiance of $600 \mu mol m^{-2} s^{-1}$ provided by a PLC6 Leaf Cuvette. Readings were taken when plants were fully induced and photosynthetic rates had stabilised. Readings were taken at $600 \mu mol m^{-2} s^{-1}$ because maximum A_a measurements stabilised around this light supply rate, as indicated by the photosynthetic light response curves that we measured for several of our species. This may have occurred because of the low peak growth light levels that we were able to supply to our plants in the greenhouse (up to $450 \mu mol m^{-2} s^{-1}$). The light-saturated A_a values we recorded (range: $1\text{--}13 \mu mol m^{-2} s^{-1}$) (Table S3) are similar to values recorded on savanna adults in the field [$3\text{--}16 \mu mol m^{-2} s^{-1}$ (Tuohy, Prior & Stewart 1991; Eamus *et al.* 1999; Prado *et al.* 2004)], but at the lower end of values recorded for seedlings of savanna and Mediterranean species under greenhouse conditions (typically between 4 and $26 \mu mol m^{-2} s^{-1}$) (Holmes & Cowling 1993; Stoneman, Turner & Dell 1994; Evans *et al.* 2000; Kgope, Bond & Midgley 2010). Our A_m values ($26\text{--}280 nmol CO_2 g^{-1} s^{-1}$) are comparable with the range presented by Wright *et al.* (2004).

STATISTICAL ANALYSIS

We used mixed models on the individual data to test whether each measured leaf trait differed between plants of different leaf habit (H, two levels: deciduous, evergreen) and different climate (C, two levels: humid, semi-arid), and between species of different leaf habits in different climate types (H \times C). Plant size (M_T) was also included in the fixed model because numerous traits have been shown to vary with plant size. Random effects included in the model were species (Species, 51 levels) and batch run in the greenhouse (Batch, three levels). Trait responses within species were also allowed to vary with plant size.

Mixed model analyses were conducted in R using the *lmer()* function of the *lme4* package (Pinheiro & Bates 2000). Output from *lmer()* includes standard deviation estimates for the random effects and regression model coefficients for the fixed effects. Ratio tests on maximum likelihoods (LRTs) were used to evaluate fixed and random effects in the model following Baayen, Davidson & Bates (2008). Random effects were first evaluated using REML in conjunction with

the full fixed effects model. Fixed effects were subsequently evaluated using ML with the chosen random model. Coefficients for parameters of the chosen models were estimated using REML (Baayen, Davidson & Bates 2008). These coefficient estimates were checked for significance using standard *t*-test procedures because we had large sample sizes ($n \geq 253$). When non-significant parameters were observed, Markov chain Monte Carlo (MCMC) sampling on the posterior distributions of the parameters was used to assess whether the probabilities changed (Baayen, Davidson & Bates 2008). Chosen models for each trait including regression model coefficient estimates for fixed effects and standard deviations for random effects are presented in Table 1.

We used PERMANOVA (Anderson 2001) on the species data (Table S3) to establish whether species of different leaf habits, different climate types and different continents could be differentiated by a combination of the considered leaf traits (number of permuted data sets = 1000). The full model tested included Leaf habit, Climate, Continent and interactions among them. Where observed *F*-values were significant for Continent (three levels) or for interactions, component groups were compared pairwise using the same PERMANOVA procedure to generate *F*-values with probabilities that could be rooted to give the *t*-values for these pairwise comparisons (Anderson, Gorley & Clarke 2008). Analyses were conducted in R using the *adonis()* command in the *vegan* package (Oksanen *et al.* 2011). As these were multiple comparison tests, we evaluated the significance of the resulting probabilities using the false discovery controlling procedure devised by Benjamini & Hochberg (1995). The results are presented in Table 2. The multivariate data were subsequently visualised using principal components analysis (PCA) to interpret the PERMANOVA results and to determine whether species groups were associated with particular traits. As we did not have complete photosynthesis data for all of the species, we conducted two multivariate analyses, the first on only those species for which we had data on all 18 traits (hereafter the 'All Traits' set) and the second on all the species with the reduced set of non-photosynthetic traits (hereafter the 'All Species' set). There were relatively more deciduous species in the *All Traits* set, which might have affected the distributions in the PCAs. Therefore, we only discuss the multivariate analyses for the *All Species* set (Fig. 1). The PCA results of the *All Traits* analyses are provided in the Supporting Information (Fig. S1). Axis scores for species and traits under each analysis are also provided in the Supporting Information (Table S4). PCAs of the *All Traits* and *All Species* datasets showed similar trait loadings for the first and second axis. Pearson correlations between all traits were also calculated to provide supplementary information on the bivariate relationships between traits (Table S5 in Supporting Information).

Results

INDIVIDUAL TRAIT VARIATION BETWEEN LEAF HABITS AND BETWEEN CLIMATE GROUPS

Fixed and random effects explained significant portions of variation in all 18 traits (Table 1). Of the fixed effects, nine traits differed significantly between leaf habits and seven traits differed significantly between climate types. In contrast to what we had hypothesised, there were no significant interactions between leaf habit and climate type for any traits. Twelve traits also varied significantly with seedling mass (M_T). Of the random effects, traits varied significantly across species in all cases and 10 morphological and leaf chemistry

Table 1. Regression results for mixed models testing for the effects of leaf habit (H) (levels: deciduous and evergreen), climate type (C) (levels: humid and semi-arid) and their interaction (H × C) on individual leaf traits of seedlings of 51 savanna tree species harvested at 20 weeks after planting as recently germinated seedlings. Leaf physiology traits were analysed for a subset of 40 species for which this information was available. The default factor levels are deciduous leaf habit and humid climate type. Model parameters are selected based on log ratio tests and Akaike information criteria (see *Methods* for details). Within the chosen models, significant coefficient estimates based on *t*-tests (5% level) are indicated in bold (*). Coefficient estimates are for the transformed variables.

Traits	Units	Transform	<i>n</i>	Random effects (standard deviation)				Fixed effects (regression estimates)				
				Species	Species × <i>M_T</i>	Batch	Residual	Intercept (Deciduous) (Humid)	<i>M_T</i>	H (Evergreen)	C (Semi-arid)	H × C (Evergreen) (Semi-arid)
Morphology												
Leaf mass fraction	g g ⁻¹	-	457	0.160	0.038	-	0.070	0.397	-	0.157	-	-
Leaf size	cm ²	ln	423	0.761	0.235	-	0.377	2.270	0.445	-	-0.558	-
Specific leaf area	mm ² mg ⁻¹	√	420	0.806	0.148	-	0.336	4.888	-0.210	-0.892	-	-
Leaf area ratio	mm ² mg ⁻¹	√	420	0.799	0.189	-	0.357	3.059	-0.156	-	-	-
Leaf dry matter content	mg g ⁻¹	√	410	2.216	0.326	0.522	0.854	18.442	-	-	0.993	-
Area-based leaf water content (LWC _a)	g H ₂ O cm ⁻²	ln	416	0.397	-	0.092	0.180	-4.908	0.078	0.502	-	-
Leaf chemistry												
<i>N_m</i>	mg N g ⁻¹	√	431	0.836	0.206	0.096	0.363	5.654	-0.183	-0.632	-	-
<i>P_m</i>	mg P g ⁻¹	ln	431	0.546	0.133	0.101	0.334	1.035	-0.180	-	0.248	-
<i>K_m</i>	mg K g ⁻¹	√	431	1.090	0.272	-	0.382	3.951	-0.166	-	0.310	-
<i>N_a</i>	g N m ⁻²	ln	416	0.337	-	-	0.202	0.322	-	0.204	-	-
<i>P_a</i>	g P m ⁻²	ln	416	0.543	0.114	0.101	0.352	-2.161	-0.095	0.612	0.253	-
<i>K_a</i>	g K m ⁻²	ln	416	0.465	0.128	-	0.246	-0.458	-	0.510	0.195	-
<i>N : P</i>	g N g ⁻¹ P	√	431	0.419	-	0.314	0.546	3.349	0.180	-0.761	-	-
Leaf physiology												
<i>A_a</i>	μmol CO ₂ m ⁻² s ⁻¹	√	254*	0.504	-	-	0.677	2.191	0.065	-	-	-
<i>A_m</i>	nmol CO ₂ g ⁻¹ s ⁻¹	ln	254	0.530	-	-	0.700	4.417	-	-	-	-
<i>g_s</i>	μmol H ₂ O m ⁻² s ⁻¹	√	254*	2.033	-	2.033	2.631	8.492	0.019	-	-	-
Photosynthetic water-use efficiency	μmol CO ₂ μmol ⁻¹ H ₂ O	ln	253*	0.211	-	0.536	0.551	-2.621	0.065	-0.300	-	-
Photosynthetic nitrogen-use efficiency	μmol CO ₂ s ⁻¹ g ⁻¹ N	√	253*	0.334	-	-	0.589	1.973	-	-	-0.261	-

*Where non-significant coefficient estimates were observed, parameter estimates were re-estimated using MCMC sampling from the posterior distributions of the parameters (Baayen, Davidson & Bates 2008).

Table 2. PERMANOVA on Euclidean distances for mean leaf traits of seedlings of 51 tree species selected from savannas in two climate groups [Humid ($n = 26$), Semi-arid ($n = 25$)] on three continents [Africa ($n = 21$), Australia ($n = 18$), South America ($n = 12$)]. Analyses were conducted on the subset of species for which all photosynthetic traits were measured ('All Traits') and for the set of traits measured on all species ('All Species'). Presented probabilities are results generated by PERMANOVA (Anderson 2001). Multiple comparison significance tests are conducted using the false discovery controlling procedure of Benjamini & Hochberg (1995), using $\alpha = 0.05$ as the critical reference level. Significant probabilities remaining after this procedure are indicated with an asterisk

Source	All Traits ($n = 40$)					All Species ($n = 51$)				
	d.f.	SS	MS	F	P	d.f.	SS	MS	F	P
Leaf habit	1	96.28	96.27	6.594	< 0.001	1	112.26	112.26	11.112	< 0.001
Climate	1	31.17	31.17	2.135	0.035	1	32.33	32.33	3.200	0.009
Continent	2	63.56	31.78	2.176	0.009	2	40.67	20.34	2.013	0.034
Residual	35	510.99	14.6			46	464.73	10.1		
Total	39	702.00				50	650.00			
Pairwise comparisons	d.f.	t	P			d.f.	t	P		
Africa vs. Australia	28	1.975	0.002	*		37	2.204	0.002	*	
Australia vs. South America	26	2.070	< 0.001	*		31	2.077	< 0.001	*	
Africa vs. South America	20	1.944	0.004	*		28	2.229	< 0/001	*	

traits also varied significantly with seedling mass among species (Species $\times M_T$). Batch effects explained a significant amount of variation for eight traits.

Species of different leaf habits differed with respect to morphological, chemical and physiological traits. Evergreen species had greater LMF but lower SLA than deciduous species, and consequently the groups did not differ significantly in LAR. Evergreen species also had greater area-based leaf water content (LWC_a) than deciduous species. Evergreen species had lower nitrogen per unit leaf mass (N_m) and N : P ratio but greater amount of nutrients per unit leaf area (N_a , P_a , K_a) than deciduous species. On average, evergreen species did not differ from deciduous species with respect to mass- or area-based assimilation rates, but they did have lower photosynthetic water-use efficiency (PWUE) than deciduous species. This was probably in part due to greater stomatal conductance for evergreen species than deciduous species, but at marginal significance level ($P = 0.070$; result *not* shown in Table 1).

Species of each climate group differed with respect to few morphological traits: species of semi-arid climates had significantly smaller LS and greater LDMC than species of humid climates. Species of semi-arid climates also had greater leaf phosphorus and potassium contents per unit mass and per unit area than species of humid environments. Species of each climate group did not differ with respect to assimilation rates, stomatal conductance or PWUE. Species of semi-arid climates had significantly lower photosynthetic nitrogen-use efficiency (PNUE) than species of humid climates.

TRAIT ASSOCIATIONS OF LEAF HABITS AND CLIMATE GROUPS

We conducted PERMANOVA on two datasets (*All Traits* set of species and the *All Species* set of traits) to evaluate whether species groups (differing in leaf habit, climate type, or continents) differed in their multivariate trait values (Table 2). All groups and all continental pairs differed significantly in their

average, multivariate traits. There were no significant interaction groups.

Principal components analysis on the seedling traits supported results from the individual traits analyses and the PERMANOVA (Fig. 1, Fig. S1). Over half of the variation in the *All Species* data set was explained by the first two principal axes (proportion of variation explained: Axis 1: 0.34; Axis 2: 0.22). Species distributions along the first principal axis of the *All Species* data indicated discrimination between species with high LWC_a and nutrient contents (N_a , P_a , K_a) and P per unit mass (P_m) vs. those with high SLA and N : P ratios (Fig. 1c). The negative relationship between leaf nutrients per unit area and SLA was supported by the pairwise negative correlations between each of these traits and SLA (Table S5). Along this axis, there was separation between leaf habits (t -test: $t = 4.54$, d.f. = 49, $P < 0.001$), with evergreen species associated with high LWC_a , N_a , P_a , K_a and P_m , and deciduous species associated with high SLA and N : P (Fig. 1a).

Species distributions along the second principal axis (Fig. 1a–c) show a trade-off between efficient leaf surface (SLA) and high leaf nutrient contents (N_m , P_m , K_m) on the one hand, vs. large leaves (LS) with high N : P ratios and high LWC_a on the other hand. Species distributions along the second axis were associated with climate type (t -test: $t = 2.44$, d.f. = 49, $P = 0.018$), such that species from semi-arid sites were associated with greater SLA and leaf nutrient contents while species from humid sites were associated with bigger LS, high LWC_a and N : P ratios.

Discussion

Savanna species that differ in leaf habit and climatic origin differed strikingly in their functional traits (Tables 1 and 2), but inconsistently with our predictions. Deciduous species were more conservative with water use than evergreen species (cf. Prediction 1), species from semi-arid and humid sites did

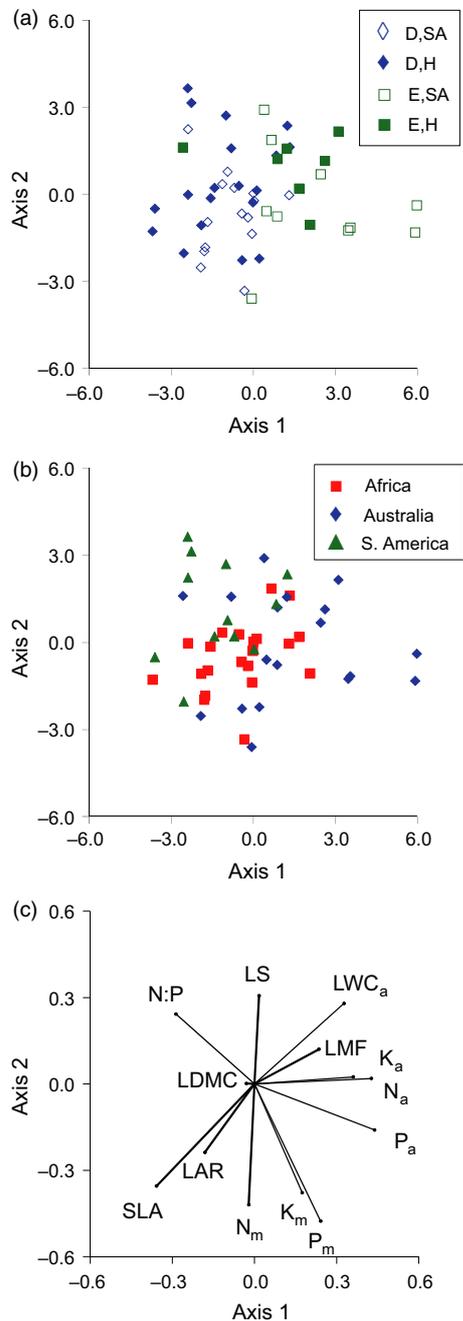


Fig. 1. Principal components analysis using mean values of seedling leaf traits of 51 savanna tree species ('All Species' set). In all, 56% of species data is explained by the first two axes (Proportion of variance explained by first two axes: Axis 1 = 0.34, Axis 2 = 0.22). Data are presented for the first and second axes. (a) Species distributions along the axes by leaf habit [symbol: diamond = deciduous (D), square = evergreen (E)] and climate type of origin [symbol fill: open = semi-arid (SA), closed = humid (H)]. (b) Species distributions along the axes by continent (symbol: square = Africa, diamond = Australia, triangle = South America). (c) Vector loadings for considered plant traits within the PC space. Acronyms are described in the text.

not differ in water use (cf. Prediction 2), and there were no significant interactions between leaf habit and climatic origin for any traits (cf. Prediction 3). Consequently, we must reject

our hypothesis. As we found no significant interactions between climate and leaf habit, we consider trait changes across species of different leaf habits and across species of different climate types separately in the sections that follow. In the process we introduce alternative hypotheses to explain our observations.

TRAIT SHIFTS BETWEEN LEAF HABITS

We found strong differences between evergreen and deciduous species, but these did not support our expectation of more conservative water used by evergreen species (cf. Prediction 1). Evergreen species had greater biomass allocation to leaves, investments in nutrients per unit leaf area (N_a , P_a , K_a) and water content per unit area (LWC_a), but lower water use efficiency (PWUE), SLA, leaf nitrogen per unit mass (N_m) and N : P ratio than deciduous species (Table 1, Fig. 1). Evergreen and deciduous species did not differ in their assimilation rates (A_m , A_a). These trait patterns instead suggest that evergreen savanna species have been selected for leaf traits that avoid excessive light and heat stress during the dry season to extend the functional longevity of their leaves, which is at the cost of water use efficiency. High temperatures can impair cellular enzymes and high light intensity stimulates the production of oxidants that can damage chloroplasts (Lambers, Chapin & Pons 1998; Logan *et al.* 2006). Deciduous savanna species may accommodate this damage because they can lose their leaves every dry season. Evergreen species, which retain their leaves through dry seasons, may have low water use efficiency to allow for evaporative cooling to pre-empt temperature-induced impairment of cellular structures, and they continue to transpire through the dry season, though with declining values (Prior, Eamus & Duff 1997; Quero *et al.* 2011). High leaf water content (LWC_a) may reduce damage to chloroplasts by converting excess energy to heat. The observed greater P_a and K_a but not N_a of evergreen species relative to deciduous species may simply reflect on their greater LWC_a : both P and K are required for cytoplasmic functioning and turgor pressure (e.g. Schroeder, Kwak & Allen 2001), whereas most leaf N is dedicated to photosynthesis (Makino & Osmond 1991). We also observed that the evergreen species orientated their leaf blades or phyllodes with steep angles to the horizontal, while deciduous species orientated their blades horizontally. Steep blade angles are well known among evergreen species of Mediterranean environments and are thought to reduce extreme heat absorption by limiting direct light interception at midday (Bombelli & Gratani 2003).

We found negative correlations between LMF with N_m and A_m (Table S5), which indicate that evergreen species produce a large amount of leaf material (high LMF; high C allocation) with low nitrogen content and photosynthetic rate per unit leaf mass (N_m and A_m) relative to deciduous species (Fig. 2). Some studies suggest that evergreen species have large LMF because their longer leaf lifespans lead to an accumulation of leaf mass (e.g. Reich, Walters & Ellsworth 1992; Lusk 2002). However, our data show that evergreen species partition more

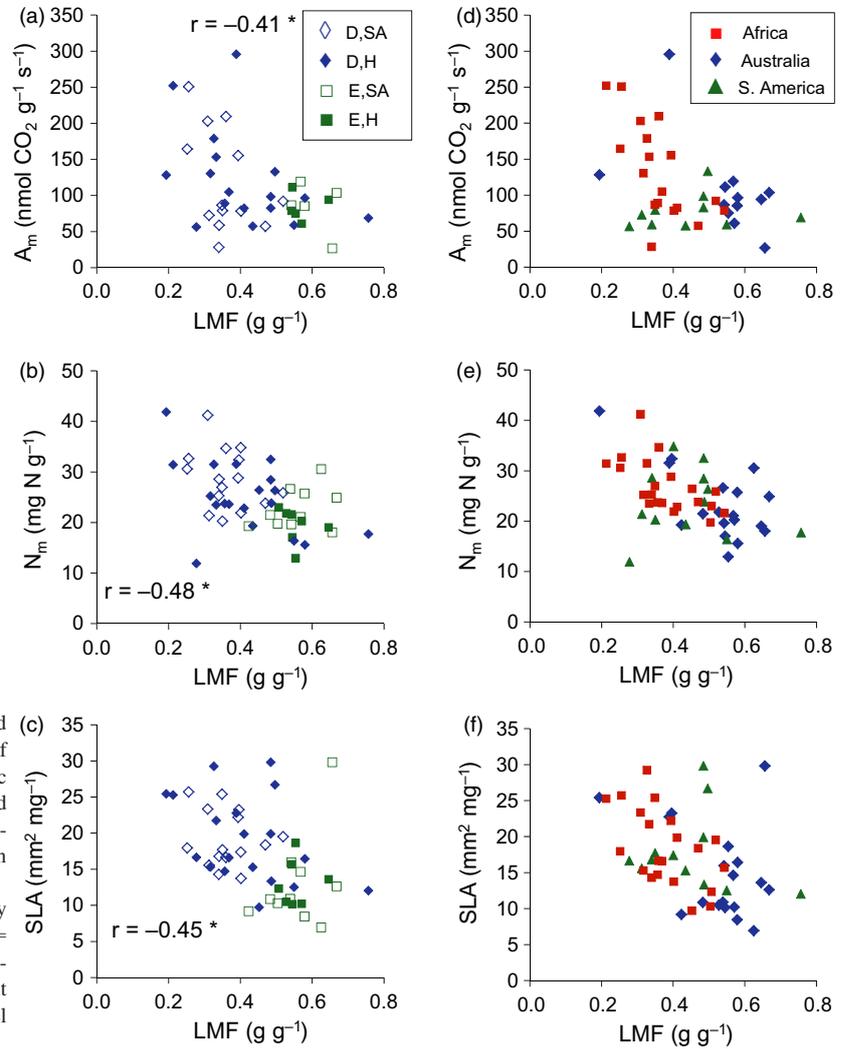


Fig. 2. Bi-plots of leaf mass fraction (LMF) and leaf assimilation rate per unit mass (A_m), leaf nitrogen content per unit mass (N_m) and specific leaf area (SLA). Panels a–c: species categorised by leaf habit [symbol: diamond = deciduous (D), square = evergreen (E)] and climate type of origin [symbol fill: open = semi-arid (SA), closed = humid (H)]. Panels d–f: species categorised by continent (symbol: square = Africa, diamond = Australia, triangle = South America). Pearson correlations between traits are presented. Significant trait correlations are indicated at the 5% level (**).

biomass to leaves than deciduous species, as hardly any leaf senescence was observed during the experiment in either leaf habit group. The low N_m of evergreen leaves relative to deciduous leaves probably reflects their greater thickness and structural mass per unit area (low SLA, high LWC_a) (Westoby *et al.* 2002). We expect that deciduous species have lower LMF than evergreen species due to leaf phenology: deciduous species allocate substantial carbohydrate resources to storage in their roots to support seasonal regrowth (e.g. Hoffmann, Orthen & Franco 2004; Kobe, Iyer & Walters 2010); this allocation is at the direct expense of allocation to leaf mass, leading to lower LMF.

TRAIT SHIFTS BETWEEN CLIMATE TYPES

Semi-arid and humid species did not differ with respect to assimilation rate, stomatal conductance, PWUE, or transpiring leaf area (LMF, SLA, or LAR). Thus, we found no evidence that species of drier environments have been selected to reduce water use (Prediction 2) within the range of water stress that we considered (MAP of 500 – 1550 mm).

The trait differences across humid and semi-arid groups that we observed are consistent with an alternative hypothesis:

semi-arid species have been selected for rapid response to soil moisture variability that allow them to maximise growth when conditions are suitable (Sterck *et al.* 2008; Birhane *et al.* 2012). In savannas, soil water is temporarily available and more so towards drier climates (Ananthakrishnan & Soman 1989; Nicholls & Wong 1990). Few tree species' seedlings seem able to use stored water to maintain photosynthetic activity through low soil water intervals (Poorter & Markesteijn 2008; De Smedt *et al.* 2012). Hence competitive advantage goes to those species that grow quickly when water is available and minimise biomass loss/damage when it is unavailable. This selects against conservative water use by plants.

So how does our data support this hypothesis? First, semi-arid species did not have lower LAR or SLA than humid species, which means they are capable of similar growth rates given suitable water conditions (Lambers, Chapin & Pons 1998; Tomlinson *et al.* 2012). Secondly, semi-arid species had smaller LSs than humid species, which has been observed across water gradients in other systems (Givnish 1987; Westoby *et al.* 2002). The decline in LS towards drier environments may result in a thinner boundary layer and therefore allow greater vapour exchange for photosynthesis (Givnish & Vermeij 1976; Givnish 1986) and greater sensible heat loss

when transpiration is limited by soil water potentials (Yates *et al.* 2010). We speculate that smaller LS may also allow species to adjust their leaf display more precisely under variable water supply conditions at low costs, by rapidly dropping or producing leaf area (Sterck *et al.* 1999). Thirdly, leaf P and K contents (P_m , K_m , P_a , K_a) of semi-arid species were greater than humid species (Table 1, Fig. 1). Both P and K are required for controlling the turgidity of stomatal guard cells that regulate gas exchange through stomata (Schroeder, Kwak & Allen 2001). Increased concentrations may allow species to close down water loss rapidly under low water conditions (Birhane *et al.* 2012; De Smedt *et al.* 2012). Maintaining cellular turgor through increased solute concentrations can also help plants to cope with water stress (see Jones 1992). More research is required to establish whether the semi-arid species have greater P and K in their palisade layers than humid species, to distinguish these possibilities.

CHANGES IN SPECIFIC LEAF AREA ACROSS WATER GRADIENTS REFLECT PLASTIC RESPONSES NOT ADAPTATION

It has been observed that certain traits can differ substantially between greenhouse-grown seedlings and in-field adult plants, notably SLA and N_m , which are both considered to be important determinants of plant and ecosystem functioning (Cornelissen *et al.* 2003a,b). Both SLA and N_m vary with local environmental conditions, particularly soil fertility for N_m and local climate for SLA (e.g. Schulze *et al.* 2006a,b). Notwithstanding that ontogenetic effects are important, it is precisely because these traits are so plastic in the field that they can only be easily tested for differences between species groups under controlled conditions. With respect to N_m , our observations on the differences between evergreen and deciduous studies match observations in adults (e.g. Westoby *et al.* 2002; Wright *et al.* 2004). However with respect to SLA across climate types, we find results that differ from field observations that we shall now discuss further.

Field investigations indicate that SLA in woody species declines towards drier environments (Wright, Reich & Westoby 2001; Wright & Westoby 2002; Santiago *et al.* 2004), leading to suggestions that lower SLA is adaptive to drier environments because it reduces transpiring leaf surfaces (Poorter *et al.* 2009). By contrast, we did not detect differences between the SLA of species of humid and semi-arid climate types in our greenhouse experiment (Table 1). We sampled the dominant species in these communities, so any adaptive benefit arising from SLA variation should have been observable in our common garden study. We conclude two points from this. First, there is a large variation and overlap in SLA of semi-arid and humid species, which supports field observations (Reich *et al.* 1999; Schulze *et al.* 2006a). Secondly, SLA is a fairly plastic trait that may depend on local environmental conditions, including levels of water stress and vapour deficit pressures (Schulze *et al.* 2006b; Poorter *et al.* 2009). Our experimental results thus imply that an observed higher SLA under drier conditions in field studies results from

a plastic response in SLA, and not from interspecific differences in SLA.

CONTINENTAL PATTERNS OF LEAF TRAITS OF SAVANNA TREES

Our multivariate analyses provide evidence that leaf traits of savanna species differ between the three continents (Table 2, Fig. 1). The first axis, explaining 34% of the across trait variation, differentiated Australian species from African and South American species. This reflects the leaf trait contrasts between evergreen species, which dominate in Australia, vs. deciduous species, which dominate in Africa and South America. These have been discussed above. The second axis, explaining 22% of the variation, is associated with differences between species of Africa and South America. The African species are characterized by higher leaf nutrients (N_m , P_m , K_m) and SLA, but lower LMF, LAR and N : P ratios than the South American species. We expect this trait differentiation represents differences in plant traits of deciduous species selected for different soil fertilities. African and Australian species were sampled over fertile and infertile geological parent materials, while South American species were sampled over infertile parent materials only, mostly from the extremely nutrient-poor Cerrado (see Table S1). Dystrophic soils in South America have 3–4 mg extractable P kg⁻¹ (Neufeldt *et al.* 2000), those in Africa have 7–13 mg P kg⁻¹ and those in Australia have 2–6 mg P kg⁻¹ (Bowman & Prior 2005). With the exception of nitrogen-fixing legumes, all species from South America had a low N_m and both legumes and non-legumes had a low P_m (Table S3), thus reflecting adaptations to low P and N supply in the soil. We thus predict that for South American savannas found on richer soils, such as those in the Andean regions (e.g. the Chaco), leaf traits of locally abundant trees will resemble the characteristics of African deciduous species more closely. Interestingly, Australian species were not distinguished even from African species on the second axis. This suggests that the proposal that differences in the abundance of evergreen and deciduous species across continents may be related to low P in Australian soils (Bowman & Prior 2005; Orians & Milewski 2007) is unlikely. The fascinating difference in the abundance of evergreen and deciduous species between continents remains a mystery.

Conclusions

Evergreen savanna species utilise water less efficiently than deciduous species (lower PWUE), possibly to protect their photosynthetic machinery against high temperatures and light during the dry season. Irrespective of leaf habit, semi-arid species appear to be selected for rapid responses to variable soil moisture conditions in order to maximise growth under transient suitable conditions. Semi-arid species had smaller leaves than humid species, which promote vapour exchange and photosynthesis and possibly rapid canopy adjustment to prevailing conditions, higher leaf P and K that may provide more effective control over transpiration and comparable SLA

and LAR that allow rapid growth when conditions are favourable. There are also significant differences between the trait suites of continental savannas. Differences in leaf traits between species from Australia vs. Africa and South America may reflect continental differences in dominance by evergreen or deciduous species respectively. Differences in leaf traits between South American vs. African species may reflect different ranges of soil fertilities of the sampled continental savannas. In summary, across continents and climate types, it appears that water stress in savannas does not select for more conservative water use, but rather for physiological and morphological adjustments to track the dynamics in the availability of water and for avoiding heat stress.

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Supporting Information

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

Figure S1. Principal components analysis for 'All Traits' data set.

Table S1. Description of the environmental characteristics of the sampled savannas.

Table S2. Qualitative data on the 51 tree species.

Table S3. Quantitative data of species' leaf traits ('species data').

Table S4. Species loadings for first two axes of PCA analyses.

Table S5. Pearson correlations of measured plant traits.