Received: 16 May 2016 | Accepted: 26 September 2018

DOI: 10.1111/1365-2745.13085

RESEARCH ARTICLE

Journal of Ecology BRITISH ECOLOGICAL SOCIETY

Seedling growth of savanna tree species from three continents under grass competition and nutrient limitation in a greenhouse experiment

Kyle W. Tomlinson[1,2] | **Frank J. Sterck**[3] | **Eduardo R. M. Barbosa**[2,4] | **Steven de Bie**[2] | **Herbert H. T. Prins**[2] | **Frank van Langevelde**[2,5]

[1]Centre for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, China

[2]Resource Ecology Group, Wageningen University, Wageningen, The Netherlands

[3]Forest Ecology and Management Group, Wageningen University, Wageningen, The Netherlands

[4]Departamento de Botânica, Laboratório de Termobiologia, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, Brazil

[5]School of Life Sciences, University of KwaZulu-Natal, Durban, South Africa

Correspondence
Kyle W. Tomlinson
Email: kyle.tomlinson@xtbg.org.cn

Funding information
Shell Research Foundation

Handling Editor: Paloma Ruiz-Benito

Abstract

1. Changes in savanna tree species composition, both within landscapes and across climatic gradients, suggest that species differ in their ability to utilize resources and cope with grass competition. Linking trait variation among species to their relative performance under resource limitation and competition treatments could provide mechanistic understanding of species' turnover across environmental gradients in savannas. We investigated how tree species differ in response to competition from grasses and nutrient supply, and whether these responses can be related to plant traits. As humid savannas are often associated with low fertility, species of humid savannas may grow more efficiently under nutrient suppression than species from semi-arid savannas. In heterogeneous grass swards, fast-growing, resource-wasteful species may be found in low grass biomass patches and slow-growing, conservative species in high biomass patches.
2. We compared seedlings of 40 tree species sampled from humid and semi-arid savannas of three continents (Africa, Australia, South America) with and without grass competition and with high and low nutrient supply. We measured traits related to soil resource capture, light capture, and growth, and tested whether these traits were related to performance under the four treatments.
3. All tree species were suppressed by grass competition and most by nutrient limitation. Only species from humid savannas in Australia grew better under nutrient limitation than their semi-arid counterparts. Deciduous species from semi-arid climates were more negatively impacted by grass competition than deciduous species from humid climates whereas the reverse pattern was observed among evergreen species. Faster growing species were more severely suppressed by grass competition and low nutrients than slower growing species. Traits associated with soil resource capture and seed mass supported growth under grass competition and nutrient limitation, but the traits differed by continent and by leaf habit.
4. *Synthesis*. We found limited evidence that tree seedlings from humid savannas grow better under nutrient limitation than species of semi-arid savannas. We confirmed that fast-growing species may be advantaged in low grass biomass patches and conservative species in high biomass patches. Traits that improved

performance under grass competition differed by continent, which may relate to differences in leaf habit and constraints on seed size.

KEYWORDS
determinants of plant community diversity and structure, grass competition, humid savanna, seedling, semi-arid savanna, soil nutrients, traits

1 | INTRODUCTION

Savannas are biomes defined by the coexistence of trees and grasses. They are found across broad regional climate and soil gradients (Lehmann et al., 2014; Sankaran et al., 2005), and tree species composition changes both across these gradients and at landscape scales (e.g., Cole, 1986; Venter, Scholes, & Eckhardt, 2003). Investigations of tree–grass coexistence have shown that the dynamics of tropical savanna systems are strongly related to tree seedling demography, as this stage is most vulnerable (Bond, 2008; Van Auken, 2000; van der Waal et al., 2011). Field experiments have demonstrated that grasses suppress tree seedling growth by competition for water (February, Higgins, Bond, & Swemmer, 2013; Kulmatiski, Beard, Verweij, & February, 2010; Mills, Rogers, Stalmans, & Witkowski, 2006; Riginos, 2009) and soil nutrients (Cramer, Chimpango, Cauter, Waldram, & Bond, 2007; Cramer, Wakeling, & Bond, 2012; February & Higgins, 2010; O'Halloran et al., 2010; van der Waal et al., 2009). However, few studies have compared the responses of multiple tree species to grass competition and resource limitation (e.g., Vadigi & Ward, 2013; Barbosa et al., 2014), and few have attempted to relate relative performance to functional trait variation across species (Barbosa et al., 2014; Varma, Catherin, & Sankaran, 2018). Barbosa et al. (2014) and Varma et al., (2018) showed that legumes and non-legumes performed differently under fertility treatments, but they did not measure functional traits that could explain this difference. In other systems, trait differences among tree seedlings are thought to explain differences in species performance across resource gradients and in response to competition (Grime, 1977; Grubb, 1977). Linking trait variation among species to their relative performances under resource limitation and competition treatments could provide a mechanistic understanding of tree species turnover across environmental gradients in savannas (Diaz & Cabido, 1997; Suding et al., 2008) and allow prediction of how this might change under future environmental conditions. These links can be explored through common garden experiments on multiple tree species sampled widely across savannas.

Across climate types, savannas in humid environments have different environmental pressures to savannas in semi-arid environments. Humid savannas are associated with low nutrient soils that distinguish them from adjacent forest communities on more fertile soils, (Lloyd et al., 2008; Murphy & Bowman, 2012), and thus, they may be more nutrient limited than water limited. By contrast, the density of woody cover in semi-arid savannas is positively related to rainfall (Sankaran et al., 2005), suggesting that semi-arid systems are likely more water limited than nutrient limited (Bond, Woodward, & Midgley, 2005; Lloyd et al., 2008). Traits which may be associated with better performance under nutrient limitation include low intrinsic nutrient requirements per unit leaf mass (N_m, P_m and K_m respectively), and traits that may improve ability to capture soil resources include greater allocation to root mass (RMF, root mass as a fraction of total plant mass), faster root extension rates (RER, length per unit time), and more efficient root allocation (STRL, taproot length per unit mass) (Tomlinson et al., 2012). Plants may also improve their seedling growth through bigger seed sizes that provide more nutrients to support first-season growth in nutrient-poor environments (Leishman, Wright, Moles, & Westoby, 2000).

Coexistence of tree species with different traits may be facilitated by spatial variation in grass competition. In both humid and semi-arid systems, grass biomass is often heterogeneous, a response to resource availability and disturbance with patches of high and low biomass, and even bare ground (Archibald, Bond, Stock, & Fairbanks, 2005; O'Connor, 1995), resulting in spatiotemporal heterogeneity in strength of grass competition. This pattern could present different opportunities for establishment if there is a trade-off between ability to grow fast and ability to tolerate competition for resources from grasses (Goldberg & Landa, 1991). Fast-growing, resource-wasteful species use water and nutrients more rapidly than slow-growing, resource-conservative species, and may therefore only achieve fast growth rates without competition from grasses. Thus, fast-growing species could grow faster in low grass biomass patches, but in competition with grass or under low nutrient supplies, they may have lower growth rates than conservative species due to more rapid loss of resources. Root traits that aid soil resource capture (RMF, RER, STRL) may also enhance growth performance under grass competition. Other traits may be more closely aligned with efficient water use: lower stomatal conductance (g_s) and greater leaf photosynthetic water use efficiency (PWUE, photosynthetic assimilation per unit water transpired) (Matzek, 2012) may each allow plants to utilize soil water availability more efficiently. Fast-growing species might be selected for traits that allow them to utilize light resources more efficiently, including greater specific leaf area (SLA), greater leaf nitrogen (N_m), greater assimilation rates (A_m) (Ordoñez et al., 2009; Wright et al., 2004), and greater stem extension rates (SER), that allow them to rapidly grow beyond hazards associated with savannas, including fire and mammalian herbivory (Hempson, Archibald, & Bond, 2015; Higgins, Bond, & Trollope, 2000).

In this study, we examine whether first-season growth of seedlings of 40 tree species taken from savannas on three continents (Africa, Australia, South America; see Supporting information Table S1) differs in response to competition from grasses and in response to nutrient supply, and whether these responses are related to plant traits that vary among species. In previous analyses with these species, we have noted that there are both evergreen and deciduous species present in our sample, and we have shown that they have distinct leaf syndromes (Tomlinson, Poorter, et al., 2013), different ontogenetic allometries (Tomlinson, Langevelde, et al., 2013), and their relative growth rates are constrained by different functional traits (Tomlinson et al., 2014). Interestingly there is a bias in distribution of leaf habits across continents; the species samples from African and South American savannas are dominated by deciduous trees whereas the samples of Australian savannas are dominated by evergreen species (Tomlinson, Poorter, et al., 2013). These samples reflect relative dominance by deciduous versus evergreen species in savannas across continents (Bowman & Prior, 2005; Cole, 1986). As the species of different leaf habits have different trait syndromes, it is plausible that evergreen and deciduous species respond differently to resource competition with grasses and with low nutrient supply. This in turn could lead to differences in patterns among continents. Several analyses comparing savannas on the southern continents have concluded that their climate space may differ and that they may function differently (Bowman & Prior, 2005; Holmgren, Hirota, Nes, & Scheffer, 2013; Lehmann et al., 2014).

Therefore, in this paper, we consider patterns of tree seedling performance in response to nutrient limitation and grass competition across climate types separately for each continent and then separately for each leaf habit, and we also evaluate the effect of traits on seedling performance separately for each continent and for each leaf habit group.

We made the following predictions:

1. Species from humid savannas grow better than species from semi-arid savannas under low nutrient conditions.
2. More rapidly growing species are more severely suppressed under grass competition and/or nutrient limitation than slower growing species.
3. Traits driving variation in growth performance under nutrient limitation and grass competition differ across continents and differ between evergreen and deciduous species.

2 | MATERIALS AND METHODS

2.1 | Species selection

We sampled dominant or 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, Grieser, Beck, Rudolf, & Rubel, 2006), which estimates the relative water deficits of particular locations by comparing a dryness threshold based on the mean annual temperature (T_{ann}, °C) with mean annual precipitation (MAP, mm) at those locations, adjusted for whether the region has predominantly winter or summer rainfall. Given a dryness threshold $P_{th} = 28 + 2\ (T_{ann})$, then semi-arid environments are defined as areas with $5\ P_{th} < \text{MAP} < 10\ P_{th}$ and humid environments are defined as areas with $\text{MAP} > 10\ P_{th}$. We sampled species from humid and semi-arid summer-rainfall savannas in eastern Southern Africa (coastal and inland savannas in South Africa and Zimbabwe), in north-eastern Australia (coastal and inland woodlands in Queensland), and in eastern South America (Cerrado and Caatinga biomes in Brazil) (Cole, 1986). The chosen species are abundant in the climate types where they were sampled. A total of 40 species were used in this experiment, including 18 species from Africa (8 humid, 10 semi-arid), 12 species from Australia (5 humid, 7 semi-arid), and 10 species from South America (7 humid, 3 semi-arid). A full species list is provided in the Supporting information (Table S1), including their family, order, continent of origin, climate type of origin, and leaf habit. Climate data and soil fertility data for the environments where seeds were collected from the tree species growing in their native ranges are provided in Supporting information (Table S2).

2.2 | Greenhouse experiment

We conducted a greenhouse experiment to evaluate the performance of the 40 savanna tree species under competition with grasses grown in a common growth environment. 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 hr (daytime) and 23°C for 12 hr (night-time), 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 seeds were collected for the experiments. Daily variation of light was from 150 to 450 $\mu\text{mol m}^{-2}\ \text{s}^{-1}$, supplied from sunshine and supplementary light (150 $\mu\text{mol m}^{-2}\ \text{s}^{-1}$), which was provided for 12–16 hr (seasonally adjusted) to ensure that the photon flux density exceeded 10 $\text{mol m}^{-1}\ \text{d}^{-1}$. This is less than one quarter of the light these species might receive in tropical savannas, where daily values range from 31.6 in winter (dry season) to 62.9 $\text{mol m}^{-1}\ \text{d}^{-1}$ in summer (wet season) (Huntley & Walker, 1982), but the plants in the greenhouse achieved substantial growth during the first growing season (Tomlinson, Poorter, et al., 2013) and this growth varied significantly among species (Tomlinson et al., 2012), indicating that available light was sufficient to distinguish growth abilities. 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).

Tree seedlings were grown in plastic tubes (10 cm diameter, 100 cm length) in a fully crossed design of high and low nutrient treatments (N_h and N_l respectively), with and without grass competition (G_1 and G_0 respectively). Pots were filled with river sand mixed with slow-release (8–9 months) fertilizer (Osmocote® 18–6-12 [N-P-K] fertilizer). Nutrient treatments were, respectively, 5 kg and 1 kg fertilizer m^{-3} river sand; the nutrient concentration levels for the high nutrient treatment were intermediate between the company specifications for low application (4.2 kg/m) and medium application (6.2 kg/m) for the product. We chose the low end of the recommended nutrient application to try to ensure that the nutrients were limiting to growth for most species. The high and low treatments were different in order to cause significant differences in growth between treatments for most species (see Results). Grass competition was imposed by growing one seedling of the C_4 grass species *Eragrostis curvula* alongside each tree seedling. *Eragrostis curvula* is native to savannas in southern and eastern Africa (Fish, Mashau, Moeaha, & Nembudani, 2015) and has been introduced as a pasture species throughout the tropics, becoming invasive across a wide rainfall range in Australia and the Americas (Csurhes, Leigh, & Walton, 2016). It grows fast and recruits tillers rapidly, making it highly competitive for resources. The grass seedlings were grown to the three-tiller stage (see Supporting information Figure S1) in seedling trays and planted at the same time as the recently germinated tree seedlings into the growing tubes. Water was supplied through irrigation at a rate of 40 ml per pot per day, equivalent to 800 mm of rainfall over 20 weeks of growth. This is similar to the amount of rainfall that humid savannas in Africa, Australia, and South America receive during the 5- to 7-month growing season (see Supporting information Table S2, for actual rainfall received and length of average growing season). However, our greenhouse plants received a much more even supply than they would receive in their natural environment.

Seedlings were grown for 20 weeks and then harvested. A maximum of 10 individuals were grown per treatment per species (range: 5–10), yielding a maximum replication per species of 40 individuals. The total dataset comprised of 1,446 individual plants: 361 in the N_hG_0 treatment, 358 in the N_hG_1 treatment, 372 in the N_lG_0 treatment, and 355 in the N_lG_1 treatment. Due to space limitations, we grew the plants in three batches with species grown in either one (10 individuals per treatment combination) or two batches (five individuals per treatment combination per batch) out of the three.

2.3 | Plant performance

We measured plant performance as plant mass gain (g) over the growing period. The mass gain of an individual plant *i* of species *j*, in a particular batch run *t*, was the difference between final dry mass of that individual (20 weeks) and an initial estimated mean dry mass for the species *j*.

$$\text{Mass gain}_{i,j,t} = \text{Mass}_{20,i,j,t} - \overline{\text{Mass}_{0,j}} \quad (1)$$

For the initial mass, which was calculated from different individuals than those used to estimate final mass, we took the average value across 10 individuals that had recently germinated and were equivalent in age to seedlings planted into the treatment pipes at the start of the growing period.

2.4 | Trait measurements

We measured morphological and physiological traits that are thought to aid plants to capture or retain soil nutrients and water or capture light for rapid growth (Cornelissen et al., 2003; Westoby, Falster, Moles, Vesk, & Wright, 2002). Measured traits that support light capture were specific leaf area (SLA, $cm^2\ g^{-1}$), leaf assimilation rate (A_m, nmol $CO_2\ g^{-1}\ s^{-1}$), leaf nitrogen content (N_m, mg N g^{-1}), and stem extension rate (SER, mm/d). We measured three root traits which may affect the ability and efficiency of plants to capture soil resources (water or nutrients), namely root extension rate (RER, mm/d), specific taproot length (STRL, m/g), and root mass fraction (RMF, g root mass/g total mass). We measured leaf traits that support efficient water use including stomatal conductance (g_s, μmol $H_2O\ m^{-2}\ s^{-1}$), plant photosynthetic water use efficiency (PWUE, μmol CO_2 $\mu mol^{-1}\ H_2O$), leaf phosphorus and potassium content (P_m, mg P g^{-1}; K_m, mg K g^{-1}). Finally, we measured traits that may support efficient growth under low nutrient supply, including leaf nutrient concentrations (phosphorus and potassium content again [P_m, mg P g^{-1}; K_m, mg K g^{-1}]), and the initial mass of recently germinated seedlings ($Mass_0$, g), which represents the initial resources allocated to a seedling in a seed. $Mass_0$ is directly related to seed mass, but is more accurate for initial resources allocated to a seedling because it only measures the living individual after emergence from the seed and therefore excludes biomass associated with the seed coat and other appendages not related to growth. To calculate $Mass_0$, we harvested 10 recently germinated seedlings, dried and weighed them, and then averaged the estimated weights. A full description of the measurement of the traits is provided in the Supporting Information and species' trait values are given in Table S3.

Although most trait measurements were collected per individual for all treatments, we calculated species-level mean trait values from across individuals of each species grown in the G_0 treatments (N_hG_0 and N_lG_0) for the analyses described below. This was because numerous traits change with plant size (McConnaughay & Coleman, 1999) and, as our grass treatment severely suppresses plant size, we could not separate treatment effects on traits from plant size effects on those traits. Therefore, we restricted ourselves to trait differences between species rather than also considering species' trait plasticity in response to treatments.

2.5 | Statistical analysis

All analyses were conducted using packages in R. The results of the models were visualized using base graphics and *ggplot2* (Wickham, 2009) in R. Some individuals (31) in the grass treatments lost mass over the duration of the experiment relative to the species' mean initial mass ($Mass_0$) with the most negative value being −0.496 g. As mass gain data were exponentially distributed, the data were normalized using the transformation $\log_e$(Massgain +0.5) prior to analysis. All independent variables were checked for normality and transformed as necessary prior to analysis.

We first checked whether the mass gain data contained any phylogenetic signal using the species' mean mass gain values for each of the four treatments. We compiled a phylogenetic tree we had previously built for these species (Tomlinson et al., 2012) using the *ape* package (Paradis, Claude, & Strimmer, 2004) and estimated Pagel's lambda on the transformed mass gain data using maximum likelihood with the function pgls() in the *caper* package (Orme et al., 2013). The ML estimate of Pagel's lambda for each of the four treatments was zero. So our subsequent analyses ignored phylogeny. We proceeded using the individual data in a mixed model framework with "species" and "batch" as random effects.

2.5.1 | Growth responses to treatments across climate groups and leaf habits

We tested whether seedling mass gain was suppressed by grass competition and nutrient limitation, and whether species from semi-arid savannas were more severely suppressed by nutrient limitation than species from humid savannas (Prediction 1). Tree seedling mass gain was modelled as a function of the grass and nutrient treatments and climate group types in a full interaction model, with "Species" and "Batch" included as random effects using the lmer() function in *lme4* (Bates, Maechler, Bolker, & Walker, 2015):

$$\text{Mass gain} \sim \text{Grass} \times \text{Nutrient} \times \text{Climate} + (1|\text{Species}) + (1|\text{Batch}) + \varepsilon \quad (2)$$

We included full interactions because we expected that the combined effects of nutrient limitation and grass competition might suppress species from semi-arid savannas more strongly than humid species. This model was applied on each continental dataset (African species, Australian species, South American species) and on each leaf habit dataset (deciduous species, evergreen species). To evaluate which terms were significant in the models, we used backward selection with likelihood ratio tests to remove non-significant terms, removing higher order interaction terms first. We used Tukey HSD tests for all *post hoc* comparisons of groups, conducted at interaction effect levels using the *glht()* and *cld()* functions in the *multcomp* package (Hothorn, Bretz, & Westfall, 2008). The fit of the full LMM models was assessed using the marginal and conditional R^2 estimators of Nakagawa and Schielzeth (2013), using the r.squaredGLMM function in the *MuMIn* package (Bartoń, 2016).

2.5.2 | Growth performance of within-region species with and without grass competition and under low versus high nutrient supply

To test whether more rapidly growing species were more severely suppressed under grass competition and/or nutrient limitation than slower growing species (Prediction 2), we tested the slopes of the individual mass gain under each of the treatment combinations against mean species' mass gain under the high nutrient, no grass treatment (N_hG_0) in each batch. A flatter slope relationship with increasing suppression (−nutrients, +grass) would indicate that fast-growing species were more suppressed by the treatment than slow-growing species.

2.5.3 | Traits that drive species performance with grass competition and under low nutrient supply

To establish which traits were related to species' performances in the presence and absence of grass competition and low nutrient supply, and whether these differed among continents and among leaf habits (Prediction 3), we tested whether variation in individual growth around the four treatment means could be statistically related with species' traits. Before using the linear mixed models, we checked Pearson correlations between the species' traits (see Supporting information Table S4). None of the traits were highly correlated with one another, with the highest correlation being rho = 0.71. For each mean trait (listed in Table 2) calculated using individuals from the "no-grass" treatments only (see above), we modelled seedling mass gain as a function of grass and nutrient treatments and that trait. As for the first analysis, we included "Species" and "Batch" as random effects in the model to account for their unexplained variation:

$$\text{Mass gain} \sim \text{Grass} \times \text{Nutrient} \times \text{Trait} + (1|\text{Species}) + (1|\text{Batch}) + \varepsilon \quad (3)$$

The terms "Grass," "Nutrient," and "Grass × Nutrient" account for the variation among treatments across all species. For this analysis, we were interested in whether any terms involving "Trait" explained significant variation, as these would indicate a positive or negative effect of the tested trait on tree seedling performance in a treatment. A significant trait main effect indicates that the trait is beneficial/inhibitory to growth under the high nutrient, no grass treatment (NhG0); a significant Grass × Trait effect indicates that the trait is beneficial/inhibitory to growth under grass competition; a significant Nutrient × Trait effect indicates that the trait is beneficial/inhibitory to growth under Nutrient limitation; and a significant Grass × Nutrient × Trait effect indicates that the trait is beneficial/inhibitory subject to the treatment combination. To evaluate which terms were significant in the models, we used backward selection with likelihood ratio tests to remove non-significant terms, removing higher order interaction terms first. To address Prediction 3, we ran the analysis separately for species from each continent and species from each leaf habit type.

3 | RESULTS

3.1 | Seedling mass gain of climate groups under treatments

Climate group comparisons for continents and leaf habits are given in Table 1 and Figure 1 and Table 2 and Figure 2 respectively. For the continental analyses, fixed effects for the Climate × Grass × Nutrient analysis accounted for 58%–63% of data variation across all three continental models (marginal R^2 values; Table 1). Climate group responses to grass or nutrient suppression varied across continents; there were significant three-way interactions between Grass, Nutrient, and Climate on mass gain of tree seedlings from Australia and South

TABLE 1 Results of ANOVA (Type III, Kenward–Rogers approximation of residual degrees of freedom) for linear mixed models testing whether tree seedling mass gain (g) differs with and without grass competition and with high or low nutrient supply, and by climate group (humid vs. semi-arid)

	Africa	Australia	South America
Sample size, *N*	645	447	354
Species number	18	12	10
Y-transformation	ln(y + 0.5)	ln(y + 0.5)	ln(y + 0.5)
Pseudo R^2: Marginal	0.60	0.63	0.58
Pseudo R^2: Conditional	0.78	0.82	0.79

Random effects	Variance	Variance	Variance
Species	0.386	0.347	0.435
Batch	0.046	0.110	0.049
Residual	0.512	0.430	0.490

Predictor	Df	Df.res	*F*	*P*	Coef. sign	Df.res	*F*	*P*	Coef. sign	Df.res	*F*	*P*	Coef. sign
Climate (SA)	1	18.4	3.7	0.071		11.9	16.1	0.002	–	9.5	9.8	0.011	+
Grass (G1)	1	620.7	551.8	<0.001	–	427.2	354.9	<0.001	–	336.1	236.0	<0.001	–
Nutrient (NI)	1	620.1	113.2	<0.001	–	427.0	4.6	0.033	–	336.8	32.5	<0.001	–
Climate: Grass	1	620.4	3.9	0.049		427.1	10.0	0.002	+	336.1	0.0	0.972	
Climate: Nutrient	1	620.1	2.0	0.159		427.0	4.1	0.043	–	336.3	0.5	0.487	
Grass: Nutrient	1	620.1	27.1	<0.001	+	427.1	3.5	0.061		336.2	10.5	0.001	+
Climate: Grass: Nutrient	1					427.0	4.0	0.046	+	336.2	9.6	0.002	–

Note. Analyses are run separately for species from three continents. Coefficient signs for the associated regression model are given for each model, but only for the significant effects. Note that the probabilities generated for the *F*-tests are identical to those generated for the *t* tests on the regression coefficients because all *F*-tests have numerator *df* = 1. The default case for the analysed models is Humid Climate, no grass and high nutrient supply.

America but not Africa. Most of the variation in the seedling growth was explained by the main effects for Grass and for Nutrients for species from Africa and South America, but Nutrients explained relatively less variation for species from Australia; for all continents, the *F*-value of the Grass main effect was much greater than other effects. In general, both grass presence and nutrient limitation reduced seedling mass gain relative to the high nutrient, no grass treatment (Figure 1), but the effect of grass competition was far more severe than nutrient limitation (see Supporting information Figure S2.

The South American and African tree species from semi-arid savannas grew more than species from humid savannas, whereas the Australian tree species from humid savannas grew more than species from semi-arid savannas (Figure 1). Among African species, there were no differences in the level of suppression by low nutrients of species from different climates (Climate × Nutrient not significant), but grass competition had a stronger negative effect on species from semi-arid areas (Climate × Grass significant). This combined evidence suggests that African species from different climate groups did not differ in their performance under nutrient stress but did differ with respect to grass competition. Among South American species, Climate × Grass and Climate × Nutrient interactions were both not significant, Grass ×Nutrient was significant and positive (similar to African species), and there was a significant Climate × Grass × Nutrient effect that was negative, indicating that species from semi-arid areas performed worse than species from humid areas under combined grass competition and nutrient stress. Among Australian species, species from semi-arid savannas were more suppressed by nutrient limitation than those from humid savannas (significant and negative Climate × Nutrient effect), but the species from semi-arid areas performed better under grass competition than species from humid areas (significant and positive Climate × Grass effect). In summary, there was evidence only from Australia that low nutrients suppressed species from semi-arid savannas more than species from humid savannas. Thus, there was

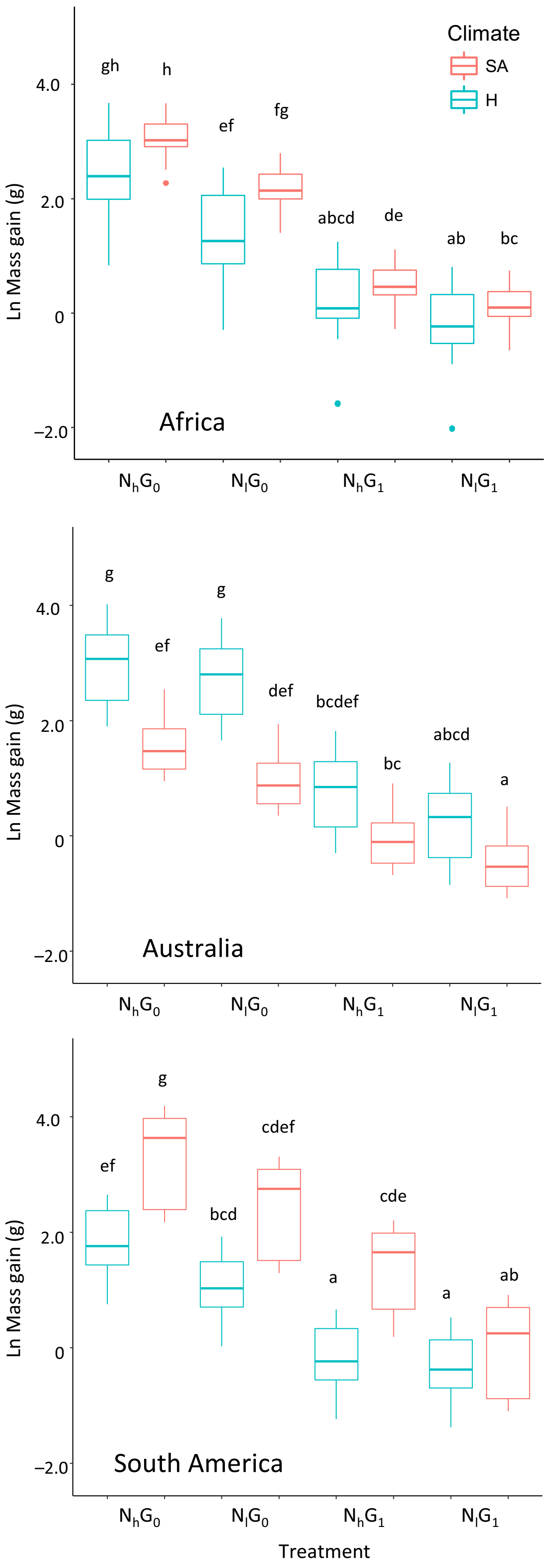

FIGURE 1 Mass gain of juvenile savanna trees from humid and semi-arid climates without and with grass competition (G_0 and G_1 respectively) and with high or low nutrient supply (N_h and N_l respectively), for different continents (Africa, Australia, South America). Post hoc comparisons across groups of each continent as indicated by letters reflect Tukey HSD tests on all groups within each continent separately [Colour figure can be viewed at wileyonlinelibrary.com]

only support from one continent for Prediction 1. There was also evidence that grass competition suppressed species from semi-arid savannas more than species from humid savannas among African and South American species, but that grass competition suppressed species from humid savannas more than species from semi-arid savannas among Australian species.

When we analysed the data by splitting the species between evergreen and deciduous leaf habits (Table 2, Figure 2), the patterns indicated that climate groups within each leaf habit were responding oppositely to grass competition but did not differ with respect to nutrient treatments. Evergreen species from semi-arid savannas had lower mass gain in the high nutrient, no grass treatment than species from humid savannas (negative climate main effect), but performed better under grass competition (positive Climate × Grass effect). By contrast, deciduous species from semi-arid savannas had greater mass gain in the high nutrient, no grass treatment than species from humid savannas (positive climate main effect), but performed worse under grass competition (negative Climate × Grass effect).

3.2 | Relative suppression of growth rate by grass competition and nutrient limitation of species from the same climate regions

Plots of individual seedling growth in each treatment combination (N_hG_0, N_lG_0, N_hG_1, N_lG_1) against mean seedling growth in the high nutrient, no grass treatment (N_hG_0) are given in Figure 3. In general, there was support for Prediction 2 that faster growing species in the least suppressed treatment (N_hG_0) were relatively more suppressed by low nutrient supply and grass competition than slower growing species. These effects were cumulative, with mass gain under the most suppressed treatment (N_lG_1) similar for fast- and slow-growing species in each climate group from each continent.

3.3 | Traits that relate to species performance with grass competition and under low nutrient supply

3.3.1 | Rapid growth traits

Across all continental analyses (see Table 3), SER was positively related to growth in the high nutrient, no grass (N_hG_0) treatment (positive main effect, "T") and was negatively related to performance under grass and under low nutrients. SLA was negatively related to growth under N_hG_0 (negative main effect, "T") and positively related to performance under grass competition among Australian and South American species, but the reverse pattern was found for

TABLE 2 Results of ANOVA (Type III, Kenward-Rogers approximation of residual degrees of freedom) for linear mixed model testing whether tree seedling mass gain (g) differs with and without grass competition and with high or low nutrient supply, and by climate group (humid versus semi-arid).

	Evergreen	Deciduous
Sample size, N	387	1059
Species number	11	29
Y-transformation	ln(y+0.5)	ln(y+0.5)
Pseudo R^2: Marginal	0.60	0.54
Pseudo R^2: Conditional	0.81	0.77

Random effects	Variance	Variance
Species	0.407	0.515
Batch	0.053	0.045
Residual	0.429	0.528

Predictor	Df	Df.res	F	P	Coef. sign	Df.res	F	P	Coef. sign
Climate (SA)	1	9.6	10.6	0.009	-	29.4	8.4	0.007	+
Grass (G1)	1	374.0	710.7	< 0.001	-	1028.3	746.2	< 0.001	-
Nutrient (NI)	1	371.1	52.9	< 0.001	-	1023.2	166.1	< 0.001	-
Climate: Grass	1	372.7	57.9	< 0.001	+	1024.6	18.1	< 0.001	-
Climate: Nutrient	1								
Grass: Nutrient	1					1023.1	13.9	< 0.001	+
Climate: Grass: Nutrient	1								

Note. Analyses are run separately for deciduous species and evergreen species. Coefficient signs for the associated regression model are given for each model, but only for the significant effects. Note that the probabilities generated for the F-tests are identical to those generated for the t-tests on the regression coefficients, because all F-tests have numerator df = 1. The default case for the analysed models is Humid Climate, no grass and high nutrient supply

African species. Leaf assimilation rate (A_m) was unrelated to performance under any of the treatments.

Among leaf habit groups (see Table 4), SER was positively related to growth under the high nutrient, no grass treatment (positive Trait main effect) and negatively related to growth under grass competition or low nutrients for both leaf habits. Among evergreen species, SLA and N_m both had positive effects on growth under grass competition, but among deciduous species, SLA, A_m, and N_m were not correlated with performance in any of the treatments (no significant Trait effects).

3.3.2 | Soil resource capture traits

Root mass fraction (RMF) was negatively related to growth under N_hG_0 and positively related to growth under grass and low nutrient supply for both African and South American species. Among Australian species, specific taproot length (STRL) was positively related to performance under grass competition, whereas among African species, STRL was negatively related to performance under grass competition and low nutrient supply and positively related to performance under N_hG_0. Root extension rate (RER) was positively related to growth under N_hG_0 among African and South American species.

The patterns observed for evergreen and deciduous species indicate that RER was positively related to growth under grass competition for both leaf habits, RMF was positively related to growth under grass competition for deciduous species whereas STRL was positively related to growth under grass competition for evergreen species.

3.3.3 | Resource utilization traits

Plant water use efficiency (PWUE) was positively related to growth under grass competition and under low nutrients for South American and African species. Among Australian species only, leaf N_m and P_m were positively related to growth under grass competition. Among leaf habits, growth under grass competition and under low nutrients for was

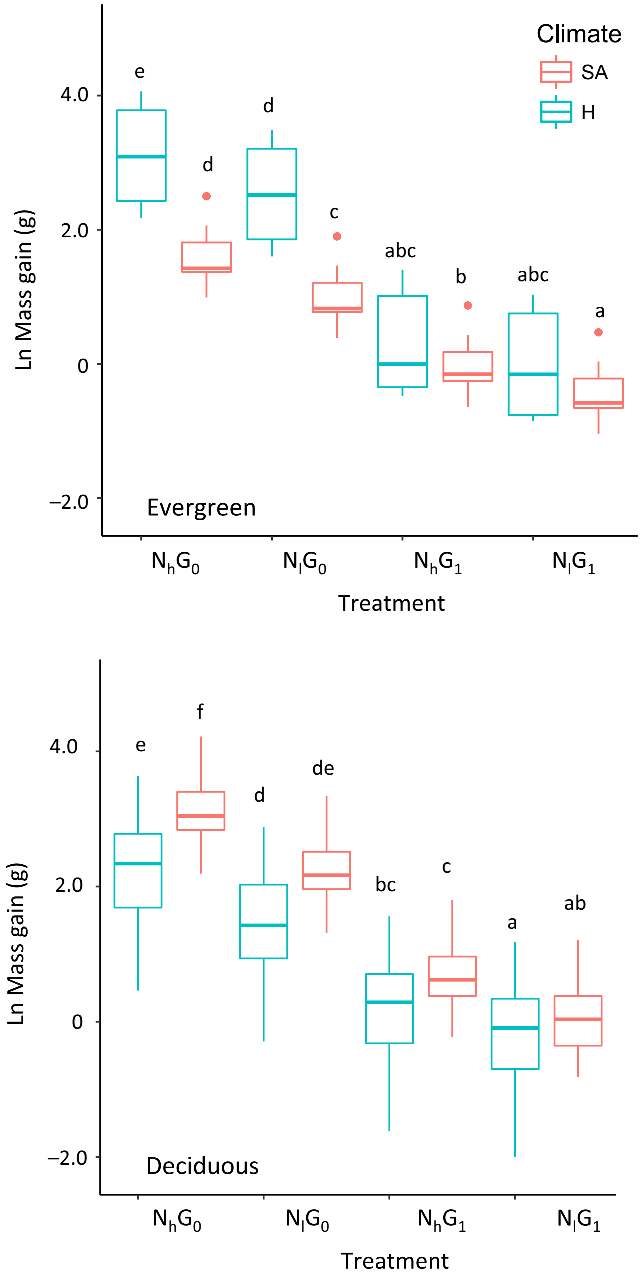

FIGURE 2 Mass gain of juvenile savanna trees from humid and semi-arid climates without and with grass competition (G_0 and G_1 respectively) and with high or low nutrient supply (N_h and N_l respectively) for different leaf habits (evergreen species, deciduous species). Post hoc comparisons across groups of each leaf habit as indicated by letters reflect Tukey HSD tests on all groups within each leaf habit separately [Colour figure can be viewed at wileyonlinelibrary.com]

positively related to PWUE for deciduous species only, whereas growth under grass was positively related leaf P_m and K_m for evergreen species only.

3.3.4 | Initial resource supply trait

Seed size was negatively related to growth under N_hG_0 and positively related to growth under grass and low nutrient supply among African and South American species. For both evergreen and deciduous groups, seed size increased performance under grass competition and under nutrient limitation. Thus, greater seed size improved seedling growth under both low nutrient conditions and under grass competition.

4 | DISCUSSION

In this paper, we tested whether species from humid savannas grow better than species from semi-arid savannas under low nutrient conditions (Prediction 1), whether more rapidly growing species are more severely suppressed under grass competition and/or nutrient limitation than slower growing species (Prediction 2), and whether there are traits that distinguish performance of seedlings in the different treatments (Prediction 3).

4.1 | Species from semi-arid and humid savannas differ in their response to grass competition, not nutrients

We found weak evidence that species from humid savannas perform better under nutrient suppression than species from semi-arid savannas (Prediction 1), as this pattern was only detected among Australian species (Table 1, Figure 1). Thus, there does not seem to be a general difference between humid and semi-arid savannas in performance under nutrient limitation. Rather performance difference may relate more directly to soil fertility differences across sites. Our Australian sampling sites in each climate type had wide ranges of fertility (see Supporting information Table S2), but our experience suggests that many more of the species from semi-arid areas we sampled were growing on heavy clay soils that were probably quite nutrient rich. We also detected a significant Climate × Grass × Nutrient effect that was negative for South American species; in other words, species from semi-arid savannas were more suppressed under combined nutrient limitation and grass competition than species from humid savannas. This suggests that the greater negative effects of nutrient deprivation on species from semi-arid areas may only be apparent under severe nutrient stress: our South American humid site was the most nutrient-poor location we sampled (see Supporting information Table S2), and thus, the species there are likely to be adapted to surviving extreme nutrient deprivation. We have already observed that these species have very low intrinsic growth rates (Tomlinson et al., 2014, 2012), which probably explains their low level of suppression. This combined evidence suggests that using actual soil fertility as a predictor in a regression model could allow us to test this more directly, but our species sample from the humid and semi-arid savannas of South America came from two locations (Brasilia DF and Patos City in Pernambuco), so our dataset is not suitable for ordinal regression. It would be worthwhile to sample species across soil gradients on a single continent to test this trend more systematically.

Unexpectedly, we observed differences across climate groups with respect to performance under grass competition that differed by continent (Table 1, Figure 1). African species from semi-arid savannas were more severely suppressed by grass treatments than species from humid savannas, and South American species from semi-arid savannas performed more poorly under grass in combination with nutrient limitation. By contrast, in Australia, species from semi-arid savannas performed better under grass competition than species from humid areas (both Climate × Grass and Climate × Grass × Nutrient interactions were significant and had positive coefficients). These patterns may be explained by the leaf habit analysis, which showed clear and highly significant patterns (Table 2). Deciduous species from semi-arid environments performed better under high nutrients and no grass competition relative to deciduous species from humid environments, but the former group were more negatively impacted by grass competition than species from humid environments. Evergreen species showed the opposite trend. This is consistent with what we observed across continents because Africa and South America are dominated by deciduous species whereas Australia is dominated by evergreen species. In the same sense, the leaf habit analysis is affected by this bias, and it would certainly be worth examining a greater sample of evergreen species taken from all continents. We think the observed patterns are at least partly an effect of different growth rates, much along the lines observed for the within-climate group analyses associated with Prediction 2 (Figure 2). Within our sample of species, relative growth rates of deciduous species from humid environments are on average lower than deciduous species from semi-arid environments, whereas relative growth rates of evergreen species from humid environments are on average greater than evergreen species from semi-arid environments (Tomlinson et al., 2014). It may be that faster growing species are more negatively impacted by grass competition in terms of their absolute growth.

4.2 | Tree species within communities perform differently under grass competition

We found that, for species from the same climate groups on each continent, grass and low nutrient supply both suppressed growth of faster growing species more than slow-growing species, to the extent that fast-growing species accumulated similar amounts of biomass to slow-growing species under combined grass competition and nutrient suppression (Figure 2). This could allow the less impacted slower growing species to perform better under grass competition in the

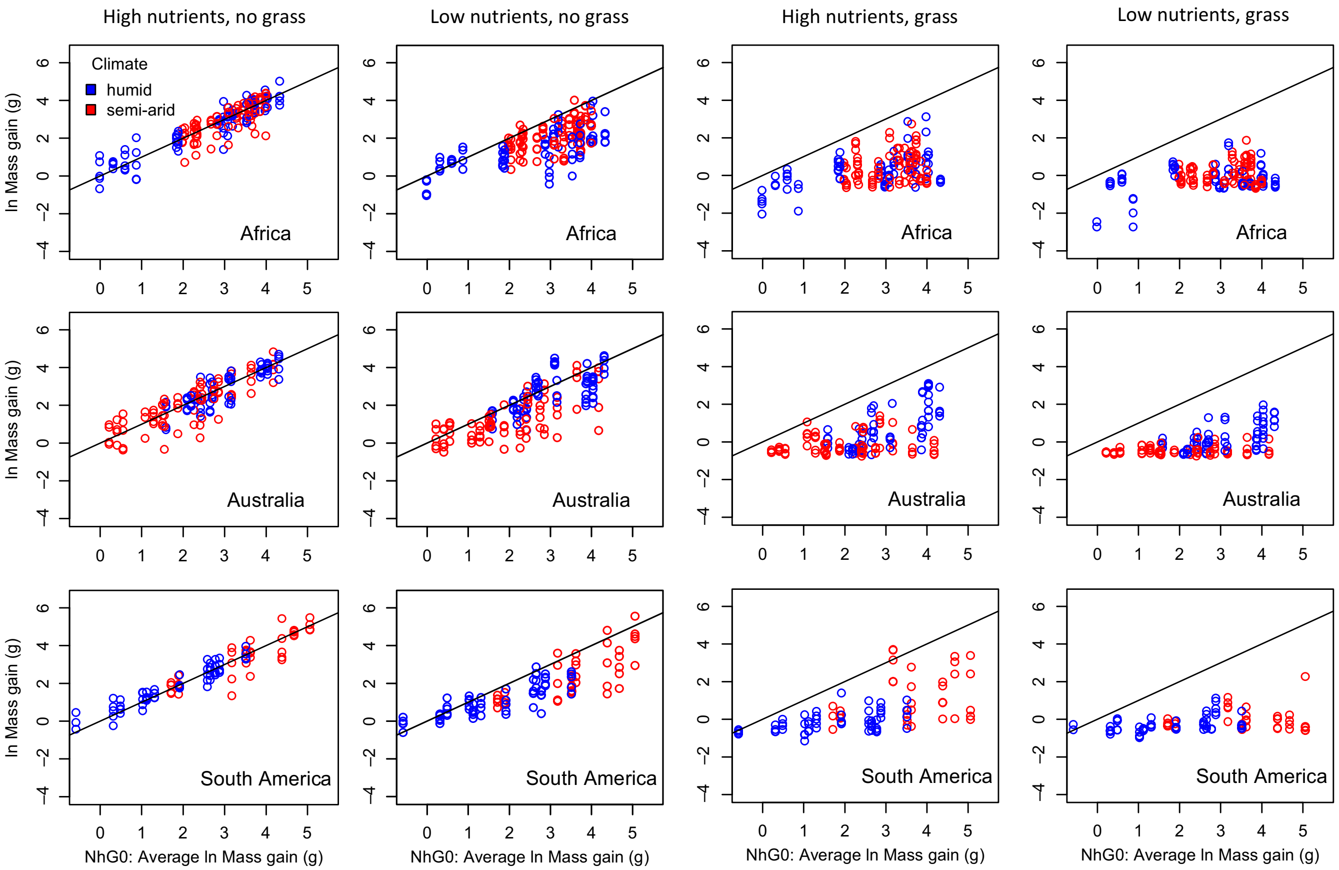

FIGURE 3 Individual mass gain under nutrient and grass suppression treatments (*y*-axis) plotted against mean species mass gain under high nutrient supply and no grass competition (N_hG_0) (*x*-axis) in the same experimental batch, plotted separately for each continent. Each column represents a different treatment combination: high nutrient supply without grass (N_hG_0), low nutrients without grass (N_lG_0), high nutrients with grass (N_hG_1), and low nutrients with grass (N_lG_1). Each row represents a different continent (from top to bottom: Africa, Australia, South America). Species from semi-arid environments (red symbols) and species from humid environments (blue symbols) are indicated in each panel. In each panel, each column of values represents the individuals of a particular species grown in a particular batch [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Effect of species-averaged plant traits (T) on tree seedling mass gain (g) under the Nutrient (N) and Grass (G) treatments. Results are for species from each continent separately

	Africa	Australia	South America
Sample size, *N*	645	447	354
Species number	18	12	10
Y-transformation	ln(y + 0.5)	ln(y + 0.5)	ln(y + 0.5)

	R^2m[a]	R^2c	G	N	G × N	T	G × T	N × T	G × N×T	R^2m	R^2c	G	N	G × N	T	G × T	N × T	G × N×T	R^2m	R^2c	G	N	G × N	T	G × T	N × T	G × N×T
Light capture																											
SLA	0.57	0.79	−	−	+	+	−	−		0.44	0.81	−	−		−	+			0.49	0.79	−	−		−	+		
A_m[b]	0.57	0.78	−	−	+					0.45	0.81	−	−						0.42	0.76	−	−					
N_m	0.57	0.78	−	−	+					0.44	0.81	−	−		−	+			0.42	0.76	−	−					
SER	0.66	0.81	−	−	+	+	−	−		0.53	0.81	−	−		+	−	−		0.67	0.81	−	−	+	+	−	−	−
Soil resource capture																											
RER	0.64	0.78	−	−	+	+		+		0.45	0.81	−	−						0.72	0.79	−	−	+	+	−	−	−
STRL[b]	0.59	0.79	−	−	+	+	−	−	+	0.58	0.81	−	−		−	+			0.42	0.76	−	−					
RMF	0.58	0.80	−	−	+	−	+	+		0.45	0.81	−	−						0.44	0.79	−	−		−	+	+	
Soil resource use																											
g_s[b]	0.57	0.78	−	−	+					0.45	0.81	−	−						0.42	0.79	+	+	−	+	−	−	+
PWUE[b]	0.58	0.79	−	−	+	−	+	+		0.45	0.81	−	−						0.42	0.78	−	−	+	−	+	+	−
P_m	0.57	0.78	−	−	+					0.58	0.82	−	−		−	+			0.43	0.77	−	+		+		−	
K_m	0.57	0.78	−	−	+					0.47	0.82	−	−		+	−			0.42	0.78	−	−		−	+		
Initial resource supply																											
Mass_0[b]	0.59	0.80	−	−	+	−	+	+		0.45	0.81	−	−						0.47	0.79	−	−		−	+	+	

Note. The table reports explained variance and records coefficient signs of significant model terms (based on likelihood ratio tests), which indicate whether the trait was beneficial (+) or negative for plant growth. The base model describing treatment effects on seedling growth is greyed out. Traits are grouped according to their possible effects on plant performance under different environmental constraints. Light capture traits are specific leaf area (SLA, $cm^2\ g^{-1}$), leaf assimilation rate (A_m, nmol $CO_2\ g^{-1}\ s^{-1}$), leaf nitrogen content (N_m, mg N g^{-1}), and stem extension rate (SER, mm d^{-1}). Soil resource capture traits are root extension rate (RER, mm d^{-1}), specific taproot length (STRL, m g^{-1}), and root mass fraction (RMF, g root mass g^{-1} total mass). Efficient resource-use traits are stomatal conductance (g_s, μmol $H_2O\ m^{-2}\ s^{-1}$), plant photosynthetic water use efficiency (PWUE, μmol CO_2 μmol^{-1} H_2O), leaf phosphorus and potassium content (P_m, mg P g^{-1}; K_m, mg K g^{-1}). Initial mass of recently germinated seedlings (Mass_0, g) is in a separate group, representing initial resource supply given to a seedling by its mother plant. Estimated species' mean trait values are provided in Supporting information Table S3.

[a] Marginal and conditional R^2 values are calculated using the marginal and conditional R^2-estimators of Nakagawa and Schielzeth (2013). [b] Variable natural log-transformed prior to analysis.

long run. However, a more severe experiment running for multiple seasons would be required to demonstrate that the growth advantage under grass shifts towards slower, more conservative species.

The observed patterns suggest that there are tree species that have been selected for growing in competition with grasses and others that perform best in the absence of grass. Hence, the pattern of tree species diversity in savannas may depend on biomass dynamics of the grass layer. Severe disturbance of the grass layer, by grazing or drought, will favour tree species with fast growth and greater aboveground allocation. Tall grass without disturbance will favour woody species with greater allocation to roots and characteristics that favour water capture and retention (Staver, Bond, Cramer, & Wakeling, 2012). Determining which of these mechanisms is at play will require careful monitoring of seedling recruitment over time in semi-arid and humid savannas in tall and short grass patches. Furthermore, changes in the amount of rainfall or the frequency of drought events could lead to differences in recruitment opportunities for different tree species and change the trees communities in savannas.

4.3 | Traits related to seedling performance under grass competition and under nutrient limitation

Some traits explained significant amounts of variation in seedling growth under the grass and nutrient treatments (Table 2). Notably, traits associated with improved/depressed performance under grass competition were mostly the same as those important for performance under nutrient limitation alone, at least among African and South American species. Furthermore, the traits that improved performance of Australian species under grass competition were mostly different from those that improved performance under grass competition among African and South American species. This was matched by differences in driving traits for evergreen species (dominant in Australia) versus deciduous species (dominant in Africa and South America).

4.3.1 | Traits related to rapid growth under high resource conditions

Across all continents, SER was positively related with growth rate in the high nutrient, no grass treatment (N_hG_0) and negatively related to performance under low nutrient supply and under grass competition. In savannas, SER is important for coping with disturbance: in fire-prone humid savannas, tree species need to raise their apical meristems above the zone where fires can damage them (the "fire trap") (Higgins et al., 2000). Stem extension rate may be equally important in semi-arid systems where herbivory by terrestrial mammals is the dominant form of disturbance to plants (Hempson et al., 2015; Olff, Ritchie, & Prins, 2002) and can be overcome when trees reach sufficient height. Our observation that SER is negatively related to performance under low nutrients and grass competition suggests that savanna tree seedlings may be subjected to a direct trade-off between ability to compete for resources and ability to overcome dominant disturbances.

Notably, SLA and leaf nitrogen content (N_m) did not have positive effects on growth under high resource supply. This contradicts plant trait orthodoxy on fast versus conservative strategies (Ordoñez et al., 2009; Wright et al., 2004). Evans' relative growth rate (RGR) formulation (Evans, 1972) (RGR = ULR × LMF) indicates that intrinsic growth rate depends on high leaf productivity (unit leaf rate, ULR) and on high proportional biomass allocation to leaves (leaf mass fraction of total biomass, LMF). Our own observations on the drivers of growth rate among these savanna species (Tomlinson et al., 2014) indicate that species achieve high growth either through high leaf productivity or high leaf allocation, not both. This may explain why SLA, A_m, and N_m were not related to performance under the N_hG_0 treatment.

Root extension rate (RER) was positively related with growth under the N_hG_0 treatment among African and South American species and very weakly among Australian species, and was unrelated or negatively related to performance under grass competition and nutrient suppression. This suggests that RER may form part of the fast growth strategy, at least among savanna species. RER describes the speed at which a seedling taproot descends through the soil, presumably towards deeper, more reliable water (Tomlinson et al., 2012). Possibly this strategy is compromised by competition from grasses, which abstract water very rapidly, and high RER is not beneficial in infertile soils, where deep penetration may not increase access to rare nutrients.

4.3.2 | Traits related to soil resource capture

Two soil resource capture traits supported greater growth in the presence of grass competition and under nutrient limitation, root mass fraction (RMF) and specific taproot length (STRL). Their importance differed by continent, with RMF being important in Africa and South America whereas STRL supported growth under grass competition in Australia. This was also matched by patterns among leaf habit groups, where STRL was positively related to growth under grass for evergreen species whereas RMF was positively related to growth under grass for deciduous species. We have previously observed that deciduous species from the most nutrient-poor soils in our sample tend to produce short taproots with substantial non-structural carbohydrate (NSC) storage (Tomlinson, Langevelde, et al., 2013). Thus, they do not appear to search the soil very extensively using their own roots. However, their high allocation to root NSC may mean that they are supporting mycorrhizal fungi that do search the soil extensively (Bâ, Garbaye, Martin, & Dexheimer, 1994; Nylund, 1988; Orians & Milewski, 2007). In this, they may differ fundamentally from evergreen species that have much thinner roots, which may allow them to search the soil volume directly. It has been shown experimentally that plants grown under water stress achieve greater biomass when inoculated with mycorrhizal fungi than without (Mirshad & Puthur, 2016; Wu, Zou, & Xia, 2006). Therefore, a future avenue of exploration would be to consider how tree seedlings of different leaf habit perform under grass with and without microbial associates.

TABLE 4 Effect of species-averaged plant traits (T) on tree seedling mass gain (g) under the Nutrient (N) and Grass (G) treatments

	Evergreen									**Deciduous**								
Sample size, *N*	387									1,059								
Species number	11									29								
Y-transformation	ln(y + 0.5)									ln(y + 0.5)								
	R^2m[a]	R^2c	G	N	G × N	T	G × T	N × T	G × N×T	R^2m	R^2c	G	N	G × N	T	G × T	N × T	G × N×T
Light capture																		
SLA	0.50	0.79	–	–		–	+	–		0.49	0.77	–	–	+				
A_m[b]	0.48	0.79	–	–		+	–			0.49	0.77	–	–	+				
N_m	0.59	0.79	–	–		–	+			0.50	0.77	–	–	+				
SER	0.54	0.79	–	–		+	–	–		0.66	0.80	–	–	+	+	–	–	
Soil resource capture																		
RER	0.52	0.79	–	–	+	–	+	+	–	0.62	0.77	–	–	+	+	–		
STRL[b]	0.54	0.79	–	+		–	+	+		0.50	0.77	–	–	+	+	–		
RMF	0.51	0.79	–	–		+	–	+		0.51	0.79	–	–	+	–	+	+	
Soil resource use																		
g_s[b]	0.50	0.79	+	–		+	–			0.50	0.78	–	+	–	–	–	–	+
PWUE[b]	0.48	0.78	–	–						0.51	0.78	–	–	+	+	+	+	–
P_m	0.63	0.80	–	–		–	+			0.52	0.77	–	–	+	+			
K_m	0.54	0.79	–	–		–	+			0.50	0.77	–	–	+				
Initial resource supply																		
$Mass_0$[b]	0.52	0.79	–	–		–	+	+		0.54	0.78	–	–	+	–	+	+	

Note. Results are for species from each leaf habit separately. The table reports explained variance and records coefficient signs of significant model terms (based on likelihood ratio tests), which indicate whether the trait was beneficial (+) or negative for plant growth. The base model describing treatment effects on seedling growth is greyed out. Traits are grouped according to their possible effects on plant performance under different environmental constraints. Light capture traits are specific leaf area (SLA, $cm^2 g^{-1}$), leaf assimilation rate (A_m, nmol CO_2 $g^{-1} s^{-1}$), leaf nitrogen content (N_m, mg N g^{-1}), and stem extension rate (SER, mm d^{-1}). Soil resource capture traits are root extension rate (RER, mm d^{-1}), specific taproot length (STRL, m g^{-1}), and root mass fraction (RMF, g root mass g^{-1} total mass). Efficient resource-use traits are stomatal conductance (g_s, μmol H_2O $m^{-2} s^{-1}$), plant photosynthetic water use efficiency (PWUE, μmol CO_2 μmol^{-1} H_2O), leaf phosphorus and potassium content (P_m, mg P g^{-1}; K_m, mg K g^{-1}). Initial mass of recently germinated seedlings ($Mass_0$, g) is in a separate group, representing initial resource supply given to a seedling by its mother plant. Estimated species' mean trait values are provided in Supporting information Table S3.

[a] Marginal and conditional R^2 values are calculated using the marginal and conditional R^2-estimators of Nakagawa and Schielzeth (2013). [b] Variable natural log-transformed prior to analysis.

4.3.3 | Traits related to soil resource utilization

Traits that improve resource use efficiency differed by continent and leaf habit. Plant photosynthetic water use efficiency (PWUE) had a positive effect on growth under grass competition among deciduous species whereas leaf phosphorus and potassium contents had positive effects on growth under grass among evergreen species. This difference is somewhat surprising as we expected that PWUE would be dependent on rapid stomatal control, itself dependent on active control by leaf potassium and phosphorus (Benlloch-González, Arquero, Fournier, Barranco, & Benlloch, 2008). That these patterns are uncoupled suggests that evergreen and deciduous species are controlling water loss using different mechanisms. Although substantial research has focussed on understanding the signalling controlling guard cell aperture (e.g., Araújo, Fernie, & Adriano Nunes-Nesi, 2011; Haworth, Elliott-Kingston, & McElwain, 2011), we could not find literature that specifically addresses whether leaf habit might alter leaf water relations (but see Niinemets, Flexas, & Peñuelas, 2011). The interactions of environmental resources and leaf habit in controlling water relations and explaining these mechanistically deserves further research.

4.3.4 | Seed mass

We found that initial mass ($Mass_0$), which directly reflects seed mass, did support improved growth under both low nutrient and grass competition conditions among African and South American species, but not Australian species. This supports the proposal that large seed size improves the ability of seedlings to establish in environments with scarce resources (Leishman et al., 2000). Both African and South American species selected here have much larger ranges and absolute values of initial mass than the Australian species (Supporting information Table S3). The implication is that seeds of most Australian species are too small to supply large amounts of resources for establishment. This may reflect other environmental constraints on seed size for Australian species, preventing seed mass allocation from being a strategy for coping with low-resource supplies.

5 | CONCLUDING REMARKS

Here we present the first study across countries on three continents to show that: (a) there is only partial evidence that species of humid savannas are more efficient at growing under nutrient limitation than species from semi-arid savannas; (b) tree species from humid savannas appear to be more efficient at growing under grass competition than species from semi-arid environments; and (c) grass competition may contribute to advantages for seedlings of different tree species within landscapes, as grass competition suppresses the biomass accumulation of fast-growing species much more severely than slow-growing species. Differences in tree species performance with and without grass competition suggest that savanna communities may develop different trajectories based on disturbance to the grass layer, with fast-growing species advantaged in short grass patches and slow-growing species advantaged in tall grass patches. We have shown that (d) different suites of traits relate to the performance rankings across species in non-competitive conditions with higher resource availability versus competition with grass and under low nutrient supply; and (e) the traits that are associated with growth performance under grass competition and nutrient limitation differ between Africa and South America on the one hand and Australia on the other hand. Our analysis of the data on the basis of leaf habit groups suggests that these continental patterns may be caused by developmental constraints associated with the deciduous versus evergreen leaf habits that dominate on the different continents, and also with constraints on seed size for Australian species.

ACKNOWLEDGEMENTS

We thank the staff of UNIFARM and the Resource Ecology Group, both of Wageningen University for technical support to the experiment, especially Jia Wu, Ainara Ballesteros-Larranaga, André Maassen, Herman van Oeveren, and Ramin Rakhshandehroo. Everardo Sampaio, Giovanna Carramaschi, Tony Grice, Xavier Poshiwa, Patricia Tomlinson, and Peggy Wiseman provided seeds for the experiment. We thank Richard Corlett for thoroughly checking the English text. The critical and helpful comments of the reviewers are gratefully acknowledged. This project and K.W.T. were funded by a research grant provided by Shell Research Foundation.

AUTHOR'S CONTRIBUTIONS

K.W.T., F.v.L., S.d.B., and H.H.T.P. conceived the project and the project design. K.W.T., F.v.L., F.J.S., and E.R.M.B. collected the data. K.W.T. analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.745 g830 (Tomlinson et al., 2018).

ORCID

Kyle W. Tomlinson http://orcid.org/0000-0003-3039-6766

Frank van Langevelde http://orcid.org/0000-0001-8870-0797

REFERENCES

Araújo, W. L., Fernie, A. R., & Adriano Nunes-Nesi, A. (2011). Control of stomatal aperture: A renaissance of the old guard. *Plant Signalling & Behaviour*, *6*, 1305–1311. https://doi.org/10.4161/psb.6.9.16425

Archibald, S., Bond, W. J., Stock, W. D., & Fairbanks, D. H. K. (2005). Shaping the landscape: Fire-grazer interactions in an African savanna. *Ecological Applications*, *15*, 96–109. https://doi.org/10.1890/03-5210

Bâ, A. M., Garbaye, J., Martin, F., & Dexheimer, J. (1994). Root soluble carbohydrates of *Afzelia africana* Sm. seedlings and modifications of mycorrhiza establishment in response to the excision of cotyledons. *Mycorrhiza*, *4*, 269–275. https://doi.org/10.1007/BF00206776

Barbosa, E. R. M., van Langevelde, F., Tomlinson, K. W., Carvalheiro, L. G., Kirkman, K., de Bie, S., & Prins, H. H. T. (2014). Tree species from different functional groups respond differently to environmental changes during establishment. *Oecologia*, *174*, 1345–1357. https://doi.org/10.1007/s00442-013-2853-y

Bartoń, K. (2016). *MuMIn: Multi-Model Inference. R package version 1.15.6*. Retrieved from https://CRAN.R-project.org/package=MuMIn.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Benlloch-González, M., Arquero, O., Fournier, J. M., Barranco, D., & Benlloch, M. (2008). K(+) starvation inhibits water-stress-induced stomatal closure. *Journal of Plant Physiology*, *165*, 623–630. https://doi.org/10.1016/j.jplph.2007.05.010

Bond, W. J. (2008). What limits trees in C 4 grasslands and savannas? *Annual Review of Ecology, Evolution, and Systematics*, *39*, 641–659. https://doi.org/10.1146/annurev.ecolsys.39.110707.173411

Bond, W. J., Woodward, F. I., & Midgley, G. F. (2005). The global distribution of ecosystems in a world without fire. *The New Phytologist*, *165*, 525–537. https://doi.org/10.1111/j.1469-8137.2004.01252.x

Bowman, D. M. J. S., & Prior, L. D. (2005). Why do evergreen trees dominate the Australian seasonal tropics? *Australian Journal of Botany*, *53*, 379–399. https://doi.org/10.1071/BT05022

Cole, M. M. (1986). *The savannas: biogeography and geobotany*. London, UK: Academic Press.

Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., … Poorter, H. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, *51*, 335. https://doi.org/10.1071/BT02124

Cramer, M. D., Chimpango, S. B. M., Van Cauter, A., Waldram, M. S., & Bond, W. J. (2007). Grass competition induces N2 fixation in some species of African Acacia. *Journal of Ecology*, *95*, 1123–1133.

Cramer, M. D., Wakeling, J. L., & Bond, W. J. (2012). Belowground competitive suppression of seedling growth by grass in an African savanna. *Plant Ecology*, *213*, 1655–1666. https://doi.org/10.1007/s11258-012-0120-7

Csurhes, S., Leigh, C., & Walton, C. (2016). African lovegrass Eragrostis curvula. In *Invasive plant risk assessment*. Brisbane, Australia: Department of Agriculture and Fisheries.

Diaz, S., & Cabido, M. (1997). Plant functional types and ecosystem function in relation to global change. *Journal of Vegetation Science*, *8*, 463–474. https://doi.org/10.1111/j.1654-1103.1997.tb00842.x

Evans, C. G. (1972). *The quantitative analysis of plant growth*. Oxford, UK: Blackwell Scientific.

February, E. C., & Higgins, S. I. (2010). The distribution of tree and grass roots in savannas in relation to soil nitrogen and water. *South African Journal of Botany*, *76*, 517–523. https://doi.org/10.1016/j.sajb.2010.04.001

February, E. C., Higgins, S. I., Bond, W. J., & Swemmer, L. (2013). Influence of competition and rainfall manipulation on the growth responses of savanna trees and grasses. *Ecology*, *94*, 1155–1164. https://doi.org/10.1890/12-0540.1

Fish, L., Mashau, A. C., Moeaha, M. J., & Nembudani, M. T. (2015). Identification guide to southern African grasses. In *An identification manual with keys, descriptions and distributions. Strelitzia 36*. Pretoria, South Africa: South African National Biodiversity Institute.

Goldberg, D. E., & Landa, K. (1991). Competitive effect and response: hierarchies and correlated traits in the early stages of competition. *Journal of Ecology*, *79*, 1013–1030.

Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist*, *111*, 1169–1194. https://doi.org/10.1086/283244

Grubb, P. J. (1977). The maintenance of species-richness in plant communities: The importance of the regeneration niche. *Biological Reviews*, *52*, 107–145. https://doi.org/10.1111/j.1469-185X.1977.tb01347.x

Haworth, M., Elliott-Kingston, C., & McElwain, J. C. (2011). Stomatal control as a driver of plant evolution. *Journal of Experimental Botany*, *62*, 2419–2423. https://doi.org/10.1093/jxb/err086

Hempson, G. P., Archibald, S., & Bond, W. J. (2015). A continent-wide assessment of the form and intensity of large mammal herbivory in Africa. *Science*, *350*, 1056–1061. https://doi.org/10.1126/science.aac7978

Higgins, S. I., Bond, W. J., & Trollope, W. S. W. (2000). Fire, resprouting and variability: A recipe for grass-tree coexistence in savanna. *Journal of Ecology*, *88*, 213–229. https://doi.org/10.1046/j.1365-2745.2000.00435.x

Holmgren, M., Hirota, M., van Nes, E. H., & Scheffer, M. (2013). Effects of interannual climate variability on tropical tree cover. *Nature Climate Change*, *3*, 755–758. https://doi.org/10.1038/nclimate1906

Hothorn, T., Bretz, F. & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*, 346–363.

Huntley, B. J., & Walker, B. H. (1982). *Ecology of tropical savannas*. Berlin, Germany: Springer-Verlag.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. (2006). World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, *15*, 259–263. https://doi.org/10.1127/0941-2948/2006/0130

Kulmatiski, A., Beard, K. H., Verweij, R. J. T., & February, E. C. (2010). A depth-controlled tracer technique measures vertical, horizontal and temporal patterns of water use by trees and grasses in a subtropical savanna. *New Phytologist*, *188*, 199–209. https://doi.org/10.1111/j.1469-8137.2010.03338.x

Lehmann, C. E. R., Anderson, T. M., Sankaran, M., Higgins, S. I., Archibald, S., Hoffmann, W. A., … Bond, W. J. (2014). Savanna vegetation-fire-climate relationships differ among continents. *Science*, *343*, 548–552. https://doi.org/10.1126/science.1247355

Leishman, M. R., Wright, I. J., Moles, A. T., & Westoby, M. (2000). The evolutionary ecology of seed size. In M. Fenner (Ed.), *Seeds: The ecology of regeneration in plant communities*, 2nd ed. (pp. 31–58). Wallingford, UK: CABI International.

Lloyd, J., Bird, M. I., Vellen, L., Miranda, A. C., Veenendaal, E. M., Djagbletey, G., … Farquhar, G. D. (2008). Contributions of woody and herbaceous vegetation to tropical savanna ecosystem productivity: A quasi-global estimate. *Tree Physiology*, *28*, 451–468. https://doi.org/10.1093/treephys/28.3.451

Matzek, V. (2012). Trait values, not trait plasticity, best explain invasive species' performance in a changing environment. *PLoS ONE*, *7*, e48821. https://doi.org/10.1371/journal.pone.0048821

McConnaughay, K. D. M., & Coleman, J. S. (1999). Biomass allocation in plants: Ontogeny or optimality? A test along three resource gradients. *Ecology*, *80*, 2581–2593. https://doi.org/10.1890/0012-9658(1999)080[2581:BAIPOO]2.0.CO;2

Mills, A. J., Rogers, K. H., Stalmans, M., & Witkowski, E. T. F. (2006). A framework for exploring the determinants of savanna and grassland distribution. *BioScience*, *56*, 579. https://doi.org/10.1641/0006-3568(2006) 56[579:AFFETD]2.0.CO;2

Mirshad, P. P., & Puthur, J. T. (2016). Drought tolerance of bioenergy grass *Saccharum spontaneum* L. enhanced by arbuscular mycorrhizae. *Rhizosphere*, *3*, 1–8. https://doi.org/10.1016/j.rhisph.2016.09.004

Murphy, B. P., & Bowman, D. M. J. S. (2012). What controls the distribution of tropical forest and savanna? *Ecology Letters*, *15*, 748–758. https://doi.org/10.1111/j.1461-0248.2012.01771.x

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*, 133–142. https://doi.org/:10.1111/j.2041-210x.2012.00261.x

Niinemets, U., Flexas, J., & Peñuelas, J. (2011). Evergreens favoured by higher responsivenesss to increased CO2. *Trends in Ecology & Evolution*, *26*, 136–142. https://doi.org/10.1016/j.tree.2010.12.012

Nylund, J. E. (1988). The regulation of mycorrhiza formation—carbohydrate and hormone theories reviewed. *Scandinavian Journal of Forest Research*, *3*, 465–479. https://doi.org/10.1080/02827588809382532

O'Connor, T. G. (1995). *Acacia karroo* invasion of grassland: Environmental and biotic effects influencing emergence and establishment. *Oecologia*, *103*, 214–223.

O'Halloran, L. R., Shugart, H. H., Wang, L., Caylor, K. K., Ringrose, S., & Kgope, B. (2010). Nutrient limitations on aboveground grass production in four savanna types along the Kalahari Transect. *Journal of Arid Environments*, *74*, 284–290. https://doi.org/10.1016/j.jaridenv.2009.08.012

Olff, H., Ritchie, M. E., & Prins, H. H. T. (2002). Global environmental controls of diversity in large herbivores. *Nature*, *415*, 901–904. https://doi.org/10.1038/415901a

Ordoñez, J. C., van Bodegom, P. M., Witte, J.-P.-M., Wright, I. J., Reich, P. B., & Aerts, R. (2009). A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography*, *18*, 137–149. https://doi.org/10.1111/j.1466-8238.2008.00441.x

Orians, G. H., & Milewski, A. V. (2007). Ecology of Australia: The effects of nutrient-poor soils and intense fires. *Biological Reviews of the Cambridge Philosophical Society*, *82*, 393–423. https://doi.org/10.1111/j.1469-185X.2007.00017.x

Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N., & Pearse, W. (2013). *CAPER: Comparative analyses of phylogenetics and evolution in* R. R package version 0.5.2. Retrieved from https://CRAN.R-project.org/package=caper.

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*, 289–290. https://doi.org/10.1093/bioinformatics/btg412

Poorter, H., & Van der Werf, A. (1998). Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance? A review of herbaceous species. In H. Lambers, H. Poorter, & M. M. I. Van Vuuren (Eds.), *Inherent variation in plant growth* (pp. 309–336). Leiden, The Netherlands: Backhuys Publishers.

Riginos, C. (2009). Grass competition suppresses savanna tree growth across multiple demographic stages. *Ecology*, *90*, 335–340. https://doi.org/10.1890/08-0462.1

Sankaran, M., Hanan, N. P., Scholes, R. J., Ratnam, J., Augustine, D. J., Cade, B. S., … Zambatis, N. (2005). Determinants of woody cover in African savannas. *Nature*, *438*, 846–849. https://doi.org/10.1038/nature04070

Staver, A. C., Bond, W. J., Cramer, M. D., & Wakeling, J. L. (2012). Top-down determinants of niche structure and adaptation among African Acacias. *Ecology Letters*, *15*, 673–679. https://doi.org/10.1111/j.1461-0248.2012.01784.x

Suding, K. N., Lavorel, S., Chapin, F. S., Cornelissen, J. H. C., Díaz, S., Garnier, E., … Navas, M.-L. (2008). Scaling environmental change through the community-level: A trait-based response-and-effect framework for plants. *Global Change Biology*, *14*, 1125–1140. https://doi.org/10.1111/j.1365-2486.2008.01557.x

Tomlinson, K. W., Poorter, L., Bongers, F., Borghetti, F., Jacobs, L., & Van Langevelde, F. (2014). Relative growth rate variation of evergreen and deciduous savanna tree species is driven by different traits. *Annals of Botany*, *114*, 315–324. https://doi.org/10.1093/aob/mcu107.

Tomlinson, K. W., Poorter, L., Sterck, F. J., Borghetti, F., Ward, D., de Bie, S., & van Langevelde, F. (2013). Leaf adaptations of evergreen and deciduous trees of semi-arid and humid savannas on three continents. *Journal of Ecology*, *101*, 430–440. https://doi.org/10.1111/1365-2745.12056

Tomlinson, K. W., Sterck, F. J., Barbosa, E. R. M., de Bie, S., Prins, H. H. T., & van Langevelde, F. (2018). Data from: Seedling growth of savanna tree species from three continents under grass competition and nutrient limitation in a greenhouse experiment. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.745g830

Tomlinson, K. W., Sterck, F. J., Bongers, F., da Silva, D. A., Barbosa, E. R. M., Ward, D., … van Langevelde, F. (2012). Biomass partitioning and root morphology of savanna trees across a water gradient. *Journal of Ecology*, *100*, 1113–1121. https://doi.org/10.1111/j.1365-2745.2012.01975.x

Tomlinson, K. W., van Langevelde, F., Ward, D., Bongers, F., da Silva, D. A., Prins, H. H. T., … Sterck, F. J. (2013). Deciduous and evergreen trees differ in juvenile biomass allometries because of differences in allocation to root storage. *Annals of Botany*, *112*, 575–587. https://doi.org/10.1093/aob/mct132

Vadigi, S., & Ward, D. (2013). Shade, nutrients, and grass competition are important for tree sapling establishment in a humid savanna. *Ecosphere*, *4*, 1–27. https://doi.org/10.1890/ES13-00239.1

Van Auken, O. W. (2000). Shrub invasions of North American semiarid grasslands. *Annual Review of Ecology and Systematics*, *31*, 197–215. https://doi.org/10.1146/annurev.ecolsys.31.1.197

van der Waal, C., de Kroon, H., de Boer, W. F., Heitkönig, I. M. A., Skidmore, A. K., de Knegt, H. J., … Prins, H. H. T. (2009). Water and nutrients alter herbaceous competitive effects on tree seedlings in a semi-arid savanna. *Journal of Ecology*, *97*, 430–439. https://doi.org/10.1111/j.1365-2745.2009.01498.x.

van der Waal, C., de Kroon, H., de Boer, W. F., Heitkönig, I. M. A., Skidmore, A. K., van Langevelde, F., … Prins, H. H. T. (2011). Scale of nutrient patchiness mediates resource partitioning between trees and grasses in a semi-arid savanna. *Journal of Ecology*, *99*, 1124–1133. https://doi.org/10.1111/j.1365-2745.2011.01832.x

Varma, V., Catherin, A. M., & Sankaran, M. (2018) Effects of increased N and P availability on biomass allocation and root carbohydrate reserves differ between N-fixing and non-N-fixing savanna tree seedlings. *Ecology and Evolution*, *8*, 8467– 8476 https://doi.org/10.1002/ece3.4289

Venter, F. J., Scholes, R. J., & Eckhardt, H. C. (2003). In J. du Toit, K. Rogers, & H.Biggs (Eds.), *The abiotic template and its associated vegetation pattern. The Kruger Experience* (pp. 83–129). Washington, DC: Island Press.

Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., & Wright, I. J. (2002). Plant ecological strategies: Some leading dimensions of variation between species. *Annual Review of Ecology and Systematics*, *33*, 125–159. https://doi.org/10.1146/annurev.ecolsys.33.010802.150452

Wickham, H. (2009). *ggplot2: Elegant graphics for data analysis*. New York, NY: Springer-Verlag.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., … Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, *428*, 821–827. https://doi.org/10.1038/nature02403

Wu, Q. S., Zou, Y. N., & Xia, R. X. (2006). Effects of water stress and arbuscular mycorrhizal fungi on reactive oxygen metabolism and antioxidant production by citrus (*Citrus tangerine*) roots. *European Journal of Soil Biology*, *42*, 166–172. https://doi.org/10.1016/j.ejsobi.2005.12.006

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Tomlinson KW, Sterck FJ, Barbosa ERM, de Bie S, Prins HHT, van Langevelde F. Seedling growth of savanna tree species from three continents under grass competition and nutrient limitation in a greenhouse experiment. *J Ecol*. 2019;107:1051–1066. https://doi.org/10.1111/1365-2745.13085