

Trait shifts associated with the subshrub life-history strategy in a tropical savanna

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Abstract Over the past 10 million years, tropical savanna environments have selected for small growth forms within woody plant lineages. The result has been the evolution of subshrubs (geoxyles), presumably as an adaptation to frequent fire. To evaluate the traits associated with the shift from tree to subshrub growth forms, we compared seed biomass, germination, survival, resprouting, biomass allocation, and photosynthesis between congeneric trees and subshrubs, and quantified phylogenetic conservatism. Despite large differences in adult morphology between trees and subshrub species, the differences are modest in seedlings, and most of the variation in traits was explained by genus, indicating considerable phylogenetic conservatism. Regardless, tree seedlings invested more heavily in aboveground growth, compared to subshrubs, which is consistent with the adult strategy of savanna trees, which depend on a large resistant-fire stem. Subshrub seedlings also invest in greater non-structural carbohydrate reserves, likely as an adaptation to the high fire frequencies typical of tropical savannas. The

modest differences as seedlings suggest that selective pressures during early development may not have contributed substantially to the evolution of the subshrub growth form and that the distinct allocation and life history must arise later in life. This is consistent with the interpretation that the subshrub growth form arose as a life-history strategy in which maturity is reached at a small stem size, allowing them to reproduce despite repeated fire-induced topkill. The convergent evolution of subshrubs within multiple tree lineages reaffirms the importance of fire in the origin and diversification of the flora of mesic savannas.

Keywords Subshrub vs tree · Brazilian Savanna · Savanna evolution · Fire adaptations · Traits phylogenetically conserved

Introduction

Evidence suggests that diversification of the tropical savanna woody flora began in the late Miocene, following the expansion of C₄ grasses and accompanying an increase in fire frequency (Maurin et al. 2014; Simon et al. 2009). This flora was assembled from multiple lineages, many of which originated as trees from evergreen or deciduous forests. Many of these lineages retained a tree growth form, but underwent natural selection for multiple traits, including thick bark, increased investment in belowground biomass and reserves, thick leaves, and reduced adult height. A more extreme reduction in adult height is observed in subshrubs (geoxyles), which have diminutive aerial stems and large investment in belowground organs (see Gottsberger and Silberbauer-Gottsberger 2006; Simon and Pennington 2012). As adults, the belowground allocation of subshrubs is often so great these subshrubs are called underground trees (Bond

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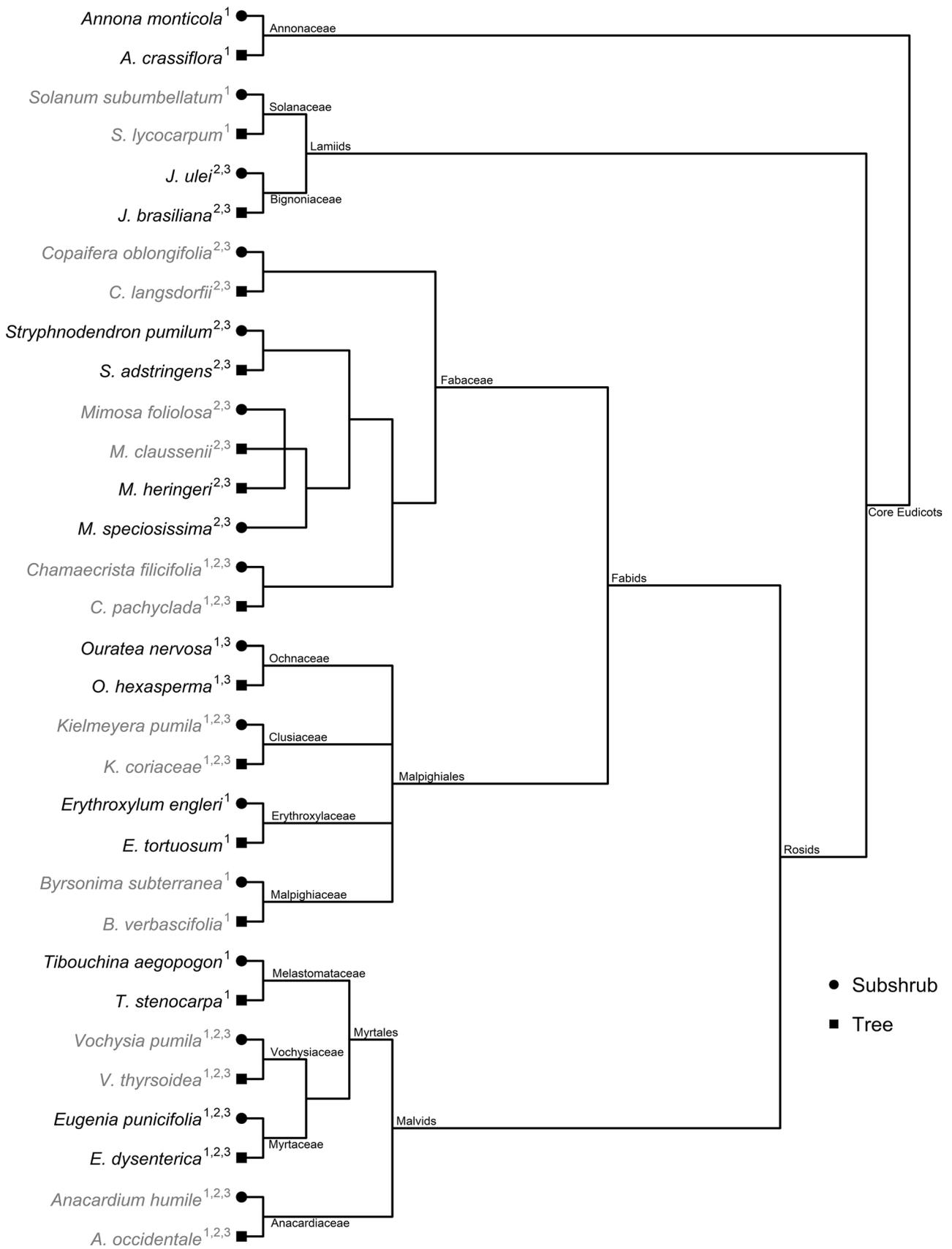


Fig. 1 Phylogenetic tree for congeneric pairs of subshrubs and trees that occur in Brazilian Savanna. Phylogenetic relation was based on the Phylomatic tree [tree R20120829—Stevens (2001 onwards)]. We improved the *Mimosa* clade using a more recent phylogeny (Simon et al. 2011). Alternating colors indicate congeneric pairs. Superscripts indicate data that were collected: ¹photosynthesis, respiration, and SLA of adult plants were measured; ²germination and seedling traits were measured; ³seed mass was measured

2016; Simon and Pennington 2012; Warming 1908; White 1976).

Trees and subshrubs represent starkly different outcomes of natural selection within the savanna environment. Although morphological differences between these growth forms are substantial as adults, it is not clear how these differences extend to physiological traits and seedling ecology, and consequently we lack a complete picture of the shift in life-history strategies that has accompanied the evolution of the subshrub habit. At first glance, subshrubs might appear to be the endpoint in a continuum of woody plant adaptation to the savanna environment. Savanna tree species tend to be shorter than closely related species typical of forest (Hoffmann et al. 2003), so the extreme reduction in height in subshrub lineages could be an extrapolation of the same evolutionary trajectory that have shaped savanna trees from forest ancestors. If this is the case, seedlings of savanna subshrubs should exhibit more extreme values of a suite of traits shown to be typical of savanna trees, including substantially lower specific leaf area (SLA), higher root–shoot ratio, and higher allocation to carbohydrate reserves (Gignoux et al. 2016; Hoffmann et al. 2003, 2004).

Alternatively, savanna trees and subshrubs might represent qualitatively distinct strategies for coping with the savanna environment (Rossatto and Franco 2017). For example, it has been argued that savanna shrubs and trees growth forms are alternate strategies for ensuring reproduction under frequent burning (Hoffmann and Solbrig 2003). That is, adult savanna trees are able to maintain reproductive size in spite of fire because their large, fire-resistant stems are largely immune to fire (Dantas and Pausas 2013; Hoffmann et al. 2009), but shrubs (and subshrubs) reproduce by resprouting vigorously and reproducing when stems are quite small (Zizka et al. 2014). These differing strategies could be manifested largely as differences in size at maturity and investment in aboveground biomass of established plants, and might not involve differences in seedling traits or in leaf physiology. In fact, since trees and subshrubs commonly coexist in the same environments, their seedlings are exposed to similar stresses and disturbances, and since their stems should be equally vulnerable to fire at this stage (Hoffmann et al. 2012; Miranda et al. 2002), they should share a similar need for sprout capacity.

Regardless of which scenario most closely describes the origin of savanna subshrubs, phylogenetic effects are likely

to be substantial. Subshrubs arose independently from multiple and diverse lineages of tropical trees (Maurin et al. 2014; Simon and Pennington 2012), so trait conservatism should ensure high functional diversity across subshrubs species and should closely mirror the underlying diversity present in their ancestral tree species (de Bello et al. 2015; Losos 2008).

To better understand the suite of plant traits associated with the subshrub growth form, the aim of this study was to compare congeneric savanna trees and subshrubs of the Brazilian Cerrado to test for convergence in seedling and leaf traits in subshrubs across multiple lineages. Furthermore, we examined the strength of phylogenetic conservatism across lineages because of its potential to shape the functional diversity of subshrub communities. This large diversity may obscure differences between trees and subshrubs, making it important to account for phylogenetic effects (de Bello et al. 2015).

Methods

Species selection and growth form classification

We selected 16 pairs of species, each containing one savanna subshrub and one savanna tree species from the same genus, both of which occur in Brazilian savanna. Using these congeneric pairs allows us to confirm that similarities found within the growth forms can be interpreted as convergent evolution, rather than shared ancestral traits (de Bello et al. 2015; Wanntorp et al. 1990). These genera represent 12 different families, well distributed across the angiosperm phylogeny (Fig. 1). We classified species based upon the criteria present in Eiten (1991, 1972) and Raunkiaer (1934). We considered a species to be a tree (phanerophyte, in the Raunkiaer system), if it commonly possesses a single, well-developed, lignified and persistent stem over 2 m tall. Species traditionally classified as subshrubs in the Cerrado include a diversity of stem morphologies that can sometimes be difficult to define in a way that is distinct from other shrubs. We considered a species to a subshrub if it possesses a lignified or partially lignified stem and is typically less than 1 m in height when mature. We also considered as subshrubs species that reach heights of 1–2 m, but only if stems are short-lived, weakly lignified, and with little branching.

Seed collection and sowing

For 11 of the species pairs, we collected seeds of both congeners. Most were collected in the vicinity of Brasília, DF, Brazil (15°57'59.76"S, 47°57'34.92"W), but seeds of *Copaifera oblongifolia* were collected in Chapada Gaúcha, MG (15°26'18.96"S, 45°27'21.96"W), *Anacardium*

humile was collected in Grão Mogol, MG (16°22'17.76"S, 43°0'36"W) and *Mimosa foliolosa* was collected in Delfinópolis, MG (20°20'17.88"S, 46°48'52.92"W). We collected seeds between November, 2013 and July, 2014. We weighed 50 seeds of each species using a precision scale (0.0001 g) to obtain mean seed mass. We sowed the species in polyethylene sacks containing approximately 12.5 l of soil (20 cm diameter × 40 cm deep), with three seeds per sack, and 60 sacks per species. Most were sown in February 2014, but *Mimosa heringeri* and *M. speciosissima* were sown in April, 2014 and the *Stryphnodendron* spp. were sown in July, 2014. *Mimosa* and *Stryphnodendron* seeds were immersed in sulfuric acid for 5 min to break physical dormancy. We used a substrate of 70% oxisol subsoil (40 cm deep or more) mixed with 30% of washed sand, without added nutrients. The experiment was conducted in a greenhouse with an automatic irrigation system supplying ~7 mm day⁻¹. Light intensity was not measured, but was close to natural daylight due to transparent walls and roof, with modest interception from the frame.

Germination, survival, resprout, biomass and reserves analysis

We monitored germination and survival at monthly intervals. At 10 months after sowing, we randomly placed the plants into three groups. One group was kept as control, the second group was clipped at soil level to assess resprout capacity, and the third group was harvested to quantify biomass and reserves. For clipped plants, the number of individuals that resprouted in each sack was assessed monthly and resprout height was measured after 6 months. Harvested plants were washed in a sieve (2 mm) to remove all soil and were then divided into shoot and roots. Roots were submerged in liquid nitrogen to stop metabolic activity and were then lyophilized and stored with silica gel until analyzed for root carbohydrate reserves. Shoots were dried in a forced air chamber at 70 °C for 72 h, separated into leaves and stems (including petiole for species with compound leaves), and weighed on a precision scale (0.0001 g).

To analyze the root reserves we randomly selected six of the harvested individuals of each species, except *Kielmeyera pumilum*, for which only five individuals were selected. When there was more than one surviving individual per sack, we analyzed the largest. Samples were ground, and soluble sugar and starch were extracted and measured using the protocol of Amaral et al. (2007).

Photosynthesis, respiration and specific leaf area of adults

We located six adult individuals of each species (Fig. 1) in natural areas with Brazilian savanna vegetation. The areas

were private properties with conserved vegetation, near Brasília, usually surrounded by other reserves (15°57'28" S, 47°55'51" W—*C. pachyclada*, *B. subterranean*, *T. stenocarpa*), by pasture, urban areas and crop fields (15°21'49" S, 48°08'32" W—*Annona* genus, *A. occidentale*; and 15°55'53" S, 48°01'24" W—all other species). We measured maximum photosynthesis rate and respiration on a leaf area basis using a portable photosynthesis system LCpro-SD (BioScientific Ltd.). Measurements were performed on a fully expanded, healthy leaf, which was exposed to direct sunlight during part of the day. We performed measurements on one leaf per individual and recorded five measurements of photosynthesis per leaf at 1 min intervals after the exchange rate stabilized. Measurements were taken between 8:30 am and 12:30 pm with a photosynthetic photon flux density (PPFD) of 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$, chamber temperature at 30 °C and open-flow mode (CO₂ concentration mean \pm SD was 373 \pm 5 $\mu\text{mol mol}^{-1}$). We then measured the dark respiration of the same leaf by turning off the LED (PPFD = 0) and covering the leaf chamber with aluminum foil. Specific leaf area (SLA) was measured in six adult individuals per species.

Statistical analysis

We used mixed-effect models with interactions to verify differences between growth forms in ecophysiological traits: seed biomass, plant germination, survival, root reserves, photosynthesis rate, respiration rate, SLA, and resprout capacity. The models included growth form as a fixed factor, and genus as a random factor. When necessary, the response variable was log transformed (Table 1) to ensure the normality of residuals. To quantify the fraction of trait variability explained by genus versus plant growth form, we calculated the fraction of the total variance that was explained by growth form (fixed effect) and by genus (random effect) using the conditional R_{GLMM}^2 , which describes the variance explained by each factor as a proportion of the sum of all the variance components (see Johnson 2014; Nakagawa and Schielzeth 2013).

While the previous analysis provides a test of phylogenetic signal across genera, we also tested for trait conservatism over the entire phylogeny using the Blomberg's K and Pagel's λ (Blomberg et al. 2003; Freckleton et al. 2002; Pagel 1999). Blomberg's K is defined as the ratio between mean squared error (MSE) of the tip data divided by the MSE of data calculated using the variance–covariance matrix derived from the phylogenetic tree (see Blomberg et al. 2003 for details) and quantifies the degree of variation in a trait that can be explained by the phylogeny. If Blomberg's $K < 1$, this indicates phylogenetic overdispersion, and traits have less phylogenetic signal than expected from Brownian motion (BM) model. If $K > 1$, there is more phylogenetic signal than expected

Table 1 Analyses of phylogenetic conservatism for ecophysiological traits in two different growth forms of Brazilian Savanna

Trait	$R^2_{\text{GLMM}_{\text{Genus}}}$	$R^2_{\text{GLMM}_{\text{Life form}}}$	K	P_1	λ	P_2
Seed mass*	0.94	0.02	0.97	0.004	1.01	<0.001
Germination rate	0.76	0.06	0.25	0.338	<0.01	>0.999
Survival rate	0.76	0.01	0.51	0.047	<0.01	>0.999
Total plant mass*	0.64	0.06	0.59	0.036	0.91	0.089
Root mass ratio	0.69	<0.01	0.64	0.012	0.84	0.101
Stem mass ratio	0.68	0.05	0.79	0.003	0.97	0.012
Leaf mass ratio	0.78	<0.01	0.80	0.005	0.85	0.070
Root: shoot ratio*	0.70	<0.01	0.74	0.010	0.96	0.020
Resprout rate*	0.57	<0.01	0.61	0.009	0.820	0.167
Photosynthesis rate	0.42	0.02	0.16	0.543	<0.01	>0.999
Dark respiration rate	0.42	<0.01	0.34	0.263	0.37	0.370
Specific leaf area	0.68	<0.01	0.12	0.856	<0.01	>0.999
Non-structural carbohydrate: structural biomass*	0.68	0.04	0.68	0.080	0.72	0.202
Root carbohydrate mass*	0.66	0.03	0.67	0.136	0.83	0.022
Root carbohydrate concentration*	0.68	0.04	0.42	0.146	0.71	0.269
Root carbohydrate mass: total plant mass*	0.75	0.02	0.34	0.246	0.79	0.251

The R^2_{GLMM} was calculated using the Johnson (2014) and Nakagawa and Schielzeth (2013) approach and represent total variance explained by genus and growth form in linear mixed models. Variables with asterisks were log transformed. The K represent the values in Blomberg's K , λ the value of Pagel's λ test, P_1 and P_2 the P value in Blomberg's K test and Pagel's λ , respectively

from BM model (Crisp and Cook 2012). The Pagel's λ compares the distribution of a trait to that expected by BM. Low λ values indicate little phylogenetic signal in a trait given, and high λ values indicate a strong phylogenetic signal (Münkemüller et al. 2012; Swenson 2014).

To perform Blomberg's K and Pagel's λ tests, we first constructed a phylogenetic tree with all 32 species. The Phylomatic tree (R20120829) was used to estimate phylogenetic distances among taxa. The tree resolution was improved using data from Hedges and Kumar (2009). We dated the nodes using the "branch length adjustment" algorithm in Phylocom (Webb et al. 2008), and we obtained the age for major nodes in the tree from Hedges and Kumar (2009).

Seedling traits are commonly correlated with seed mass, so differences in traits between growth forms could result from differences in mean seed size. Therefore, we also compared growth forms using analysis of covariance (ANCOVA) with seed mass as a covariate. Similarly, we used ANCOVA to test for differences in photosynthetic traits with SLA as a covariate. All analyses were conducted in *R program* (R Core Team 2016), with the packages *lme4* (Bates et al. 2015), *car* (Fox and Weisberg 2011), *branching* (Chamberlain 2016), *phytools* (Revell 2012), *rncl* (Michonneau et al. 2015), and *ape* (Paradis et al. 2004).

Results

On average, seeds of trees were 36% heavier than seeds of subshrubs ($F_{1,10} = 6.015$, $P = 0.034$, Fig. 2a). At an age of 10 months, total seedling biomass was 72% higher in trees than in subshrubs ($F_{1,9} = 5.242$, $P = 0.048$, Fig. 2d). Similarly, trees had greater biomass of stems ($F_{1,9} = 8.968$, $P = 0.015$), leaves ($F_{1,9} = 8.921$, $P = 0.038$), and shoots ($F_{1,9} = 7.031$, $P = 0.026$), but not of roots ($F_{1,9} = 4.071$, $P = 0.074$). When compared in context of biomass allocation, the only significant difference between tree and subshrub was stem mass ratio ($F_{1,9} = 32.788$, $P < 0.001$, Fig. 2g), which was 31% higher in trees than in subshrubs. Root mass ratio ($F_{1,9} = 0.426$, $P = 0.531$), leaf mass ratio ($F_{1,9} = 0.093$, $P = 0.767$) and root:shoot ratio ($F_{1,9} = 0.476$, $P = 0.508$) were not significantly different between growth forms. Seed biomass was not correlated with total plant biomass ($F_{1,16} = 2.001$, $P = 0.176$) or with root biomass ($F_{1,16} = 4.040$, $P = 0.062$).

Germination success did not differ significantly between subshrubs and trees ($F_{1,9} = 3.171$, $P = 0.109$); with means of $73 \pm 17\%$ (mean \pm SD) and $62 \pm 27\%$, respectively (Fig. 2b). Survival after 10 months was similar between forms ($F_{1,9} = 0.656$, $P = 0.439$), with survival of $81 \pm 18\%$ for trees and $76 \pm 22\%$ for subshrubs (Fig. 2c). Resprout capacity did not consistently differ between life forms

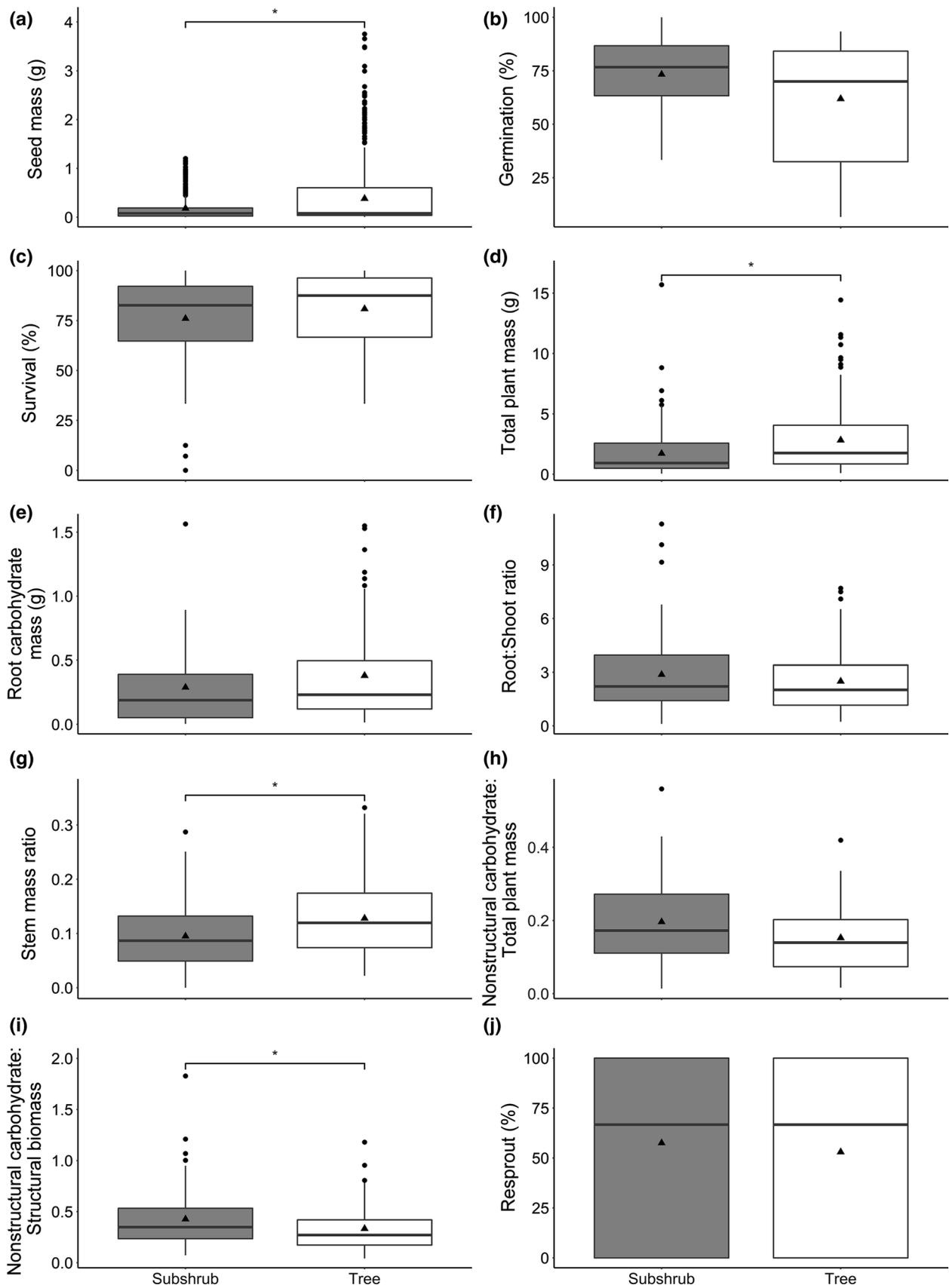


Fig. 2 Seed mass (a), germination (b), survival (c), total plant mass (d), root carbohydrate mass (e), root:shoot ratio (f), stem mass ratio (g), non-structural carbohydrate:total plant mass (h), non-structural carbohydrate:structural biomass (i) and resprout (j) of congeneric pairs of subshrubs and trees in Brazilian Savanna. The box plots indicate median, quartiles, and data range. Dots denote outliers and triangles means. An asterisk indicates a significant difference ($P < 0.05$)

($\chi^2 = 0.023$, $P = 0.878$, Fig. 2j) with mean resprouting of 57.5% for subshrubs and 53.0% for trees. Total root carbohydrate mass did not differ between the growth forms ($F_{1,9} = 1.944$, $P = 0.197$, Fig. 2e), but non-structural carbohydrate:structural biomass ratio of roots was 37% higher in subshrubs than trees ($F_{1,9} = 5.830$, $P = 0.039$, Fig. 2i). Root carbohydrate mass:total plant mass ratio did not differ between trees and subshrubs ($F_{1,9} = 3.479$, $P = 0.095$, Fig. 2h). There was a relationship between root carbohydrate mass and seed biomass ($F_{1,16} = 13.457$, $P = 0.002$), and root carbohydrate concentration was not correlated with seed biomass ($F_{1,16} = 3.209$, $P = 0.092$).

In adults, there was no difference between growth forms in light-saturated photosynthesis ($F_{1,10} = 0.053$, $P = 0.823$) dark respiration rate ($F_{1,10} = 0.001$, $P = 0.968$) or SLA ($F_{1,10} = 0.077$, $P = 0.787$), and no correlation was detected between rates of photosynthesis ($F_{1,18} = 0.519$, $P = 0.481$) or respiration ($F_{1,18} = 0.040$, $P = 0.843$) with SLA.

Overall, phylogeny explained much more of trait variation than did growth form. That is, genus explained more than 67% of the total variance in most traits. In contrast, the maximum variance explained by growth form was 6% for the traits total biomass and germination rate (Table 1). Even so, not all traits had a significant phylogenetic signal when the full phylogeny was considered. While seed mass, stem mass ratio, root:shoot ratio and root carbohydrate mass were phylogenetically conserved, the others 12 analyzed traits were not (Table 1).

Discussion

Although the differences in aboveground biomass and leaf mass between trees and subshrubs are large and evident as adults (Díaz et al. 2016; Poorter et al. 2012), there are surprisingly few consistent differences in seedling characteristics (Fig. 2). Instead, variation in seedling traits was consistently found to be the most strongly explained by genus (Table 1, R^2_{GLMM}) than by growth form. Similar patterns were observed for leaf traits of adult plants.

Overall, our results suggest that savanna subshrubs should not be considered simply an extreme endpoint along the same axis of specialization observed in savanna trees that arose from forest ancestors. Although such a continuum is suggested by adult heights (subshrubs < savanna trees < forest trees), similar rankings are not consistent for

several other traits. Specifically, subshrubs did not exhibit lower SLA, higher A_{max} , nor higher seedling root:shoot ratio, as would be expected based on comparisons of savanna and forest trees (Hoffmann and Franco 2003; Rosatto et al. 2013).

Of all the seedling traits studied, investment in stems and carbohydrate reserves were the only ones that hinted at the remarkable divergence between trees and subshrubs that develops later in life. As seedlings, trees consistently invested a modestly larger fraction of their biomass in stems (13 versus 9%, Fig. 2g) while subshrubs have greater investment in carbohydrate reserves (39 versus 29% of root structural mass, Fig. 2i). These differences should have important implications for post-fire recovery of seedlings because even at this early stage, subshrubs have less stem biomass to replace following topkill, and they have higher root carbohydrate concentrations with which to recover. Although significant, these differences were small compared to the variation across genera (Table 1).

The modest differences in seedling allocation between savanna trees and subshrubs suggest that natural selection during establishment and early growth might not be a primary factor driving the divergence of these growth forms. At this stage, seedlings of both growth forms are subject to an environment characterized by long dry seasons, low nutrient availability, and high risk of fire. In particular, fire is a particularly important factor in savannas, and may occur about once every 2–5 years (Archibald et al. 2013; Gottsberger and Silberbauer-Gottsberger 2006). The herbaceous aboveground layer is reduced by more than 90% after fire (Miranda et al. 2002), and seedlings of trees and subshrubs are topkilled, requiring them to regrow from soil level. We found that seedlings of both subshrubs and trees recover similarly well following biomass loss (Fig. 2j), and exhibit similar functional traits. Our experiment, however, may have been insufficient to detect the true differences in fire response of seedlings; clipping does not reproduce the complex physical and chemical effects of fire, while differences in resprout ability may become evident only after repeated topkill events (Bellingham and Sparrow 2000; Fensham et al. 2003; Vesk and Westoby 2004).

The differences in seedling traits, particularly in allocation to stem biomass, accrue further as plants approach maturity, when subshrubs and trees exhibit starkly different strategies. As adults, subshrubs and trees have sharply different heights (Díaz et al. 2016), and exhibit distinct strategies to cope with fire. Savanna trees exhibit an escape strategy (Zizka et al. 2014) in which adults are largely resistant to fire, allowing them to retain reproductive size by avoiding biomass loss during fire. In contrast, subshrubs are able to reproduce sexually despite repeated loss of aboveground biomass because they recover reproductive size quickly after fire (Bond 2016; Zizka et al. 2014).

Since our allocation and growth data are limited to seedlings, it is not clear how or when these different adult strategies arise during development. However, as seedlings grow, eventually a stem size is reached at which subshrubs are reproductively mature, while trees are neither mature nor large enough to resist fire (Fig. 3, Hoffmann and Solbrig 2003). It is not clear whether both growth forms would maintain similar growth rates until this time, nor if they would exhibit similar allocation patterns. More importantly, however, is that this point marks the divergence between growth forms in their demographic responses to frequent fire. At this

point, trees are susceptible to being maintained in a fire-trap of repeated topkill and resprouting, and may be maintained indefinitely in this suppressed state without an opportunity to reach reproductive maturity (Bond and van Wilgen 1996; Hoffmann et al. 2009). Meanwhile, subshrubs are generally able to recover reproductive size quickly between fires, thereby being able to produce seeds in the intervals between fires (Fig. 3). Allocation patterns may have diverged substantially prior to reaching this size, but perhaps the most essential difference between these growth forms is the stem size at sexual maturity.

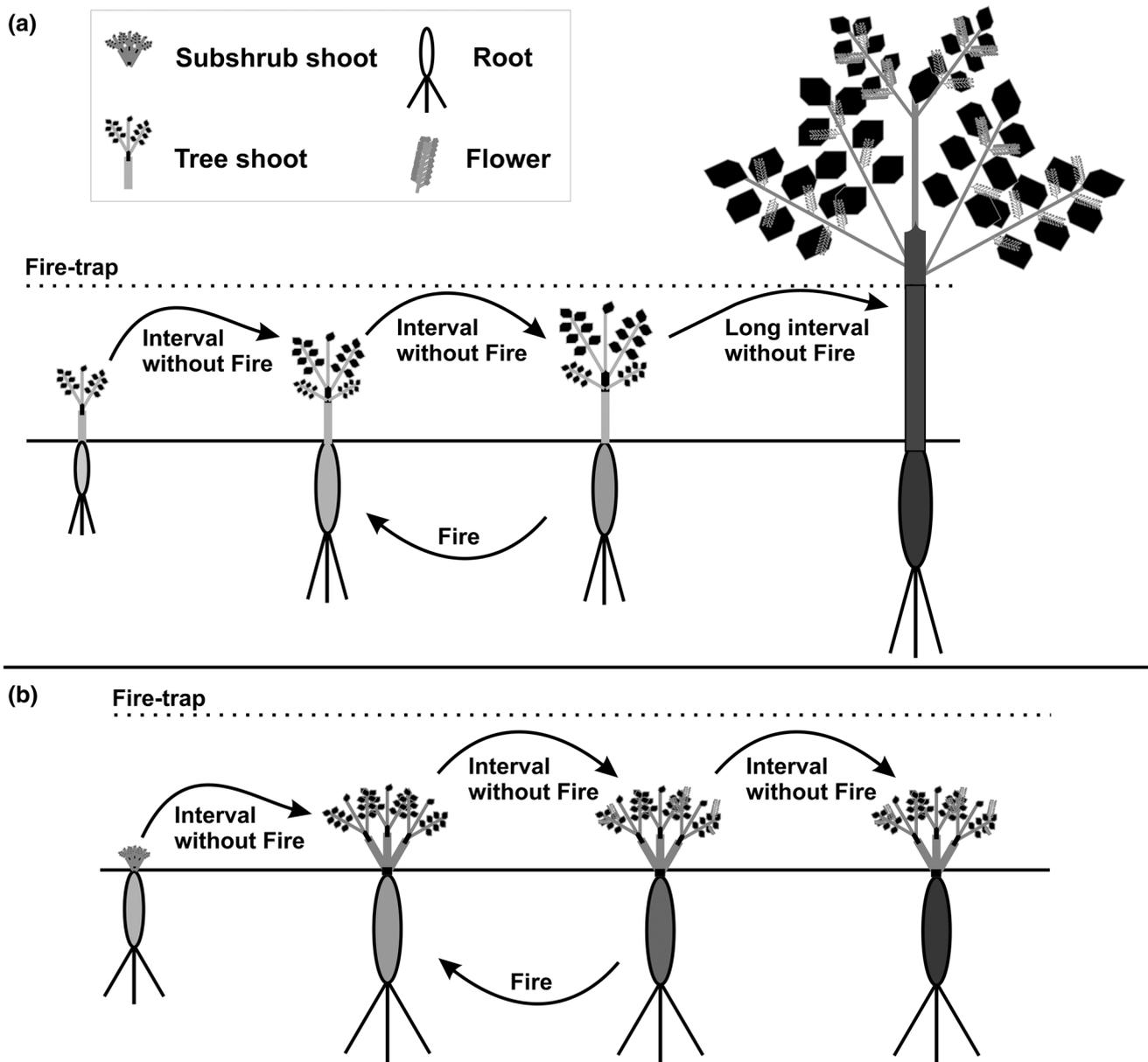


Fig. 3 Schematic of the life histories of a tree (a) and a subshrub (b), showing the differences in development and reproduction and response to the frequent fire. The root colors indicated theoretical dif-

ferences in reserves content (*light colors* less reserves, *dark colors* more reserves). The fire-trap line could be equally applied to bark thickness as well

This may have indirect effects on evolution of maximum size because evidence suggests a tradeoff between maximum size and ability to reproduce at small size (Aarssen 2015). Thus, the ability to reproduce while suppressed by frequent fires may preclude the ability to become a large, fire-resistant tree, even if considering reproduction of the tallest individuals in a cohort (Pilon and Durigan 2017).

The reduction of growth form might have also had indirect consequences for the evolution of seed size. Even though seed mass exhibited strong phylogenetic conservatism, we found evidence for directional selection towards smaller seeds in subshrubs within genera. This may simply arise from the general tendency for small growth forms to produce small seeds (Díaz et al. 2016). Regardless, the reduction of seed size will have consequences for seedling establishment and success because small seeds are more vulnerable to heat shock (Ribeiro et al. 2015) and smaller seedlings are more vulnerable to fire and drought (Lahoreau et al. 2006). On the other hand, the smaller seeds should be more easily dispersed and should be more easily buried, which would aid in protection from fire and predation (Leishman et al. 2000; Moles and Westoby 2004; Smith and Fretwell 1974).

One remarkable feature of subshrub evolution is the fact that they arose independently within a large number of tropical tree lineages (see examples in Gottsberger and Silberbauer-Gottsberger 2006; Maurin et al. 2014; Simon and Pennington 2012). These multiple origins suggest that the conquest of fire-prone ecosystems may require simple, easily acquired, genetic changes, perhaps involving gene regulation and rather than structural mutation (Simon and Pennington 2012). This could involve, for example, downregulation of gibberellin pathways, which is known to induce both dwarfism and flowering at smaller stem sizes (Davies 2010; Gupta and Chakrabarty 2013), two characteristics of subshrubs (Fig. 3).

Regardless of the genetic mechanism, the evolutionary shift from tree to subshrub has relatively little impact on many species traits. In fact, for most traits, genus explained >65% of the total interspecific variation across the study species, revealing strong phylogenetic conservatism of these traits (Table 1). Each of these independent origins occurred within a lineage that possesses a unique suite of plant functional traits. This, combined with substantial conservatism of these traits at the genus level, has given rise to a functionally diverse community of subshrubs in the Cerrado, which largely mirrors the diversity of lineages from which the subshrubs evolved.

Conclusions

In conclusion, these findings have multiple implications for the ecology and evolution of the subshrub (geoxyle) growth form, which is a diverse component of mesic savannas of South America and Africa. As seedlings, functional traits among our study species were more strongly determined by phylogeny than by growth form, but presumably, this pattern is inverted later in development as plants differentiate into the highly distinctive tree and subshrub growth forms. Even so, leaf traits of adult plants continue to show little difference between growth forms (Electronic Supplementary Material (ESM)—Fig. S1, Rosatto and Franco 2017), emphasizing the importance of allocation and reproductive traits that characterize these growth forms.

Nevertheless, there were significant differences in several traits between subshrubs and trees across multiple independent lineages, revealing an influence of natural selection on these traits. Overall, subshrubs had significantly smaller seeds, greater investment in carbohydrate reserves and less investment in aboveground growth during early development, all of which are consistent with a life history strategy involving adults that invest little in permanent aboveground structures and reproduce quickly after fire. On the other hand, trees are comparatively less adapted to habitats with high fire frequency. Nonetheless, they can remain as “gullivers” (Bond and van Wilgen 1996), this life-history strategy depends on longer free-fire intervals or alternatively many years of accumulating reserves for fueling sustained rapid growth to become fire resistant. The subshrub life-history strategy reaffirms the importance of fire events in the origin of savanna biodiversity during the recent evolution and diversification of Brazilian savanna flora. Subshrubs comprise an important component of this flora, which by means of extreme reduction in growth form have converged on an effective strategy for the savanna environment.

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Author contribution statement ABG conceived, designed and performed the experiments with contributions from all authors; ABG executed the field work; ABG and WAH performed the statistical analysis. ABG, AS, WAH wrote the manuscript and provided editorial advice.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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