Biomass partitioning and root morphology of savanna trees across a water gradient

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Summary

1. Plant organ biomass partitioning has been hypothesized to be driven by resources, such that species from drier environments allocate more biomass to roots than species from wetter environments to access water at greater soil depths. In savanna systems, fire may select for greater allocation to root biomass, especially in humid environments where fire is more frequent. Therefore, species from drier environments may have been under selection pressure to reach deeper soil water more effectively than species from humid environments, through faster root extension, more efficient depth penetration, and faster plant growth rates to respond rapidly to variable rainfall events.

2. We compared biomass partitioning, root morphology traits [root extension rate, RER; specific taproot length (STRL)] and relative growth rate (RGR) of seedlings of 51 savanna tree species, sampled from three continents (Africa, Australia and South America) in a greenhouse experiment. We used phylogenetically corrected and uncorrected analyses to compare the traits of the groups. We conducted a PERMANOVA on the combined traits to establish whether species could be distinguished on the basis of their combined traits.

3. On average, species from humid environments allocated more biomass to roots and less to stems than species from semi-arid environments, consistent with the expectation that fire pressure selects for greater allocation to roots in humid environments. However, some species from humid environments had fast growth rates instead of high allocation to roots. Both RER and STRL were greater among species of semi-arid environments than among species of humid environments, and also differed between continents. Differences between strategies under each climate type appear to be associated with leaf habit.

4. *Synthesis.* Plant biomass partitioning has been selected by defoliation pressure and the effects of this selection pressure can supersede any selection in response to local water constraints. Root morphological adaptations, but not plant growth rate, of tree seedlings, have been selected in response to water deficits.

Key-words: Africa, Australia, fire, plant–climate interactions, resource allocation, root morphology, root traits, South America

Introduction

Biomass partitioning to leaves, stems and roots varies among plant species. This partitioning among vegetative organs has been considered of adaptive value (Tilman 1988) because plants trade-off between above- and below-ground resource acquisition (Brouwer 1962; Thornley 1972; Bloom, Chapin & Mooney 1985). Consequently, biomass partitioning has been hypothesized to vary predictably among species along resource gradients such that locally dominant species allocate more

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heavily to the organ that captures the most limiting resource at that location (Tilman 1988; Chapin, Autumn & Pugnaire 1993). These predictions for biomass partitioning have frequently been falsified when species dominant at different positions along resource gradients have been grown in common garden experiments (Berendse & Elberse 1989; Campbell, Grime & Mackey 1991; Ryser & Eek 2000). Most common garden experiments of biomass partitioning by species growing across resource gradients have concentrated on nutrients and light, whereas water has rarely been tested. It has been hypothesized that species from drier environments allocate more to roots [greater root mass fraction (RMF), g g^{-1} plant mass] than species from wetter environments (Tilman 1988), and there are some field data supporting this (Markesteijn & Poorter 2009). Comparative experiments are required to confirm whether or not biomass partitioning to roots is higher in species from more water-stressed conditions.

Defoliation pressure is one selective force that may affect biomass allocation to organs. In environments subjected to repeated, severe defoliation, biomass partitioning away from the plant organs subjected to defoliation may be selected for, given that plants with less resource loss to defoliation have a competitive advantage over individuals that do not partition resources in this manner (see Raunkiaer 1934; Bellingham & Sparrow 2000).

Our own research focuses on the growth characteristics of tree species from savannas, where fire is a major cause of defoliation (Scholes & Archer 1997; Bond, Woodward & Midgley 2005; Bond 2009). Fire is a non-selective defoliator that removes shoot biomass. Tree juveniles and seedlings rarely have sufficiently large stems to resist fire events and die back at or near ground level (Bond, Woodward & Midgley 2005). Many savanna species overcome this 'fire trap' (Trollope & Tainton 1986; Higgins, Bond & Trollope 2000) by accumulating growth reserves underground, beyond the reach of fires, from which they can develop new shoots after fire events (Raunkiaer 1934; Bell, Pate & Dixon 1996; Hoffmann & Franco 2003; Hoffmann, Orthen & Franco 2004; Schutz, Bond & Cramer 2009; Wigley, Cramer & Bond 2009). Across continents, fire frequency and intensity in savanna systems is highest in humid environments (fire interval between 1–3 years) and declines towards semi-arid and arid environments (fire interval > 3 years) (Barbosa, Stropianna & Grégoire 1999; Ramos-Neto & Pivello 2000; Russell-Smith *et al.* 2003; Bravo *et al.* 2010). As the relative selection pressure by fire decreases towards drier environments, it is plausible that species' biomass partitioning to roots declines from humid to semi-arid environments. Importantly, this prediction is opposite to the expectation based on resource capture theories (Tilman 1988; Chapin, Autumn & Pugnaire 1993) that partitioning to roots is greater among species from semi-arid environments than species from humid environments because water limitation increases towards semi-arid environments.

Species growing under water-stressed conditions may optimize their foraging strategies for water resources in the soil through root morphology instead of root mass partitioning. Drier environments are also characterized by greater rainfall variability and more frequent drought events during the growing season than wetter environments (Ananthakrishnan & Soman 1989; Nicholls & Wong 1990; Ward 2009). As water recedes down the soil profile, an important characteristic of species growing in drier environments may be the ability of recently germinated seedlings to access deeper, more reliable water resources to avoid early death due to water stress (Bond 2008). Two traits may be important for accessing deeper water: fast root extension rates (RER, mm day^{-1}) and efficient depth penetration per unit cost in root biomass, achieved by producing longer and thinner taproots. We call this second trait specific taproot length (STRL), defined as the taproot depth penetration per unit cost in root biomass (m g^{-1}). It is similar to, but distinct from, specific root length (m g^{-1}), which is measured on secondary roots and is used to describe the ability of species to search soil volume per unit mass invested (Nicotra, Babicka & Westoby 2002; Cornelissen *et al.* 2003). RER and STRL might both be greater among species abundant in drier environments than species abundant in wetter environments. Because RER and STRL reflect particular organ growth rates, species with high RER and STRL might also have high total plant relative growth rates (RGR, g g^{-1} day^{-1}). High RGR may be beneficial for species from drier environments because it allows them to respond rapidly to periods of resource availability, which are intermittent in these environments and driven by rainfall events (Ananthakrishnan & Soman 1989; Nicholls & Wong 1990; Ward 2009).

In this paper, we present a large-scale study of biomass partitioning and root traits of 51 tree species dominant in humid and semi-arid savannas of Africa, Australia and South America, grown under common conditions. Savanna vegetation is widely distributed in the tropical and sub-tropical parts of the world (Cole 1986). Species compositions change across rainfall gradients in savannas (Cole 1986), which suggests that there has been environmental niche specialization along moisture gradients. Thus, any biomass allocation shifts associated with niche specialization along moisture gradients should be apparent in comparisons between species from semi-arid and humid savannas grown under common conditions.

We concentrated on seedlings because this is the stage at which plants are most sensitive to environmental impacts (Bond 2008; Van Langevelde *et al.* 2011), and hence, strategies evolved in response to water deficits should be apparent at this stage. We tested two hypotheses on root adaptations of species distributed across water availability gradients:

1 Species from semi-arid environments have greater RMF than species from humid environments.
2 Species from semi-arid environments forage more efficiently for deeper water and have faster growth rates than species from humid environments.

We used phylogenetically corrected and uncorrected analyses to compare the traits of the groups.

Materials and methods

SPECIES SELECTION

We identified semi-arid and humid environments in equatorial and warm-temperate climates, using the Köppen-Geiger climate classification (Kottek *et al. 20*06). This system estimates the relative water deficits of particular locations by comparing a dryness threshold (P_{th}), based on the mean annual temperature (T_{ann}, °C), with mean annual precipitation (MAP, mm) at those locations. The dryness threshold assumes that annual evaporative demand is related to mean annual temperature and it is adjusted for seasonal variation in rainfall distribution, which in savannas falls mainly in the summer months: $P_{th} = 28 + 2 \times T_{ann}$. The dryness threshold estimates critical MAPs below which environments are considered to be semi-arid and arid, respectively. Arid environments are defined in the range MAP $< 5 P_{th}$, semi-arid environments are defined in the range $5 P_{th} <$ MAP $< 10 P_{th}$ and humid environments are defined as areas where MAP $> 10 P_{th}$. We sampled dominant or very abundant tree species from humid and semi-arid 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 1 provides descriptive information for the sampled environments. The South American species were all sampled over low-fertility soils, while 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 (8 humid, 4 semi-arid), representing nine Angiosperm orders (APGIII), 13 families and 28 genera. A full species list is provided in Table S1 (see Appendix S1 in Supporting Information), including their family, order, continent of origin and climate type of origin. The sampled species reflected continental biases in dominant plant families (Cole 1986): Australian savannas are dominated by Myrtaceae and the Fabaceae sub-family Mimosoideae, African savannas are dominated by Combretaceae and two subfamilies of Fabaceae, Mimosoideae and Caesalpinioideae, while humid South American savannas are composed of a large number of co-dominant families and semi-arid savannas are dominated by Fabaceae (Mimosoideae and Caesalpinioideae). Consequently, two plant orders formed the bulk of the species sample, namely Fabales and Myrtales.

GREENHOUSE EXPERIMENT

We conducted a greenhouse experiment to evaluate the effect of climate type on root traits of seedlings of the 51 savanna tree species selected from the three continents. 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 (day) and 23 °C for 12 h (night). Supplementary light (150 μmol m^{-2} s^{-1}) was provided for 12–16 h (seasonally adjusted) to ensure that the daily supply of photon flux density exceeded 10 mol m^{-2} day^{-1}.

Tree seedlings were grown in plastic tubes of 10 cm in diameter and 100 cm in length, allowing the roots more space to grow in an effort to reduce pot limitation. Pots were filled with river sand mixed with slow-release fertilizer [Osmocote 18-6-12 (N-P-K) fertilizer (8–9 month mixture)] at a concentration of

Table 1. Description of the environmental characteristics of the sampled savannas

Location	Vegetation type	Climate			Soils		
		Mean annual precipitation (MAP) (mm)	Mean annual temperature (°C) (min, max)	Koppen-Geiger aridity index (MAP/P_{th})	Parent material	Clay content (%)	CEC (cmol kg^{-1})
Australia							
Humid	North-eastern Queensland	898–1156	16.6, 29.0	12.2–17.1	Basalt Alluvium	8.0–43.0	2.0–20.6
Semi-arid	North-central Queensland	525–663	16.6, 33.3	6.5–8.7	Basalt Sandstone Mudstone	8.0–57.0	2.0–42.8
Africa							
Humid	KwaZulu-Natal, South Africa, Zimbabwe	840–998	12.7, 26.0	12.8–14.1	Alluvium Shale Granite	8.0–44.0	1.6–20.5
Semi-arid	Limpopo, South Africa	502–630	12.0, 29.5	6.8–8.7	Basalt Granite	10.0–51.5	2.0–38.0
South America							
Humid	Brasilia, Brazil	1552	16.1, 26.6	21.9	Granite	64.0–74.0	0.4–8.1
Semi-arid	Paraiba, Pernambuco, Brazil	517–858	18.3, 30.4	6.5–10.3	Granite	8.4–15.7	8.1–25.6

Data sources: Climate: 1. Australia: Bureau of Meteorology, Australian Government, http://www.bom.gov.au, 2. Africa: South African Weather Service, http://www.weathersa.co.za, 3. South America: NOAA Global Climate Normals 1961–1990 http://www.climate-charts .com. Soils: 1. Australia: ASRIS, http://www.asris.csiro.au, 2. Africa: Venter, Scholes & Eckhardt 2003; AGIS, http://www.agis.agric.za, 3. South America: Furley 1999; Moreira 2000Leal, Wirth & Tabarelli 2007; de Luna, Coutinho & Grisi 2008.

5 kg fertilizer m^{-3} river sand. 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 far more regular water supply than most of these species would receive in their natural environment, but the intent 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.

Seedlings were planted into pots following germination, and then grown for a further 5 or 20 weeks before harvesting, to measure different functional traits of the species (described below). A maximum replication of 10 individuals per species was grown for each time interval. Due to limitations on space in the greenhouse compartment, it was necessary to grow plants in three batch repetitions of the experiment to cover all species evaluated. Individual species were therefore grown in one (10 individuals per time interval) or more usually two (five individuals per time interval) of the three batch repetitions. Some seedlings did not thrive after transplanting into pots and those individuals were destroyed. Actual replications per species are provided in Table S1 (Appendix S1).

DATA COLLECTION

Seedlings were harvested at 0, 5 and 20 weeks after planting to measure biomass allocation and root functional traits. We predicted species of semi-arid savannas to have greater RMF than species from humid savannas. Therefore, total seedling mass (g dry matter) and organ mass fractions of plants [g g^{-1} dry matter; leaf mass fraction (LMF); stem mass fraction (SMF); RMF] were estimated using plants harvested at 20 weeks. We tested whether allocation to roots (RMF) was at the expense of leaf allocation (LMF) or stem allocation (SMF).

Seedlings harvested at 5 weeks were used to measure root length and root dry mass to obtain estimates of (RER, mm day^{-1}) and STRL (m g^{-1}) per species. These root traits describe the plant's efficiency at searching for deeper water resources. RER was calculated as the difference between final taproot length (RL$_{final}$) and mean initial taproot length (RL$_{initial}$), measured on seedlings harvested at 0 weeks, divided by the number of days of growth (d).

$$RER = \frac{RL_{final} - \overline{RL_{initial}}}{d} \qquad \text{eqn 1}$$

Specific taproot length was calculated as the taproot length divided by root dry mass. Only individuals with no major secondary roots were included in this calculation. Both RER and STRL were expected to be respectively faster and longer for species from semi-arid environments than for species from humid environments as a response to more intermittent water availability. Most species produced only tap roots with little side root development during the first 5 weeks of growth and roots of the fastest species reached the bottom of the 1-m tubes at about this time, allowing reasonable estimates for these two parameters. RER and STRL were not measured on plants older than 5 weeks, both because in many species, taproot growth was limited by pipe length, which skewed estimates of RER, and because many species showed substantial tap root thickening, which would have skewed estimates of STRL.

Relative growth rate was calculated over 5–20 weeks. RGR was calculated as the difference between the logged final mass at 20 weeks (lnMass$_{final}$) and the mean logged initial mass at 5 weeks (lnMass$_{initial}$) of individuals of the species, divided by the interval of growth (days) (adapted from Hoffmann & Poorter 2002).

$$RGR = \frac{lnMass_{final} - \overline{lnMass_{initial}}}{d} \qquad \text{eqn 2}$$

Mean trait values per species are provided in Table S1 (Appendix S1).

STATISTICAL ANALYSIS

We assessed the effect of climate type (binary variable: H = humid; SA = semi-arid) on biomass partitioning, root morphological traits and RGR of savanna tree seedlings using species' mean estimates of each organ mass fraction (RMF, SMF and LMF), root traits (RER, STRL) and RGR in ANCOVA. As plant biomass partitioning can change with ontogeny and plant size (Gedroc, McConnaughay & Coleman 1996; McConnaughay & Coleman 1999; Enquist & Niklas 2002; Sack, Maranon & Grubb 2002), the natural logarithm of species mean seedling mass at the time of measurement (g) was included as a covariate to correct for size effects on partitioning within species (Mass$_5$ for STRL, RER and RGR; Mass$_{20}$ for RMF, SMF and LMF). Mean seedling mass did not differ between climate groups at 5 weeks (Mass$_5$) or 20 weeks (Mass$_{20}$) [independent samples *t*-test with probabilities for differences between means of 0.698 for ln(Mass$_5$) and 0.582 for ln(Mass$_{20}$)]. *Continent* was included as a blocking variable to account for potential differences between continents with respect to the parameters measured. Interactions between Mass and Continent, Mass and Climate, and Continent and Climate were also included. The interaction effects involving Mass were all non-significant and these results are not reported here. Hence, the full ANCOVA model tested on all variables reported here was as follows:

$$y = \beta_0 + \beta_1 \ln(Mass) + \beta_2 \text{Continent} + \beta_3 \text{Climate} \\ + \beta_4 \text{Continent} \times \text{Climate} + \varepsilon \qquad \text{eqn 3}$$

Continent was included as a random effect because we had no hypotheses as to how measured variables might change across the continents. Models were first tested with the interaction term included. If the interactions were non-significant, these were dropped and the models were rerun using only the main effects.

As our target species represent a wide phylogenetic range across Eudicots, we tested for phylogenetic bias in the statistical analyses of the traits data (see Appendix S2 for a full description of methods and results). A first set of ANCOVA models was run on each variable using ordinary least squares regression without phylogenetic correction. A second and third set of models used a generalized least squares regression to impose a phylogenetic correction on the data, representing an additive tree structure and an ultrametric tree structure of the measured species (Grafen 1989; Martins & Hansen 1997; Garland & Ives 2000). All models were analysed using the Regressionv2.m program (Lavin *et al.* 2008).

We used PERMANOVA (Anderson 2001), implemented in the adonis() command in vegan package of R (Oksanen *et al.* 2011), to establish whether species of different climate types and from different continents could be differentiated by a combination of the considered traits (RMF, SMF, LMF, RER, STRL, RGR) (number of permuted data sets = 1000) and therefore followed different rooting strategies. The full model tested included Continent, Climate and the interactions between Continent and Climate. Where the observed *F*-values were significant, component groups were compared pairwise using the same PERMANOVA procedure to generate *F*-values with probabilities that could be rooted to estimate the *t*-values for these pairwise comparisons (Anderson, Gorley & Clarke 2008). 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 multivariate data were subsequently visualized using principal components analysis (PCA) to interpret the PERMANOVA results and to determine whether species groups were associated with particular traits. Pearson correlations between all traits and mean masses were also calculated to provide supplementary information on the relationships between traits.

Results

REGRESSION MODELS

The statistical results obtained under all three models for each variable are presented in Table S2 and Appendix S2. Evidence for phylogenetic signal on the trait distributions was mostly quite weak. Exceptions were the RMF and SMF, which were significantly better supported by the phylogenetic models. In general, significant factor effects detected by models were similar for non-phylogenetic and phylogenetic models, but phylogenetic models detected a significant mass effect more frequently. The Continent × Climate interaction effect was usually non-significant, with the exception of SMF, where significant differences were found between South American humid and semi-arid species under the phylogenetic models. As phylogenetic models did not change the detected main effects, results in Fig. 1 are presented for the non-phylogenetic model.

Biomass allocation among species did not match our hypotheses and each organ type was significantly associated with a different set of model predictors (Fig. 1). First, species from semi-arid environments did not allocate more biomass to roots than species from humid environments. Allocation to roots differed significantly among continents, such that African species had greater RMF than Australian species (Fig. 1a). Allocation to stem (SMF) was greater among species from semi-arid environments than species from humid environments and increased with plant mass, but did not differ across continents (Fig. 1b). Allocation to leaves (LMF) differed among continents, being greater among Australian species than African or South American species, but did not differ across climate types (Fig. 1c).

Patterns of root morphological traits across climate groups supported our second hypothesis: both RER and STRL were significantly greater for species from semi-arid environments than species from humid environments (Fig. 1d,e). In addition, RER and STRL both differed across continents: RER was greater among African and Australian species than among South American species. STRL was greater among Australian species than among either African or South American species. Both RER and STRL were also significantly related to species' mean seedling mass at 20 weeks (positively for RER and negatively for STRL), but they were uncorrelated with one another (Table 2). RGR did not differ between climate groups or continents (Fig. 1f).
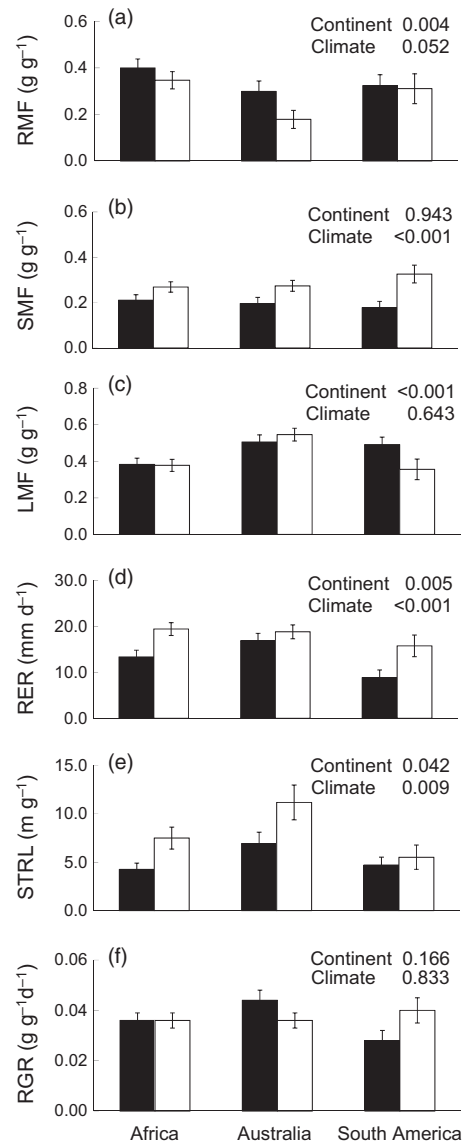

Fig. 1. Estimated marginal means of (a) root mass fraction (RMF, g g^{-1} total plant dry mass), (b) stem mass fraction (SMF, g g^{-1} total plant dry mass), (c) leaf mass fraction (LMF, g g^{-1} total plant dry mass), (d) root extension rate (RER, mm day^{-1}), (e) specific taproot length (STRL, m g^{-1}) and (f) relative growth rate (RGR, g g^{-1} day^{-1}) of seedlings of sampled savanna tree species stratified by climate groups (■ humid, □ semi-arid), under the non-phylogenetic model. Single standard errors for each estimate are included. RMF, SMF and LMF values presented are estimated for the covariate ln Mass = 2.638. RER, STRL and RGR values presented are estimated for the covariate ln Mass = −1.053.

MULTIVARIATE ANALYSIS

PERMANOVA on the full model confirmed that species group could be distinguished at both main effect (Continent and Climate) and interaction effect (Continent × Climate) levels (Table S3, Appendix S3). Species from humid environments were significantly distinguished from species from semi-arid environments. Australian species were significantly different from African and South American species, but the latter groups did not differ from one another. After controlling for

Table 2. Pearson correlations of measured plant traits (RMF, root mass fraction; SMF, stem mass fraction; LMF, leaf mass fraction; STRL, specific taproot length; RER, root extension rate; RGR, relative growth rate) and mean biomass estimates at 5 and 20 weeks (Mass$_5$, Mass$_{20}$) on 51 savanna tree species

	RMF	SMF	LMF	STRL (ln)	RER	RGR	Mass$_5$ (ln)
SMF	−0.473***						
LMF	−0.760***	−0.210					
STRL (ln)	−0.514***	0.223	0.413**				
RER	0.098	0.389**	−0.375**	0.002			
RGR	−0.429**	0.370**	0.207	0.427**	0.004		
Mass$_5$ (ln)	0.366**	0.165	−0.518***	−0.632***	0.685***	−0.233	
Mass$_{20}$ (ln)	−0.154	0.469**	−0.161	−0.035	0.526***	0.678***	0.517***

Non-normal variables that have been natural log-transformed to improve normality are indicated. Significant correlations are indicated (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

false positives, six pairs of Continent × Climate sub-groups differed significantly from one another (Table S3, Appendix S3). Australian species from semi-arid environments were significantly different from all other groups except Australian species from humid environments. African and South American species from semi-arid environments were both significantly different from South American species from humid environments.

Principal components analysis on the seedling traits supported results from the ANCOVA and PERMANOVA (Fig. 2). Species data were substantially explained by the first and second principal axes (43% and 28%, respectively). Along the first axis, species were discriminated between those with high STRL, LMF and RGR on the right-hand side and those with high RMF on the left-hand side. Species with high STRL and high LMF were predominantly Australian species and South American species from humid environments, confirming the PERMANOVA results. Of these species, Australian species from semi-arid environments were placed most extremely, distinguishing them significantly from all other interaction groups in the PERMANOVA. Species with high RMF were predominantly African and South American species from humid environments, confirming that high allocation to roots is a strategy associated with species from humid environments, not species from semi-arid environments. However, numerous species from humid environments from these continents were located away from RMF along the first and second axes, indicating that high allocation to roots is not the only viable strategy in humid savanna communities. Discrimination along the second axis appeared to be greatest between species with high RER and SMF vs. species with high LMF. There also appeared to be some discrimination between humid and semi-arid species, as species from semi-arid environments were predominantly found along the upper half and species from humid environments predominantly along the lower half of the axis, confirming the PERMANOVA result. Most species associated with high RER and SMF were species from semi-arid environments from Africa and South America, explaining their significant separation from most other groups in the PERMANOVA. The overall placement suggested that Australian species were more closely associated with high STRL than species from other continents (Table S3, Appendix S3).

Discussion

In this study, we compared biomass allocation and root morphology of seedlings of tree species found across water gradients in savannas on three continents. Our results suggest that most traits measured do distinguish savanna tree species from different climate groups, but not always in accordance with our hypotheses. In addition, there was strong evidence that some traits differed across continents, suggesting that species from different continents have on average responded differently to the selection pressures imposed in semi-arid and humid savanna environments.

BIOMASS PARTITIONING ACROSS CLIMATE TYPES: RESPONSE TO WATER SUPPLY OR TO FIRE?

We tested the hypothesis that species from semi-arid environments would allocate more to roots than species from humid environments (Tilman 1988; Chapin, Autumn & Pugnaire 1993; Markesteijn & Poorter 2009) (Hypothesis 1). Instead, we observed that species from humid environments allocated more to roots and significantly less to stems than species from semi-arid environments, but they did not differ with respect to leaf partitioning (Fig. 1a). These patterns can be explained as evolved adaptations of species under different fire pressures, as fire frequency is higher in more humid savannas (Barbosa, Stropianna & Grégoire 1999; Ramos-Neto & Pivello 2000; Russell-Smith et al. 2003; Bravo et al. 2010). Young seedlings growing in fire-prone humid savannas have a high probability of shoot removal by fire events, which prevent them from progressing to reproductive maturity (Trollope & Tainton 1986; Higgins, Bond & Trollope 2000). Low investment in stems but unchanged investment in leaves allows the plants to build structures cheaply but sufficiently to carry leaf canopies that provide photosynthates for storage in roots (Schutz, Bond & Cramer 2009; Wigley, Cramer & Bond 2009; Kobe et al. 2010), maximizing their retention of resources beyond fire impacts. In this way, savanna trees can increase the rate at
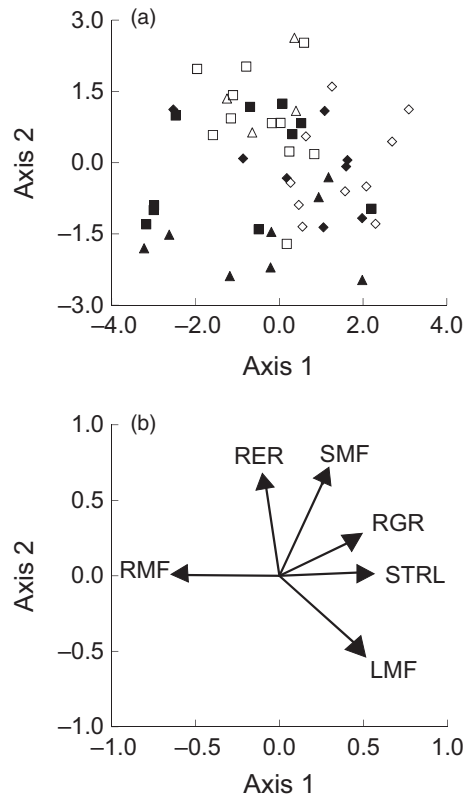
Fig. 2. Principal components analysis using mean values of seedling traits of 51 savanna tree species (non-phylogenetic data). 71% of species data is explained by the first and second axes (Eigenvalues: Axis 1 = 0.43, Axis 2 = 0.28). (a) Species distributions along the first and second axes. Species' symbols distinguish continent of origin (square = Africa, diamond = Australia, triangle = South America) and symbol fill represents climate type of origin (open = semi-arid, closed = humid). (b) Vector loadings for considered plant traits within the PC space of the first and second axes. (Trait acronyms: RMF: root mass fraction (g g^{-1} total plant dry mass); SMF: stem mass fraction (g g^{-1} total plant dry mass); LMF: leaf mass fraction (g g^{-1} total plant dry mass); RER: root extension rate (mm day^{-1}); STRL: specific taproot length (m g^{-1}); and RGR: relative growth rate (g g^{-1} day^{-1}).)

which they build sufficient reserves and size to overcome the 'fire trap' (Trollope & Tainton 1986; Higgins, Bond & Trollope 2000). Resource storage in roots is a successful species' functional trait to survive and overcome shoot removal by fires (Bell, Pate & Dixon 1996; Hoffmann, Orthen & Franco 2004; Schutz, Bond & Cramer 2009; Wigley, Cramer & Bond 2009). Many of the species from humid environments in our study produced swollen tap roots as seedlings, which is common among woody species in humid savannas and which appears to occur for storing carbohydrates and other nutrient reserves against fire events (Bell, Pate & Dixon 1996; Hoffmann & Franco 2003).

SPECIES FROM SEMI-ARID ENVIRONMENTS USE MORE EFFICIENT ROOT MORPHOLOGY FOR WATER CAPTURE

Species from semi-arid environments had more efficient root morphology for searching for deep water than species from

humid environments (Hypothesis 2): they had faster RER and greater STRL than species from humid areas (Fig. 1d,e). This is consistent with the idea that plant survival under drier conditions depends on root placement and rapid adjustment (Walter 1939; Nicotra, Babicka & Westoby 2002) when water is available rather than on greater biomass partitioning to roots.

We found no evidence that species from semi-arid environments have faster growth rates (RGR) than species from humid areas to increase access to soil water resources, so RGR does not appear to aid species in accessing water. We suggest that drought is a stronger (and more direct) selector on seedling survival than the total amount of water resources available over the period. Root placement prior to drought events is probably a more crucial response to conditions in dry systems.

PLANT SYNDROMES IN HUMID AND DRY SAVANNAS

At least four plant syndromes could be distinguished among seedlings of savanna trees from the PCA, and that these have climatic and continental biases (Fig. 2; Table S3, Appendix S3). First, species adaptations for dry environments were associated with two different rooting syndromes that had clear continental biases. Species from semi-arid environments from Africa and South America were grouped with high RER while species from semi-arid environments from Australia were grouped with high STRL. STRL and RER were uncorrelated (Table 2). In the PCA (Fig. 2), STRL occurred in the opposite direction to RMF, which suggests that species with thin tap roots during early growth did not subsequently develop thickened tap roots, while many species with high RER were able to do so. Within our data set, the two rooting syndromes appear to be closely associated with leaf habit. This is indicated first by the fact that the Australian species in our sample are predominantly evergreen (14 of 18 species) while African and South American species are predominantly deciduous (18 of 21 species and 12 of 12 species, respectively), matching known continental savanna patterns (Bowman & Prior 2005) (see PCA loadings in Table S4, Appendix S3). Second, of the African species associated with high STRL, *Brachylaena discolor* is evergreen, while the two Australian species associated with high RER, *Erythrina vespertilio* and *Acacia bidwillii*, are deciduous. Thus, our results suggest that evergreen species use finer root structures (high STRL) for searching for water while deciduous species use rapid root extension (high RER) to search for water.

There appear to be two different strategies for coping with fire in humid environments, which are also partly associated with leaf habit. One group of deciduous African and South American humid savanna species were associated with high allocation to roots (Table S4, Appendix S3), which presumably helps them to survive shoot removal by fires. A second group of species from humid environments were associated with high allocation to leaves (high LMF) or to stems (high SMF) and with high RGR. Those species with high allocation to leaves were predominantly Australian and evergreen species, although there were also some deciduous species among them, while the species associated with high allocation to stems were

predominantly deciduous. The fact that these species from humid environments were not associated with high allocation to roots but were associated with rapid growth rates and high allocation to leaves suggests that their strategy for coping with fires may depend on rapid growth during fire-free intervals to reach a size where they are large enough to withstand fire events (Trollope & Tainton 1986; Higgins, Bond & Trollope 2000). Evergreen species may be excluded from the strategy to extensively store resources below-ground because their persistent leaf habit prevents them doing so. This further suggests that vegetation structure in humid savannas may differ substantially in Australian savannas, compared to African and South American savannas, because their selected strategies for coping with fire differ. African and South American savannas may include large numbers of juvenile trees that follow the root storage strategy with a wide range of ages, repeatedly suppressed by consecutive fires ('Gullivers', Bond & Van Wilgen 1996), while Australian savannas may contain few cohorts of juveniles that recruited during very wet years or near the start of long fire-free intervals.

Conclusion

Our study shows that water stress does not drive biomass partitioning differences among species of semi-arid and humid savannas. We suggest that fire is a more probable selective factor for biomass partitioning across moisture gradients in savannas, because of its severe consequences for plant resource retention and hence for long-term growth. Root trait differences between species from humid and semi-arid climates seem to indicate differences in efficiency at searching for deep water, achieved through producing thinner roots and through rapid root extention.

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Supporting Information

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

Appendix S1. Species data.

Appendix S2. Phylogenetic analyses.

Appendix S3. Multivariate analyses.

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