African savanna grasses outperform trees across the full spectrum of soil moisture availability

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Summary

- Models of tree–grass coexistence in savannas make different assumptions about the relative performance of trees and grasses under wet vs dry conditions. We quantified transpiration and drought tolerance traits in 26 tree and 19 grass species from the African savanna biome across a gradient of soil water potentials to test for a trade-off between water use under wet conditions and drought tolerance.
- We measured whole-plant hourly transpiration in a growth chamber and quantified drought tolerance using leaf osmotic potential (Ψ_{osm}). We also quantified whole-plant water-use efficiency (WUE) and relative growth rate (RGR) under well-watered conditions.
- Grasses transpired twice as much as trees on a leaf-mass basis across all soil water potentials. Grasses also had a lower Ψ_{osm} than trees, indicating higher drought tolerance in the former. Higher grass transpiration and WUE combined to largely explain the threefold RGR advantage in grasses.
- Our results suggest that grasses outperform trees under a wide range of conditions, and that there is no evidence for a trade-off in water-use patterns in wet vs dry soils. This work will help inform mechanistic models of water use in savanna ecosystems, providing much-needed whole-plant parameter estimates for African species.

Introduction

Explaining the coexistence and relative abundance of trees and grasses in grassy biomes poses an ongoing ecological challenge (Bond, 2008). These two plant functional types (C_3 trees and mostly C_4 grasses) exhibit broad differences in structure and function (Scholes & Archer, 1997), so it can be difficult to identify the specific traits that matter most for determining tree : grass biomass ratios under a given set of environmental conditions. It is well established that the upper limit of tree cover is constrained by mean annual precipitation (Sankaran *et al*., 2004; Staver *et al*., 2011), suggesting that water availability is a fundamental factor governing tree–grass interactions. Given this, much of the literature on tree–grass coexistence theory has focused on differences in ecohydrological traits between trees and grasses (Walker *et al*., 1981; Rodríguez-Iturbe *et al*., 1999; van Wijk & Rodriguez-Iturbe, 2002; Xu *et al*., 2015). In particular, a number of coexistence models make specific assumptions about the relative performance (in terms of water use, growth, recruitment, etc.) of each functional type under wet vs dry conditions (Scholes & Walker, 1993; van Wijk & Rodriguez-Iturbe, 2002; Xu *et al*., 2015), but many of these assumptions have yet to be rigorously tested. Moving the field forward requires new datasets that compare tree and grass performance across gradients of soil moisture availability.

An early model of tree–grass coexistence was the two-layer model, which assumes that grasses are superior competitors for water in topsoil layers, while trees escape competitive exclusion through access to subsoil moisture (Walter, 1971; Walker & Noy-Meir, 1982). This model does not require a trade-off between performance and soil moisture availability, although it categorizes trees as drought avoiders (Pivovaroff *et al*., 2016). Several subsequent models de-emphasized the role of niche partitioning along a rooting depth axis and focused on other trait differences. The temporal niche partitioning model (Scholes & Walker, 1993), for example, proposes that trees and grasses coexist through phenological differences, with grasses dominating during periods of high soil moisture during the wet season, and trees dominating both early and late in the dry season, when grasses are dormant and soils tend to be dry. As a second example, Xu *et al*. (2015) proposed a model that minimizes the role of rooting depth, positing that coexistence results because, while grasses have higher transpiration rates and water-use efficiencies (WUEs) than trees, trees outperform grasses during dry periods as a result of their lower respiration rates (Simioni *et al*., 2004). In a third example, van Wijk & Rodriguez-Iturbe (2002) proposed a coexistence model based on a persistence-colonization trade-off: trees are assumed to be more sensitive to stress and experience higher mortality rates than grasses under drought conditions, but this is assumed to be offset by a higher colonization potential in trees under wet conditions. These various models provide examples of widely divergent assumptions about whether trees or grasses are dominant under wet vs dry conditions due to different mechanisms. All of the above models are purely resource-based models. Other models of tree–grass coexistence have been proposed that place greater emphasis on the roles of herbivores and fire (Higgins *et al.*, 2000; Van Langevelde *et al.*, 2003; D'Odorico *et al.*, 2006). Although we acknowledge the role of these processes in savanna dynamics, our focus here is on the resource side of the equation, given that disturbance-based explanations alone are insufficient to explain the existence of savannas under all conditions, especially in drier climates (Sankaran *et al.*, 2004)

A key step toward resolving the tree–grass coexistence debate, from the resource perspective, can be taken by comparing the relative performance of tree/grass species assemblages across a range of soil moisture conditions. Among other metrics, this involves quantifying the relative drought tolerance of the two functional types, as well as rates of transpiration under both dry and wet conditions. Many models have drawn parameter estimates from Scholes & Walker (1993), who synthesized a rich body of savanna research conducted at the Nylsvley field site in South Africa. This synthesis, while comprehensive, often had to combine a number of datasets that relied on different methods for quantifying ecohydrological metrics in trees and grasses (Scholes & Walker, 1993). As a result, it can be difficult to directly compare tree and grass leaf trait values (e.g. the point of stomatal closure, transpiration rates, and WUE) under a common set of conditions. For example, the Nylsvley data suggest that savanna trees and grasses have comparable leaf-level transpiration rates and wilting points (Scholes & Walker, 1993), findings that have guided model assumptions (Xu *et al.*, 2015). There remains considerable uncertainty, however, about the relative values of key traits for tree and grass species for any given savanna ecosystem (Sankaran, 2019). Stomatal conductance, xylem conductance, photosynthetic pathway, and root morphology all contribute to overall drought resistance of a species (Tyree, 2003; Anderegg *et al.*, 2016). Trees have a higher carbon demand as a result of denser tissue and greater rate of photorespiration at high temperatures than C_4 grasses (Craine & Dybzinski, 2013; Greenwood *et al.*, 2017). Furthermore, trees typically exhibit tighter controls on stomatal conductance and lower xylem conductivity than grasses (Wolf *et al.*, 2016; Choat *et al.*, 2018; O'Keefe *et al.*, 2022). However, tree species vary widely in these traits, and therefore in their ability to tolerate drought conditions. The additional option of avoiding drought via rooting depth also appears relevant in many ecosystems, depending on edaphic conditions (Rice *et al.*, 2004). It is difficult, however, to measure these traits in isolation and accurately estimate their combined effects on transpiration and carbon acquisition during drought.

Even when leaf-level trait data are available, other challenges remain. Most empirical data on plant ecohydrological traits are collected at the organ level (leaf, stem, or root), yet models of tree–grass dynamics ultimately require an understanding of whole-plant behavior (Eagleson & Segara, 1985; Scholes & Walker, 1993; van Wijk & Rodriguez-Iturbe, 2002). Data on stomatal and root/shoot xylem conductance can be used in combination with modeling to infer whole-plant transpiration (WPT) for a given set of environmental conditions (Sperry & Love, 2015; Sperry *et al.*, 2017). This approach, however, suffers from two shortcomings: First, the scaling-up process adds model uncertainty; second, it requires a full suite of parameter estimates and submodels of conductance collected for every organ/tissue within a given species (Meinzer *et al.*, 2010; Venturas *et al.*, 2017). An alternative approach is to circumvent the scaling-up process by collecting whole-plant data where possible.

The primary objective of this study was to quantify and compare: the transpiration rates of coexisting savanna tree and grass species across a range of soil moisture conditions; and tree vs grass drought tolerance. In addition, we quantified the WUE and relative growth rate (RGR) of trees and grasses under well-watered conditions to tie together transpiration and carbon assimilation in these species. We relied on a whole-plant approach for most of these metrics. We focused on the seedling demographic stage, given that this is a critical stage in tree–grass dynamics: trees and grasses compete for moisture in the upper soil layers, and ecohydrological differences unrelated to rooting depth are most likely to play a dominant role at this stage (Cramer *et al.*, 2012; Tedder *et al.*, 2014). Our study aims to resolve a key question in the tree–grass coexistence debate: do trees and grasses exhibit a tradeoff in water use under wet and dry conditions, or does one functional type consistently outperform the other across a wide range of soil moisture conditions?

Materials and Methods

Plant seeds and growing conditions

We obtained seeds of 17 tree species from a commercial supplier (Silverhill Seeds, Cape Town, South Africa) in 2017 and 2018, and collected seeds of nine additional tree species (for a total of 26) and 19 grass species at Wits Rural Facility (WRF, Limpopo Province, South Africa) in early 2018 and 2019. The majority of our species are native to southern African Lowveld savanna (MAP = 500–700 mm). We used seeds from this pool (either all species or subsets of them) to obtain three sets of measurements: (1) WPT across a range of soil water potentials; (2) whole-plant WUE; and (3) intrinsic whole-plant RGR. We grew three separate cohorts of tree seedlings and grasses over two different periods (May–October 2019 for (1) and (3) above, and February–September 2020 for (2)) for each set of glasshouse measurements (Supporting Information Table S1). In all cases, we grew plants from seed at the Botany Greenhouse at the University of Georgia, under similar environmental conditions. We soaked tree seeds in tap water for 48 h after scarifying the seeds of hard-coated species using a scalpel in a small X pattern. Both tree and grass seeds in starter trays filled with soilless media (Fafard 3B; Sun Gro Horticulture, Agawam, MA, USA), watering daily to saturation. As soon as germinated seedlings formed one fully expanded true leaf, we transplanted them into pots, where we continued to water daily until the experimental measurements for each study were conducted. There were slight variations in planting media and pot size used depending on the requirements for each dataset, which we detail below.

To document the glasshouse growing environment, we measured glasshouse temperature and relative humidity with a VP–V4 humidity and temperature sensor, and quantified shortwave radiation (spectral range = 380–1120 nm) with a PYR pyranometer, both connected to an Em50 datalogger (Meter Group Inc., Pullman, WA, USA). For the period May–October 2019, mean minimum and maximum temperatures were 25.0 and 33.4°C, respectively, and mean daily relative humidity was 79%. The mean daily shortwave radiation (08:00–20:00 h) was 490 W m^{-2} (*c.* 2240 μmol m^{-2} s^{-1}) with an estimated PPFD of 1075 μmol m^{-2} s^{-1} based on an integration of our pyranometer's spectral response.

Glasshouse experiment 1: whole-plant transpiration as a function of soil moisture potential

In our first set of measurements, we used a gravimetric method based on sealing plants and pots to measure WPT (Holdo & McHargue, 2020). We took a time course of weights for our 26 tree species and 19 grass species over 7–14 d dry-down in our growth chamber (mean *c.* 10 d). Six individuals could be measured in the chamber at a time, and we recorded data for at least three individuals per species (Fig. S1). Before insertion in the chamber, we photographed a vertical profile of the plant's photosynthetic biomass and took a soil sample for gravimetric water content at field capacity. We did not consider self-shading as a potential confounding effect; however, because the trees are seedlings and the grasses single tussocks, the chance of consequential self-shading is far lower than in a crowded understory.

When the plants stopped meaningful transpiration (< 10% of day one change in mass), we harvested aboveground dry biomass and took another soil sample for gravimetric water content. Then, converted gravimetric water content (GWC) to soil water potential (Ψ_S) using a water retention curve specific to our soil (Fig. S2). We fit a van Genuchten function (van Genuchten, 1980) to the data ($n = 48$ measurements, adj. $R^2 = 0.92$) and used it to transform our GWC values into Ψ_S time series for each pot (Methods S1).

Glasshouse experiment 2: whole-plant WUE and RGR

To measure whole-plant WUE, we used the wrapped-pot approach in the glasshouse over a 6-wk period (to allow meaningful plant growth), adding and recording known quantities of water to individual pots to maintain a constant soil moisture regime. For this longer-duration study, we used a reduced set of species, focusing on five common grass and five common tree species all grown from seed (Table S1). We calculated whole-plant WUE by dividing plant mass gain by water mass transpired (Methods S1). To compare the intrinsic RGR of trees and grasses, we grew 17 grass and 15 tree species from seed (between four to six individuals per species) and transplanted into individual pots between May and October 2019 (Table S1). We calculated RGR as $(M_{\text{final}}) - (M_{\text{initial}})/t$, where M_{initial} and M_{final} are plant initial and final mass (g), and t is time (d) between transplantation and harvest (median: 50 d, range: 21–134 d; Methods S1).

Field measurements: drought tolerance

In conjunction to our glasshouse measurements of WPT, we analyzed previously collected drought tolerance data from tree and grass species in field plots at WRF, the source for our grass seed and several of our tree seeds. This provided an opportunity to compare patterns in linked physiological traits between glasshouse and field conditions. In March 2018 (during the wet season), we collected fresh leaves from between one and seven individuals (median and target number = 5) per species of 19 grass and 33 tree species (Table S1). We calculated leaf osmotic potential (Ψ_{osm}), measured with a dew-point osmometer, as an index of drought tolerance with the following equation $\Psi_{\text{osm}} = \text{osmolarity} \times -2.3958/1000$, from Griffin-Nolan *et al.* (2019). Ψ_{osm} represents the internal solute potential of the leaf tissue. Leaf osmotic potential is closely correlated to turgor loss point; the water potential at which a plant cannot maintain cell turgor. Lower values of both are linked to resisting leaf dehydration and maintaining transpiration during periods of water stress (Ashraf & Mehmood, 1990; Zhu *et al.*, 2018).

Data analysis

We conducted all analyses in R v.4.0.4 (R Development Core Team, 2011). For mixed models with a single random effect, we used the NLME R package (Pinheiro & Bates, 2000). In most of our analyses, we had multiple crossed random effects, which are easier to specify in the LME4 package (Bates *et al.*, 2015), so we used the latter package for those analyses. We first converted transpiration to hourly values of transpired water mass on a leaf-mass basis (in units of g g^{-1} of H_2O h^{-1}). In grasses, we considered leaf mass as comprising all shoot photosynthetic tissues. We lacked leaf-mass data for a few individuals ($n = 5$) that had lost all of their leaf mass by the end of their growth chamber run. In these cases, we used stem mass data in combination with shoot mass data (leaf and stem) from the same species collected in a separate experiment (in the same glasshouse) to estimate leaf mass. We chose this leaf-mass approach because we did not collect a full root : shoot ratio for every individual in our growth chamber. We calculated four transpiration variables across contrasting values of soil water potential Ψ_S: daily mean (E_{mean}) and daily maximum (E_{max}) hourly transpiration, within-day slope of hourly transpiration from 06:00 to 18:00 h (E_{wd}), and the between day differences in transpiration from 18:00 to 06:00 h the following day (E_{bd}) (Fig. 1). The first two variables captured overall differences in daily transpiration, while the second two reflected within and between daily water-use changes, respectively. We used E_{wd} to measure changes in water use over the course of a single daily cycle, while E_{bd} quantified patterns of overnight recovery following a dark cycle, for example, reflecting nightly recharge of water from the soil into the rooting zone. We tested for differences between trees and grasses for each variable across a range of soil water potentials, which we classified into four bins: −0.0 to > −0.5, −0.5 to > −1.0, −1.0 to > −1.5, and ≤ −1.5 MPa. For each Ψ_S bin, we used linear mixed models implemented using the *lmer* function in the LME4 package,

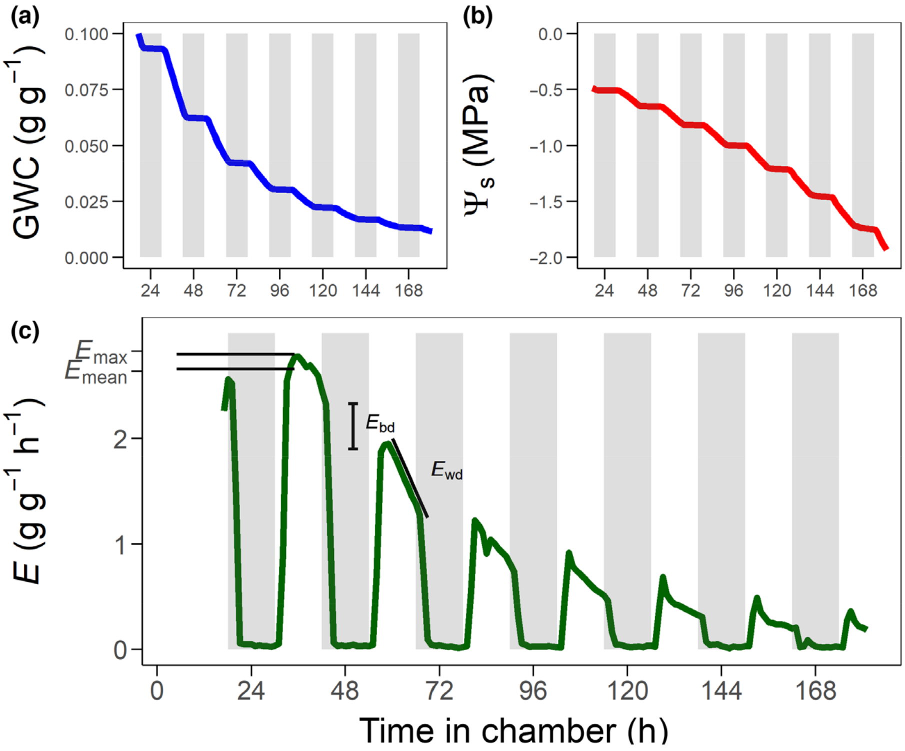

Fig. 1 Soil water potential and transpiration of a *Vachellia grandicornuta* tree seedling over the course of 7 d inside a growth chamber. (a) Decline in gravimetric water content (GWC) and (b) soil water potential (Ψ_S) over time in the growth chamber. (c) Depiction of four variables used to compare plant water use against soil water potential: E_{mean} and E_{max} are the mean and maximum hourly transpiration values, E_{wd} represents the slope (negative, in this case) of within-day decline in hourly transpiration between morning and evening, and E_{bd} equals the difference between the final daytime mean hourly transpiration and the first mean hourly transpiration the following morning. Shaded regions represent dark (12-h) periods.

treating functional type as a fixed effect and species and growth chamber batch as random effects. We also tested for potentially confounding effects of light and vapor pressure deficit (VPD) on transpiration rate inside the chamber. These confounding effects could arise if size or other differences between functional types and/or species were to lead to systematic differences in the chamber environment or incident radiation (e.g. within a growth chamber, radiation levels decline as a function of distance to the light source, so shorter plants may respond differently from taller plants). For the light effects, we calculated a weighted value of incident radiation for each individual plant by combining the leaf height profiles we obtained photographically with a growth chamber light attenuation curve. Our analysis of possible systematic differences between functional types in terms of incident radiation focused on height differences rather than other effects such as possible self-shading differences. The latter would be difficult to quantify, but we also considered them to be of lower importance than height differences because of the sparse nature of seedling canopies. We calculated VPD from the weather station temperature and relative humidity data. To test for these effects, we fit four *lmer* models (a null model, a VPD model, a VPD plus weighted radiation model, and a soil water potential model) using E_{mean} as the response variable and compared their relative fit using AIC. We used individual hourly values of E_{mean} as the unit of observation, with species, growth chamber batch, day, and individual plant ID as random effects.

For the WUE dataset, we calculated WUE by dividing shoot dry mass gain by water mass transpired (given by the sum of all mass losses corrected for plant mass gain). To estimate plant mass gain, we needed an estimate of initial and final belowground mass, which we did not quantify in this experiment. We therefore used measurements of root mass ratio for our study species obtained in a separate glasshouse experiment (Wargowsky *et al*., 2021) to estimate the initial and total final mass of each plant on the basis of initial and final shoot mass. To test for tree–grass differences in whole-plant WUE, RGR, and Ψ_{osm}, we used mixed models implemented with the *lme* function in the NLME package (Pinheiro & Bates, 2000) treating species as a random effect.

Results

Our analysis of the effects of growth chamber environment suggested that variation in soil moisture was the dominant driver of plant transpiration, with transpiration declining sharply as soil moisture potential declined (Fig. S3). Our comparison of the four transpiration models found very strong support (ΔAIC = 744.6 relative to the null model) for the soil water potential model (Table S2), and although there was some support for a VPD model (ΔAIC = 5.8 relative to the null model), the effect was relatively weak (Table S2). Importantly, there was no evidence for an effect of plant height on transpiration mediated by light, given that the VPD plus weighted radiation model did not improve fit over the VPD model (Table S2).

Our gravimetric method effectively captured diurnal cycles of transpiration driven by the light : dark phases used in the growth chamber (Fig. S4). Transpiration rates during dark periods were close to zero, suggesting that evaporative and leakage losses were negligible and that fluctuations in pot mass over time were essentially capturing transpiration losses (Fig. S4). Trees had significantly lower mean (E_{mean}) and maximum (E_{max}) transpiration rates than grasses at nearly every level of soil water availability (Fig. 2a,b). In most cases, tree transpiration

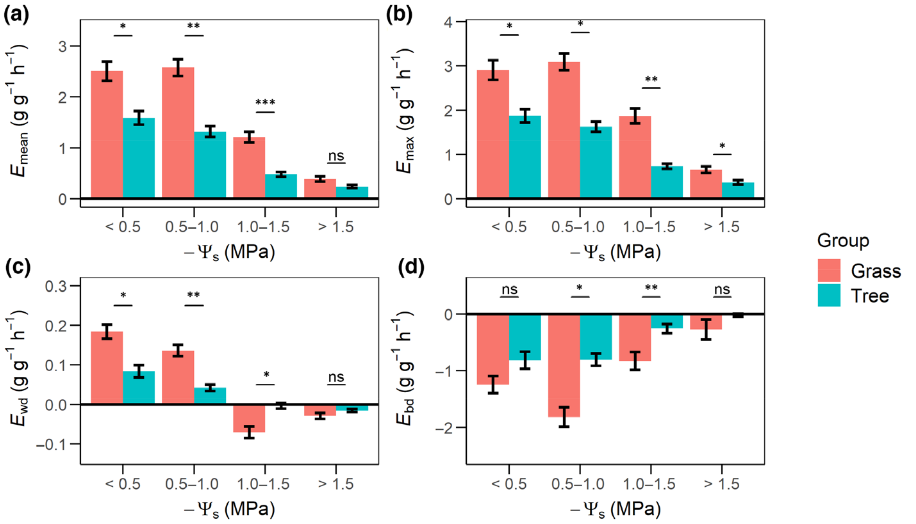

Fig. 2 Plant water use (mean ± SE) as a function of soil water potential (Ψ_S). (a) Mean (E_{mean}) and (b) maximum (E_{max}) transpiration, (c) slope of within-day decline in transpiration (E_{wd}), and (d) difference between the final daytime mean hourly transpiration and the first mean hourly transpiration the following morning (E_{bd}). Mean daily soil water potential Ψ_S is binned into four categories. All values are shown as per gram of photosynthetic mass. Statistically significant differences between trees and grasses within each bin were determined via linear mixed effects model (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

rates were roughly half those of grasses on a leaf-mass basis. As soils dried down, the magnitude of this difference remained the same or increased until $\Psi_S < -1.5$ MPa (Fig. 2). Under the driest conditions, meaningful transpiration (> 12% of maximum) did not occur in either trees or grasses, although grasses still maintained a significantly higher maximum daily transpiration than trees (Fig. 2). At higher soil water potentials, both grasses and trees transpired a higher proportion of their daily water budget in the afternoon, as evidenced by the positive slope found in within-day transpiration patterns (Fig. 2c). Grasses showed a significantly faster increase in transpiration than trees over the course of the day (E_{wd}) at high water potentials (Fig. 2c), suggesting a progressive ramping-up of water use following the initiation of transpiration. As soils dried down, however, the directionality of this effect was inverted, with grasses tending to use water at a declining rate over the course of the day (Fig. 2c). By contrast, trees tended to maintain low but steady rates over time (Fig. 2c). Grasses also showed a significantly greater difference between evening and morning transpiration than trees at all levels of soil water availability (Fig. 2d), except under very dry conditions ($\Psi_S < -1.5$ MPa). In nearly all cases, the first hour of morning transpiration was higher than the last hour of the previous evening (Fig. 2d).

Grasses had a significantly lower leaf osmotic potential (Ψ_{osm}) than trees (mixed model: $F_{51,197} = 8.95$, $P = 0.004$; Fig. 3a), suggesting a greater drought tolerance than the former under field conditions. When transpiration rates (E_{max} under well-watered conditions) and drought tolerance are examined jointly, grasses and trees tend to occupy distinct sections of trait space, with grasses being both more drought tolerant and able to rapidly exploit available water than trees (Fig. 3b).

Under well-watered conditions, grasses (mean = 180 g of H_2O transpired per g of shoot mass growth) had a mean whole-plant WUE that was almost 50% higher (linear mixed model: $F_{7,39} = 16.3$, $P < 0.005$; Fig. 4a) than that of trees (mean = 337 g of H_2O per g of shoot mass growth). Under similar conditions, grass mean RGR was *c.* 3 times higher than tree RGR (linear mixed model: $F_{27,41} = 83.6$, $P < 0.0001$; Fig. 4b).

Discussion

In this study, we were able to quantify key functional traits for a large suite of co-occurring tree and grass species from a widespread savanna type, thus allowing us to develop unprecedented insight into the comparative performance of these two functional types. Our results suggest that, at least under controlled conditions, savanna C_4 grasses outperform co-occurring C_3 tree seedlings across a wide range of soil moisture conditions. C_4 grasses have often been characterized as being effective exploiters of soil moisture when it is readily available (i.e. at high water potentials; Nippert & Knapp, 2007). Our results both reinforce this finding and quantify the extent of the water uptake advantage that grasses possess over trees. More importantly, we show that this advantage does not necessarily diminish when conditions become stressful, as demonstrated by the fact that grasses are able to use significantly more water per unit leaf mass than trees even below the conventional wilting point of −1.5 MPa (Fig. 2b). This dry-condition advantage is reinforced by leaf-level measures of the osmotic potential, which in some grass species (e.g. *Perotis patens*) can be below −5 MPa. Overall, our results convincingly suggest that no fundamental trade-off exists between transpiration rate and drought tolerance. Furthermore, along a soil moisture gradient these two functional traits are positively related (Fig. 3b, where it should be noted that drought tolerance declines along the *x*-axis).

In addition to their higher transpiration rates, grasses translated their higher water-use rates into higher whole-plant biomass gains than trees did. By multiplying the mean grass : tree transpiration ratio (1.57 when soil moisture was not limiting) with the mean whole-plant grass : tree WUE ratio (1.87), we obtain an expected growth ratio of 2.95, which is very close to the actual measured RGR grass : tree ratio of 3.56. Although crude, it is striking that the combination of whole-plant parameters obtained from separate datasets agreed (consisting of different species mixtures and growing conditions) reinforce the tight coupling between water use and growth. As soil moisture declines, it is likely grasses continued to maintain a higher growth rate than

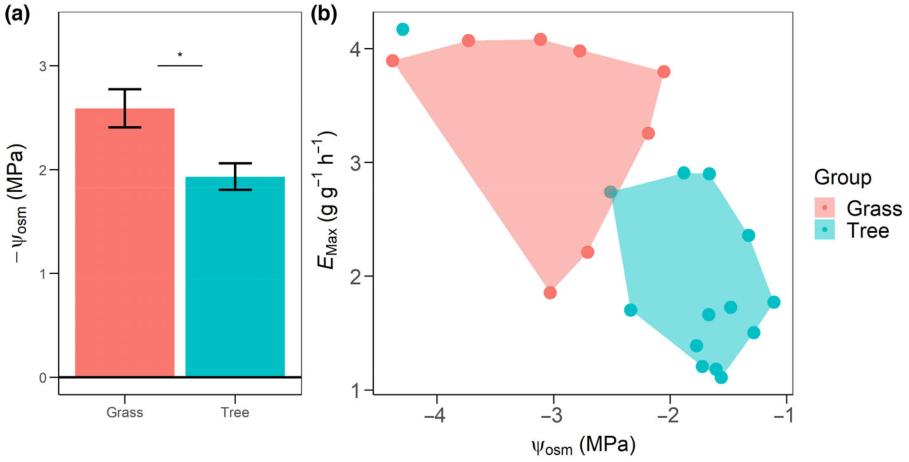

Fig. 3 (a) Difference in osmotic potential (mean ± SE) between trees and grasses and (b) species means (a species subset with greater than two replicates for both variables) for leaf osmotic potential (Ψ_{osm}) vs maximum daily transpiration (E_{max}) across trees and grasses. A single outlying tree species (*Vachellia tortilis*) is omitted from the tree polygon. Statistically significant differences between tree and grass Ψ_{osm} were determined via linear mixed effects model (*, $P < 0.05$).

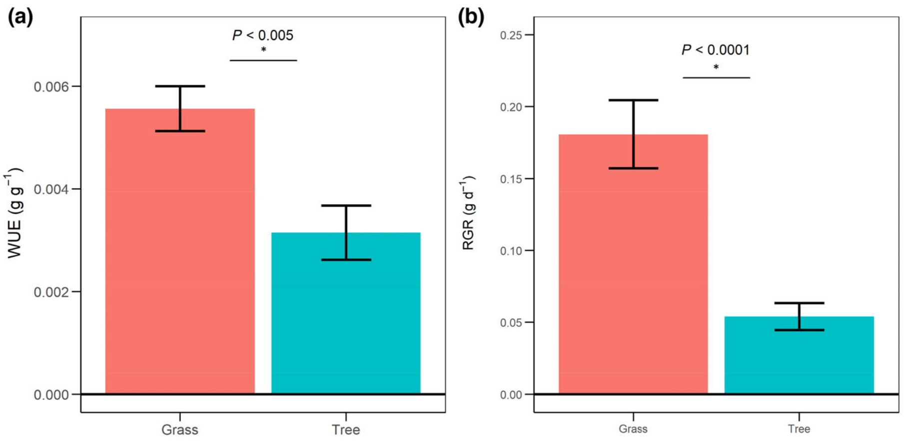

Fig. 4 Differences (mean ± SE) between trees and grasses in (a) whole-plant water-use efficiency (WUE) and (b) relative growth rate (RGR). Statistically significant differences between tree and grass leaf osmotic potential (Ψ_{osm}) were determined via linear mixed effects model (*, $P < 0.05$).

tree seedlings, due to the strong correlation between transpiration and internal CO_2 concentrations. Additionally, grasses' lower leaf osmotic potential supports a lower risk of hydraulic failure during drought due to the strong link between Ψ_{osm} and P50 (Bartlett *et al.*, 2012, 2016).

The twofold advantage of grasses, efficiently exploiting soil resources when abundant (and translating these resources into fast growth), while also showing high tolerance to resource limitation, has been recognized in temperate grasslands (O'Keefe & Nippert, 2018), but has not been as clearly articulated in tropical savannas, where water use and niche partitioning have been the focus of many efforts to explain how trees and grasses coexist (Walker *et al.*, 1981; Xu *et al.*, 2015; Yu & D'Odorico, 2015). A key question arises: if grasses outperform trees under both dry and wet conditions, how do these two functional types coexist? A possible answer is provided by the model proposed by Xu *et al.* (2015), who suggested that grasses do indeed transpire more than trees under both wet and dry conditions, but grasses lose biomass at a faster rate than trees under dry conditions. Under a dynamic soil moisture regime, this can theoretically lead to coexistence (Xu *et al.*, 2015). Alternatively, rooting depth differences that allow niche partitioning can provide a mechanism for coexistence even in the absence of functional trait trade-offs in surface soil water use (Holdo, 2013). Having established that grasses are dominant in terms of water use, resolving this issue requires a better understanding of whole-plant carbon (C) assimilation patterns and changes in tree/grass water use efficiency across gradients of soil moisture availability.

In general, by demonstrating grass dominance under both wet and dry conditions, our results are not consistent with models that assume a tree 'advantage' over grasses under conditions of moisture limitation. To various degrees, the Xu *et al.* (2015) model, the persistence-colonization, and the temporal niche separation model all make this assumption. By contrast, the two-layer model allows trees to persist in the face of strong grass competition by accessing subsoil water that infiltrates below the soil layers where grassroots dominate water uptake (Holdo & Nippert, 2023). Under this model, a performance trade-off across a soil moisture gradient is not required. In a recent review, Holdo & Nippert (2023) argued that savanna trees are prevented from eventually outshading grasses by ecohydrological limitations on tree leaf area (in dry savannas), or by herbivores and fire (in wet savannas), but that grasses are consistently dominant to trees in terms of competition for soil moisture.

Our WUE and RGR datasets only captured wet conditions, so it is indeed possible that a trade-off could result from differences in net carbon balance across soil water availability between these two functional groups. A key to understanding how tree vs grass C balance patterns change with soil moisture is the nature of stomatal control in the two functional types. Recent work assumes that plants juggle the risk of C starvation against the risk of hydraulic failure across gradients of moisture stress (both the supply and demand sides) via stomatal control (Sperry *et al.*, 2017), but little is known about how stomatal control strategies might differ in a C_3 tree and a C_4 grass. Trees may tend to minimize the risk of hydraulic failure and gain an advantage under dry conditions as a result of the strength of their stomatal control mechanism. Their lower transpiration rates may be controlled by a more conservative isohydric strategy, even when soil water potential is relatively high (Meinzer *et al.*, 2017). Trees in neotropical savannas are known to impose these tighter stomatal control strategies when compared to grasses or herbaceous species (Rossatto *et al.*, 2013). Isohydric species typically have an advantage during the dry-down between rain events when compared to anisohydric species (Manzoni *et al.*, 2015). In addition to differences in stomatal control, we require better estimates of tree/grass differences in respiration rates (since C starvation ultimately results when respiration depletes C reserves) across soil moisture gradients. Current assumptions about these differences tend to rely on leaf-level measurements, which show a higher cost of dark respiration in grasses owing to their higher light-compensation point (Scholes & Walker, 1993; Simioni *et al.*, 2004). A per leaf-mass approach to quantify C balance across a range of environmental conditions, similar to this study, would address these uncertainties. This scale is ecologically relevant when describing plant community dynamics and more easily measured in the field and scaled to an entire community. Notably, these estimates do not capture the net contribution of nonphotosynthetic tissues toward respiration, such as fine roots. The respiratory demands of woody species, which have a greater proportion of nonphotosynthetic tissue, may be underestimated as a result.

It is also important to note that extrapolating our measurements to make inferences about tree/grass competitive dynamics in real communities is impeded to an extent by the fact that growing seedlings in isolation is not necessarily equivalent to growing them in direct competition with each other. Previous work, both under glasshouse (Holdo & Brocato, 2015; Campbell & Holdo, 2017) and field conditions (February *et al.*, 2013), has shown that tropical savanna grasses are competitively dominant to tree seedlings, and that competition is highly asymmetric (Campbell & Holdo, 2017), in agreement with the differences in tree and grass performance we report here. It is important to note, however, that plants growing under field conditions may exhibit traits that we did not observe under growth chamber conditions, possibly as a result of the presence of neighboring plants. Some C_4 grass species have been found to have high rates of nocturnal transpiration under conditions of high soil moisture availability (O'Keefe & Nippert, 2018). It has been suggested that this may serve as a competitive strategy for grasses growing in the presence of neighbors within their rooting zone (O'Keefe *et al.*, 2016) and would suggest that patterns of transpiration and WUE (which declines with nocturnal transpiration) inferred from isolated plants may not necessarily predict performance in competitive scenarios.

In addition to tree/grass differences in mean and maximum transpiration from day to day, our results shed insight into differences in within-day fluctuations in water use, and these similarly support the idea that grasses exhibit a more 'aggressive' water use strategy than tree seedlings. Grass water use increased over the course of the day when soil water potential was high, suggesting these grasses are increasing transpiration despite little change to the surrounding VPD. We hypothesize that this increase in transpiration throughout the day was likely due to an increase in stomatal conductance. This trend flipped when soil water potential fell below −1.0 MPa, likely due to rapid depletion of soil moisture within the rooting zone throughout the day, as documented by previous work (O'Keefe & Nippert, 2018). Additionally, this explains why grasses experienced larger overnight differences in transpiration than trees. Due to faster water uptake, grasses rapidly depleted moisture adjacent to absorptive roots, and therefore benefited more than trees from overnight water redistribution across the soil profile. To the best of our knowledge, these within- and between-day differences in water-use patterns between trees and grasses have not been document before. They are important, because they vary over the course of the day, and in aggregate they account for daily quotas of water consumption. They imply that, over the course of a season, variation in daylength can lead to contrasting nonlinear effects on cumulative daily water use in the two functional types.

These findings will help inform key assumptions made in current mechanistic models of tree–grass coexistence. Our conclusions strengthen the two-layered model's assumption that trees are unable to compete with grasses in the surface soil, at least for water resources. This also leads to a contradiction with the temporal niche partitioning model if they are occupying the same soil layers. We found that trees were unable to transpire as efficiently as grasses at low soil water potentials. Unless they are using water from deeper soil layers as a mechanism of drought avoidance, as with the two-layered hypothesis, they would not be at a carbon advantage during the early and late dry season when compared to grasses. While we did not measure colonization rates of tree and grass species, our findings are at odds with the assumptions made in the colonization-trade-off model. For colonization to occur under wet conditions, tree seedlings would need to outcompete grasses. These data show that even at field capacity, trees are unable to keep up with grasses' ability to exploit shallow soil water. If trees are better colonizers, they may outcompete grasses using other mechanisms, such as nitrogen-fixation mutualisms or allelopathy.

Our results suggest that, when savanna tree seedlings and grasses are exploiting similar soil volumes, grasses consistently outperform trees, extracting more water under all conditions, and using this water more efficiently to produce more biomass. Much of our understanding of and ability to predict tree–grass dynamics under novel climates and rainfall regimes relies by necessity on ecophysiological models of plant performance. A key challenge for these models is obtaining relevant parameter estimates to inform key processes such as transpiration and growth. Our whole-plant approach helps to address important gaps for this process in tropical and subtropical savanna ecosystems. In addition, our study helps resolve a key source of conflict about the underlying mechanism of tree–grass coexistence: whether there is a trade-off in tree vs grass performance across gradients of soil moisture availability. Our results show that, at least in terms of water use, there is no evidence for such a trade-off.

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Competing interests

None declared.

Author contributions

MWB, JEN, and RMH planned and designed the experiment. MWB, JEN, and JBN performed the study and collected data. MWB and RMH analyzed the data and wrote the manuscript. JEN and JBN provided feedback and edited the manuscript.

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Data availability

The data that support the findings of this study are openly available in Zenodo at https://github.com/mbelovitch/NPH-MS-2023-42507/tree/v1.0.0, reference doi: 10.5281/zenodo.7764603.

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Supporting Information

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

Fig. S1 Six plants at the start of a run of growth chamber measurements.

Fig. S2 Soil water retention curve calculated using a gravimetric approach for this study's soil.

Fig. S3 Hourly transpiration (normalized by leaf mass) as a function of soil water potential (Ψ_S), incident radiation weighted by plant biomass distribution as a function of height, and vapor pressure deficit for savanna tree seedlings and grasses under growth chamber conditions.

Fig. S4 Hourly transpiration time series (normalized by leaf mass) for four savanna tree seedlings and four grass tussocks under growth chamber conditions.

Methods S1 Supplementary methods describing whole-plant transpiration measurements, water-use efficiency, and relative growth rate measurements.

Table S1 Sample sizes for tree and grass species used for analyses of whole-plant transpiration, leaf turgor loss point, whole-plant water-use efficiency, and relative growth rate.

Table S2 Model fits (given by AIC, the Akaike information criterion) for four models of hourly transpiration (normalized by leaf mass) for savanna tree seedlings and grasses under growth chamber conditions.