# Root-niche separation between savanna trees and grasses is greater on sandier soils

Madelon F. Case[1]*, Jesse B. Nippert[2], Ricardo M. Holdo[3], and A. Carla Staver[1]

[1] Yale University, Department of Ecology and Evolutionary Biology, New Haven, CT 06511 USA

[2] Kansas State University, Division of Biology, Manhattan, KS 66506 USA

[3] University of Georgia, Odum School of Ecology, Athens, GA 30602 USA

* Corresponding author: madelon.florence@gmail.com

165 Prospect St, New Haven, CT 06511 USA

## Abstract

1. In savannas, partitioning of belowground resources by depth could facilitate tree-grass coexistence and shape vegetation responses to changing rainfall patterns. However, most studies assessing tree vs. grass root-niche partitioning have focused on one or two sites, limiting generalization about how rainfall and soil conditions influence the degree of rooting overlap across environmental gradients.
2. We used two complementary stable isotope techniques to quantify variation (a) in water uptake depths and (b) in fine root biomass distributions among dominant trees and grasses at eight semi-arid savanna sites in Kruger National Park, South Africa. Sites were located on contrasting soil textures (clayey basaltic soils vs. sandy granitic soils) and paired along a gradient of mean annual rainfall.
3. Soil texture predicted variation in mean water uptake depths and fine root allocation. While grasses maintained roots close to the surface and consistently used shallow water, trees on sandy soils distributed roots more evenly across soil depths and used deeper soil water, resulting in greater divergence between tree and grass rooting on sandy soils. Mean annual rainfall predicted some variation among sites in tree water uptake depth, but had a weaker influence on fine root allocation.
4. *Synthesis*. Savanna trees overlapped more with shallow-rooted grasses on clayey soils and were more distinct in their use of deeper soil layers on sandy soils, consistent with expected differences in infiltration and percolation. These differences, which could allow trees to escape grass competition more effectively on sandy soils, may explain observed differences in tree densities and rates of woody encroachment with soil texture. Differences in the degree of root-niche separation could also drive heterogeneous

responses of savanna vegetation to predicted shifts in the frequency and intensity of rainfall.

**Keywords:** niche partitioning, plant-climate interactions, rooting depths, savanna, soil texture, stable isotopes, tree-grass coexistence

## Introduction

Shifts in the frequency and intensity of rainfall are widely predicted as an outcome of global climate change, even where mean annual rainfall does not change (Fischer, Beyerle & Knutti 2013; Westra *et al.* 2014), with impacts on diverse ecosystems (Knapp *et al.* 2008). Savannas – defined by the co-dominance of trees and grasses – could be particularly responsive to changes in rainfall patterns, because belowground competition for water has been widely observed to mediate productivity in these systems (Riginos 2009; February *et al.* 2013b; Kulmatiski & Beard 2013b; Holdo & Nippert 2015). Shifts to fewer, larger rain events could change the vertical distribution of soil moisture to the potential benefit of deeper-rooted plants (Knapp *et al.* 2008). As a result, rooting patterns could be key to predicting competitive outcomes between plant species and functional groups. If trees have access to deeper soil water than grasses, then rainfall intensification could favor savanna trees (Kulmatiski & Beard 2013b; Holdo & Nippert 2015; Berry & Kulmatiski 2017). Conversely, if trees are competing directly with grasses for resources within the same soil layers, infrequent, intense rainfall may instead competitively favor grasses which are often the more aggressive water users (Xu, Medvigy & Rodriguez-Iturbe 2015; O'Keefe & Nippert 2018). Variation in the degree of root-niche separation along environmental gradients could lead to heterogeneous responses to rainfall intensification. However, while root-niche separation has been widely hypothesized as a process underlying savanna vegetation dynamics (Walter 1939; 1971; Walker & Noy-Meir 1982; Ward, Wiegand & Getzin 2013), comparative studies of root-niche separation across more than a single site are rare, and the environmental determinants of tree and grass rooting strategies remain poorly constrained.

The idea that savanna trees use deeper water than grasses is an old one: Walter's two-layer hypothesis (Walter 1939) and later interpretations (Walter 1971; Walker & Noy-Meir 1982; Ward *et al.* 2013) proposed that vertical resource partitioning between shallow-rooted grasses and deeper-rooted trees could promote their coexistence by minimizing direct competition for water and nutrients. Evidence for belowground resource partitioning has been mixed, with some studies claiming that substantial overlap between tree and grass rooting strategies refutes the two-layer hypothesis (Mordelet, Menaut & Mariotti 1997; Le Roux & Bariac 1998; Hipondoka *et al.* 2003; Verweij *et al.* 2011) and others emphasizing differences between trees and grasses, even where some overlap is present (Knoop & Walker 1985; Kulmatiski *et al.* 2010; Kambatuku, Cramer & Ward 2013; Ward *et al.* 2013; Holdo 2013; Holdo & Nippert 2015; Holdo, Nippert & Mack 2018). Experiments have consistently shown that grasses do competitively suppress trees (Kraaij & Ward 2006; Riginos 2009; Ward & Esler 2011; February *et al.* 2013b; Vadigi & Ward 2014), suggesting that their resource-use niches are not completely separate, but even some degree of niche partitioning by depth could still contribute to tree growth and establishment in a grass-dominated environment (Holdo 2013).

Crucially, if the degree of overlap in rooting niches varies with environmental factors such as soil texture and rainfall, we could expect context-dependent responses to environmental change. Indeed, our previous work has found that savanna tree cover is higher under infrequent, intense rainfall on sandy soils and at low mean annual rainfall, but decreases with rainfall intensity on clayey soils (Case & Staver 2018). One hypothesized mechanism for this pattern is that trees have deeper roots on sandy soils (Zhou *et al.* 2020), such that they overlap less with grasses and may more effectively exploit deep percolation during larger rainfall events (Case & Staver 2018). It makes sense theoretically that rooting depths and the extent of root-niche differentiation between trees and grasses would vary with soil texture and rainfall (Holdo 2013) – and a few studies have found empirical support for this (Knoop & Walker 1985; Schenk & Jackson 2002b; Holdo *et al.* 2018; Zhou *et al.* 2020). One experimental comparison between two sites under identical climates found that trees on the sandier site were less limited by competition from grasses and used more subsoil water (Knoop & Walker 1985), while another experiment found that *Acacia mellifera* seedlings reached greater biomass on sandy than rocky soils (Ward & Esler 2011). However, most studies evaluating belowground niche partitioning in savannas have been conducted at just one or two sites at a time – or, in previous work by Holdo *et al.* (2018), across variation in rainfall but not soils – making broad generalization challenging. A rigorous comparative approach employing the same methods across several sites at once is necessary to reach broader conclusions about how root-niche partitioning varies across environmental gradients, especially with respect to soil texture.

Studies also vary in terms of the metrics used for determining and comparing tree and grass rooting niches. The most extensive global syntheses have emphasized maximum rooting depth (Schenk & Jackson 2002a; b), although this can present a limited picture of the overall pattern of root distribution throughout the soil column. Many site-specific studies in savannas, by contrast, have considered an integrative picture of fine or coarse root biomass allocation by depth (Mordelet *et al.* 1997; Hipondoka *et al.* 2003; February & Higgins 2010; February, Cook & Richards 2013a; Zhou *et al.* 2020). Still others have pointed out that the distribution of root biomass does not necessarily match the functional rooting depth, which describes actual water uptake patterns (Kulmatiski *et al.* 2010; Nippert & Holdo 2015; Holdo *et al.* 2018). Variation in root functional traits, such as specific root length, root tissue density, and hydraulic conductivity, further determine variation in root function with depth (Nippert & Holdo 2015; Valverde

Barrantes *et al.* 2017; McCormack & Iversen 2019). Measurements of water uptake and biomass allocation are both relevant to understanding and contrasting tree and grass rooting strategies and can illuminate different time scales of observation. Water uptake depths, measured here using natural-abundance stable isotopes of water as tracers, provide a snapshot of where plants are actively obtaining water at the time of sampling. Fine-root biomass distributions, measured here using root cores, indicate longer-term patterns of carbon allocation and investment.

In this study, we asked whether soil texture and rainfall influence the degree of root-niche separation between trees and grasses in savannas. Our comparative approach utilized a naturally occurring rainfall gradient crossed with a soil texture contrast in Kruger National Park in South Africa, with a total of eight study sites paired by mean annual rainfall and distributed across sandy and clayey soils. We used stable isotope techniques to characterize water uptake depths and fine-root biomass distributions of the dominant tree and grass species at each site, and compared across sites to determine whether rooting patterns and the degree of root-niche overlap varied predictably with environmental context.

## Methods

### *Study sites*

Kruger National Park (hereafter, Kruger) is a large protected area in northeastern South Africa (22°20’ to 25°30’ S; 31°10’ to 32°00’ E). Kruger comprises nearly 20,000 $km^2$ of tropical and subtropical savanna. Rainfall is concentrated mainly between November and April, with a gradient of mean annual rainfall spanning approximately 350 mm in the north to over 750 mm in the south (Figure 1). The park is dominated by two major soil types, largely orthogonal to the north-south rainfall gradient, which differ markedly in texture, hydraulic properties, and nutrient content. Granite-derived soils in the west are sandy and nutrient-poor, whereas basalt-derived soils in the east are clayey and nutrient-rich (Figure 1) (Buitenwerf, Kulmatiski & Higgins 2014; Staver, Botha & Hedin 2017). Fires are frequent, with an average return interval of about 3.5 years (Govender, Trollope & van Wilgen 2006), and the park is home to a diverse community of African savanna wildlife. Tree densities are variable but tend to be higher on the sandy granitic soils and where maximum intervals between fires have been longest, while grass biomass is higher on clayey basaltic soils and increases with mean annual rainfall (Staver *et al.* 2017). The flora of the park comprises > 200 $C_4$ grass species and > 400 tree and shrub species, with dominant woody species including several within the Fabaceae (*e.g.*, *Senegalia* and *Vachellia* spp., *Dichrostachys cinerea*, and *Colophospermum mopane*) and Combretaceae (*Terminalia* and *Combretum* spp.).

For this study, we examined tree and grass rooting depths at a total of eight sites: four on sandy granitic soils and four on clayey basaltic soils, paired by mean annual rainfall. Sites on granitic soils were a subset of those established for a previous study of functional rooting depths along the rainfall gradient (Holdo *et al.* 2018). We paired these locations with sites on basaltic soils at equivalent mean annual rainfall, as near to the granitic sites as permitted by road access and the spatial distribution of soils and rainfall. All sites were located on topographic crests and were 100 to 200 m from roads. Mean annual rainfall at sites ranged from ~470 mm for the driest pair to ~600 mm for the wettest pair, with site-specific rainfall values extracted from inverse-distance-weighted interpolation of long-term rain gauge records (Staver, Wigley-Coetsee & Botha 2019). Although there are wetter areas in Kruger, they are restricted to granitic soils, so 600 mm was the highest mean annual rainfall where a site pair across soil types could be sampled. Sampling occurred in May and early June 2018, just as wet-season rains were ending, with all sites sampled at least a week after the most recent rainfall event to allow for evaporative enrichment and the development of gradients in soil water isotopes with depth.

*Field collection*

At each site, we identified individuals of the 2-3 dominant grass species and 2-4 dominant tree species within our target size (trees between 1.5 and 2.5 m in height) for collection of xylem water. We clipped fresh twig samples from three individuals of each tree species and immediately placed them inside 12-ml Exetainer vials (Labco, UK), with each vial containing several twigs from multiple branches of a single individual. For grasses, we clipped non-photosynthetic aboveground stems (culms), and pooled samples from several grass tufts into two vials per species. To minimize the occurrence of evaporative fractionation, we immediately sealed vials with Parafilm, placed them on ice, and kept them frozen until water extraction could be conducted in the laboratory. To obtain end-member values for fine roots of $C_4$ grasses and $C_3$ trees to parameterize carbon-isotope mixing models, we excavated and clipped representative fine root samples from each dominant tree and grass species at each site.

We collected soil samples and fine root depth cores from soil pits. At each site we dug three soil pits to a depth of at least 50 cm below the surface, unless soils were too shallow or stony to reach that depth. We collected soil samples for soil water extraction from depths of 5, 15, 30, and 50 cm (or 40 cm in shallower pits), sealed them in Exetainer vials, and placed them on ice. We chose these sampling depths based on previous work in the region showing that the gradient of evaporative enrichment is steepest near the soil surface (Holdo & Nippert 2015; Holdo *et al.* 2018). We collected soil cores for fine-root quantification by pounding a metal soil corer (AMS 2” diameter x 6” long cylindrical soil core sampler [5.08 cm diameter x 15.24 cm long], for a fixed soil volume of 308.9 $cm^3$ per sample) horizontally into the wall of each pit at depths of 5, 15, 25, 35, and 45 cm.

*Sample processing and laboratory analysis*

After field collection, we removed and washed all fine roots from each soil core, and dried these samples in a drying oven at 65° C. In the laboratory at Yale University, root samples were further rinsed with deionized water, dried again at 65° C, and ground to a fine powder with a mortar and pestle (homogenizing all root fragments found within a single core). This yielded a total of 104 mixture samples from soil cores and 49 end-member samples for stable carbon isotope analyses. We analyzed these samples for $\delta^{13}C$ values using a Costech ECS 4010 Elemental Analyzer with Conflo III interface in the Yale Analytical and Stable Isotope Center. The $\delta^{13}C$ values were expressed as deviations from an international standard (VPDB) in parts per thousand (‰) using $\delta$-notation:

$$\delta = \left[\left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000\right]$$

where R is the ratio of the rare to the common isotope for the sample and standard. The long-term standard deviation measured for in-house quality-control standards was 0.2 ‰ for $\delta^{13}C$.

We used cryogenic vacuum distillation to extract water from all stem and wet soil samples. We conducted water extractions at Kansas State University using an open manifold system that allowed for removal of non-condensable gases and organic contaminants under vacuum (Nippert & Knapp 2007; Holdo *et al.* 2018). Water extractions yielded a total of 76 xylem water samples from trees, 44 from grasses, and 92 soil water samples. We analyzed these samples for $\delta D$ and $\delta^{18}O$ values using a Picarro L1102-*i* CRDS analyzer at the Stable Isotope Mass Spectrometry Laboratory at Kansas State University. Of the soil water samples, 14 could not be analyzed reliably due to insufficient sample amount, but we still had at least duplicate coverage of each standard soil depth sampled at each site. We assessed the potential for organic contamination of plant water samples using ChemCorrect software, and discarded any flagged samples. The δD and $\delta^{18}$O ratios were calculated as deviations from an international standard (V-SMOW) in parts per thousand (‰) using $\delta$-notation, as above. The long-term standard deviation for these measurements using in-house quality-control standards was <0.3 ‰ for δD and <0.15 ‰ for $\delta^{18}$O.

*Statistical analyses*

We conducted all statistical analyses in R version 3.3.2 (R Core Team 2016). To compare functional rooting depths based on water isotope values across sites, we modeled the relationship between soil water isotopes and depth, accounting for site-specific differences in soil water isotope profiles, to generate predicted estimates of mean water uptake depth from xylem water isotope values. This approach diverges from traditional isotopic mixing models (Ogle, Wolpert & Reynolds 2004; Parnell *et al.* 2010), but is nonetheless useful here, where the four "sources" at each site (depths from 5 to 50 cm) are not independent pools but rather fall along a monotonic continuum of isotopic variation, and where some sample values fell outside of the bounds of the sources, suggesting water uptake that was either shallower than 5 cm or deeper than 50 cm (Holdo & Nippert 2015; Holdo *et al.* 2018). Values of $\delta^{18}$O and δD were highly collinear across soil water profiles (Supplemental Figure S1), such that the first principal component (PC1) accounted for 91% of the variation in the two isotope values, so we used PC1 as a univariate indicator of isotopic variation (Holdo *et al.* 2018). PC1 declined linearly with log-transformed soil depth (Supplemental Figure S2). We modeled soil water depth as a function of PC1, site, and the interaction between PC1 and site, in a generalized linear modeling framework with a Gaussian log link (Supplemental Figure S3). By including both site and an interaction term between PC1 and site as predictors, we accounted for variation in the slope and intercept of enrichment profiles among sites. We used this model to infer average water uptake depth based on $\delta^{18}$O and $\delta$D for all tree and grass xylem water samples.

Because of substantial species turnover among sites, and a flexible sampling strategy that focused on whatever the dominant species were at each site (Table 1), there was only limited overlap in species identities across environmental gradients; we also note that species turnover could in itself be a signal of shifts in dominant rooting strategies, though this is difficult to disentangle. We statistically analyzed differences in plant water uptake depths across sites in two ways: first, a community-level approach ignoring species identity and analyzing water uptake depth in a linear modeling framework as a function of plant type (tree vs. grass), soil type, mean annual rainfall, and interactions between all predictor variables; and second, as a linear mixed effects model considering the same suite of predictor variables as fixed effects but adding species as a random effect. For both approaches, we selected the best set of predictor variables by comparing second-order Akaike Information Criterion ($AIC_c$) values of models containing all possible subset combinations of predictors. Mean annual rainfall was treated as a continuous variable and centered on the mean for all analyses, while plant type and soil type were treated as categorical predictors.

To determine proportional contributions of tree and grass fine roots to homogenized soil core samples, we used a simple two-end-member linear mixing model, calibrated for the dominant species present at each site. Within each site, all local grass and tree $\delta^{13}$C values were averaged to yield one mean end-member value per functional type. We assumed that the isotopic signature of each homogenized core sample (*M*) was a mixture of mean tree (*T*) and grass (*G*) isotopic signatures associated with the site, and thus calculated the proportional contribution of each on the basis of the mixing equation:

$$M = pT + (1-p)G$$

where $p$ is the proportion of the sample (by biomass) comprised of tree fine roots and $1-p$ is the proportion comprised of grass fine roots. While nearly all of the mixture values fell within the bounds of the end members, four were slightly outside the bounds of site-specific end-member means, yielding tree proportion values that were negligibly negative (between 0 and -0.032); these were set to zero (representing 100% grass content) for analysis. To estimate grass and tree fine-root biomass values, we multiplied these proportions by the total dry biomass found for each depth sample, and scaled up to units of g $m^{-3}$. We analyzed determinants of fine-root depth distributions in a linear modeling framework, with sample biomass modeled as a function of depth, soil type, mean annual rainfall, and interactions between soil and depth and rainfall and depth. We constructed separate models for total biomass, tree root biomass only, and grass root biomass only, and selected the preferred combination of predictor variables for each by comparing $AIC_c$ values of models.

## Results

### *Water uptake depths*

At all sites, soil water isotope composition showed consistent patterns with depth. Water from deeper soil layers was isotopically lighter, with more depleted (more negative) values of both $\delta^{18}O$ and $\delta D$, while shallow soil water had higher values of $\delta^{18}O$ and $\delta D$, suggesting evaporative enrichment at the soil surface (Figure 2). These consistent, monotonic gradients allowed for the use of natural abundances of soil water isotopes as a tracer for water uptake by depth, as described in Methods. To compare tree and grass functional rooting depths across sites, we used the isotopic composition of tree and grass xylem water to infer mean depths of water uptake, based on our modeled log-linear relationship between soil water isotope composition and depth (Supplemental Figures S2-3).

At all eight sites, tree xylem water was isotopically lighter than grass xylem water, indicating deeper soil water uptake by trees (Figure 2). Analyzed across sites, mean inferred tree functional rooting depths were always deeper than those of grasses, whether models explicitly accounted for species identity or not (Figure 3, Table 2, Supplemental Table S1). For both trees and grasses, functional rooting depths were generally deeper on sandy than clayey soil (Table 2, Supplemental Table S1). However, the degree of divergence between tree and grass roots varied with soil texture and mean annual rainfall. Tree water uptake was deeper and more distinct from shallow-rooted grass on sandy soils, and especially so at the wettest sites, as indicated by the three-way interaction between plant type, soil, and rainfall in community-level analyses that ignored species identity (Figure 3, Table 2, Supplemental Table S1). This three-way interaction term was not included in the best model when species identity was accounted for as a random effect, suggesting a potential confounding influence of species turnover on overall community-level patterns (Table S1, Figure S5). Both statistical modeling approaches favoured the inclusion of a two-way interaction between plant type and soil, indicating that trees consistently have a stronger deep-rooting response in sandy soils compared to coexisting grasses (Table 2, Supplemental Table S1).

### *Fine-root biomass allocation*

Total fine root biomass extracted from soil cores was higher per unit volume on clayey soils than sandy soils, but did not vary predictably with rainfall (Table S2). In general, total fine root biomass declined substantially with depth, from a mean of 2,238 ± 217 g $m^{-3}$ at 5 cm depth to 885 ± 103 g $m^{-3}$ at 45 cm on clayey soils, and from a mean of 1,742 ± 233 g $m^{-3}$ at 5 cm to 832 ± 115 g $m^{-3}$ at 45 cm on sandy soils.

Carbon-isotope signatures of homogenized roots collected from soil cores fell in between the isotopic values found for $C_3$ tree and $C_4$ grass end-members at each site, indicating a mixture of tree and grass fine roots throughout the soil column (Supplemental Figure S4). However, the proportional contribution of tree vs. grass varied widely across samples. We used these estimates of proportional contributions, multiplied by measurements of total fine root biomass by depth, to generate estimates of tree and grass fine root biomass in each sample (Figure 4).

Trees and grasses differed in how their fine-root distributions responded to soil texture. Regardless of soil texture or mean annual rainfall, grass fine-root biomass declined sharply with soil depth, with most grass roots concentrated close to the surface. Total grass fine-root biomass was higher on clayey soils than sandy soils, but soil texture did not meaningfully influence the slope of the biomass-depth relationship (Figure 4, Table S2). Total tree fine-root biomass was also higher on clayey than sandy soils. By contrast, however, while tree fine-root biomass showed a sharp decline from shallow to deep layers in clayey soils, similar to grass, tree fine roots were more evenly distributed throughout the soil column in sandy soils, indicating greater relative allocation of fine roots to deeper layers (Figure 4, Table S2). Neither tree nor grass fine-root distributions responded consistently to mean annual rainfall in the best statistical models, despite some apparent variation among sites (Figure 4, Table S2).

## Discussion

We found predictable relationships between environmental context and tree and grass rooting niches across Kruger National Park. In terms of functional rooting depth (corresponding to actual water uptake), trees consistently used deeper water than grasses, and trees and grasses both shifted their water use deeper on sandy soils. However, trees varied more with environmental context than grasses: on sandy soils and at wetter sites, trees used deeper water and differed more from grasses than on clayey soils and at drier sites. In terms of fine root biomass allocation (*i.e.*, where in the soil roots were located), soil texture determined not only the overall amount of fine root biomass (with greater total biomass on clayey soils), but also patterns of allocation by depth. Grasses consistently allocated most fine roots to shallow soil layers regardless of soil texture. By contrast, tree rooting varied depending on soil type, with shallow allocation of fine roots on clayey soils but a more even distribution of fine roots across soil depths on sandy soils. Meanwhile, mean annual rainfall had little effect on total biomass or allocation of fine roots. In sum, our results suggest that, while grasses in this system largely take up shallow water, trees employ divergent strategies depending on soil texture: competing more directly with grasses for shallow soil water on clayey soils, but accessing deeper soil moisture when available on sandy soils (Knoop & Walker 1985).

Savanna ecologists have long been interested in the extent to which trees and grasses overlap in their rooting niches. Though some studies have not found separation between tree and grass belowground resource use, particularly in mesic savannas (Ward *et al.* 2013), a common finding in semi-arid savannas is that, while trees and grasses may overlap in root distributions and water uptake, trees tend to use deeper soil water on average (Knoop & Walker 1985; Kulmatiski *et al.* 2010; Kambatuku *et al.* 2013; Ward *et al.* 2013; Holdo 2013; Holdo & Nippert

2015; Holdo *et al.* 2018). Theoretically, even subtle niche differences could stabilize coexistence (Chesson 2000; Adler, HilleRisLambers & Levine 2007; Holdo 2013). Here, our findings support the idea of niche differentiation between trees and grasses. Both water uptake and fine root allocation suggest that trees exploit deeper soils than grasses do. Across clay soils, the average water uptake depth of grasses and trees was 6 cm and 20 cm, respectively, while across sandy soils it was 9 and 36 cm. Moreover, where most previous assessments of savanna tree and grass root-niche differentiation have focused on one or two sites, our more extensive comparative approach allowed us to assess how these patterns vary across environmental gradients. By systematically comparing across eight sites, we found that soil texture and, to a lesser extent, mean annual rainfall structured the relative rooting strategies of trees and grasses in semi-arid savannas. This suggests that some unexplained heterogeneity between previous studies, which have disagreed widely about the extent and importance of root-niche differentiation (Ward *et al.* 2013), could be attributable to soil texture. It also suggests that the importance of root-niche differentiation may vary substantially across sites as a mechanism stabilizing tree-grass coexistence.

Theory predicts that mean annual rainfall should also influence optimal rooting strategies (Laio, D'Odorico & Ridolfi 2006; Holdo 2013), so it is notable here that soil texture mattered more than mean annual rainfall in determining differences in rooting strategy, particularly fine root allocation. This is consistent, however, with some other observations of Kruger vegetation, specifically that tree densities across the park vary predictably with soils but not with rainfall (Staver *et al.* 2017), and that total fine-root biomass responds much more strongly to soil type than to rainfall (Kulmatiski, Sprouse & Beard 2017). Recent observational work across southern Africa has also found that tree coarse root investment with depth responds to soil texture but not mean annual rainfall (Zhou *et al.* 2020). In our consideration of tree and grass rooting strategies here, it could be that the rainfall gradient sampled was simply too narrow to observe meaningful differences. Mesic savannas are more limited by disturbance feedbacks than by water availability (Sankaran *et al.* 2005; Bond 2008; Staver, Archibald & Levin 2011), such that disturbances may play a stronger role in shaping rooting strategies (Tomlinson *et al.* 2012; Bhattachan *et al.* 2012; Wigley *et al.* 2019), likely resulting in departures in root allocation by depth from those we observed across this semi-arid rainfall gradient (Ward *et al.* 2013). But there are other possibilities. Given high inter-annual rainfall variability in savannas, soil texture is a more consistent feature of the environment from year to year than rainfall, and thus perhaps more likely to structure lasting community-level differences in root biomass allocation. The north of Kruger receives less rainfall on average, but rainfall is nonetheless highly variable, and some years, the rainfall gradient inverts (such as during the 2014-2016 drought, but also within the bounds of more typical rainfall variation; Staver *et al.* 2019).

In contrast to the fine-root biomass results, we did find that water-uptake patterns varied along the rainfall gradient, with the deepest water uptake by trees at the wetter sites on sandy soils. It is worth considering in some detail the fact that this contrasts with previous work by Holdo *et al.* (2018) in Kruger, which examined water-uptake patterns across the rainfall gradient on sandy soils only, and found instead that trees used shallower water at sites with higher mean annual rainfall. A few factors could be driving these differences. First, the previous study sampled more extensively across the rainfall gradient, including sites in the wettest parts of the park, which were not considered here because they lacked a soil-texture contrast. That study also focused on intraspecific variation in three focal species, whereas the community-level approach used here incorporates turnover in dominant species between sites; when we accounted for species identity, the interactive effect of rainfall was less important. Temporal variability also likely contributes to divergent observations of rooting strategy, both between studies of water uptake conducted in different years, and between the water uptake and fine-root biomass observations reported here. Water-uptake measurements provide a snapshot of functional rooting depth (Nippert & Holdo 2015), which depends on root activity and root traits as well as contemporaneous soil-moisture availability, and has been found to fluctuate seasonally in trees (Kulmatiski & Beard 2013a). Though fine-root biomass distributions can also shift temporally, they integrate root allocation over longer time scales, without distinguishing which roots are most often active, such that a mismatch between these measurements is not surprising (Kulmatiski *et al.* 2010; Nippert & Holdo 2015). In the long term, allocation of roots throughout the soil profile will constrain the extent to which plants can adopt a flexible strategy of shifting to deeper water uptake depending on soil moisture conditions (Kulmatiski & Beard 2013a). Our results suggest that trees on sandy soils, which invest more heavily in an infrastructure of deep roots (Zhou *et al.* 2020), are best equipped to deploy such flexibility. Trees on clayey soils, and grasses at all sites, invested predominantly in shallower fine roots, and also varied less widely across sites in water uptake depths. Further monitoring of seasonal variation in root activity across sites, paired with explicit measurements of soil moisture and transpiration, could provide more detailed insight into these dynamics (Mazzacavallo & Kulmatiski 2015).

The major finding here, that soil texture is a key determinant of root-niche separation between trees and grasses in savannas, can help explain existing savanna vegetation patterns, while also improving predictions of vegetation change. A better ability to use deeper soil moisture and mitigate competition with grass could enhance tree performance on sandy soils, likely contributing to widely observed patterns of higher tree cover (Williams *et al.* 1996;

Sankaran, Ratnam & Hanan 2008; Fensham, Butler & Foley 2015; Staver *et al.* 2017) and faster rates of woody encroachment (O'Connor, Puttick & Hoffman 2014; Case & Staver 2017) on sandy soils, though rocky soils can also have higher rates of encroachment (Britz & Ward 2007). Soil texture also mediates the relationship between tree cover and rainfall intensity, where trees respond more positively to infrequent, intense rainfall regimes on sandy soils (Case & Staver 2018); this, too, could be an outcome of variation in the degree of tree vs. grass root-niche differentiation with soil texture. As climate change causes shifts in the frequency and intensity of rainfall (Fischer *et al.* 2013; Westra *et al.* 2014), differences in rooting strategies could drive heterogeneous vegetation responses, with trees increasingly favored on sandy soils, but less able to maintain populations on clayey soils where competition with grass is more intense.

## Acknowledgements

We gratefully acknowledge the support of South African National Parks staff, especially Tercia Strydom, in making this work possible. We also thank Phumlani Zwane and Fergus O'Brien for field assistance, Tom Murray and Zachary Gold for laboratory assistance, Brad Erkkila for help with stable isotope analyses, and members of the Staver lab at Yale University as well as two anonymous reviewers for feedback on this manuscript. MFC was supported by a National Science Foundation Graduate Research Fellowship and by grants from Yale University (MacMillan International Dissertation Fellowship, E&EB Graduate Student Chair's Fund, YIBS Doctoral Dissertation Improvement Grant, and Yale Analytical and Stable Isotope Center Matching Funds). JBN was supported by NSF-DEB 1928875.

## Author contributions

All authors contributed to conceiving ideas and designing methodology. MFC collected field samples; MFC and JBN performed laboratory analyses; and MFC and ACS analyzed data. MFC led the writing of the manuscript, and all authors contributed feedback on drafts and gave final approval for publication.

## Data availability

Data will be made publicly available via the South African National Parks Data Repository upon publication.

**Table 1.** Site characteristics and dominant species sampled at each site.

| **Site code** | **Soil type** | **Mean annual rainfall (mm)** | **Tree species** | **Grass species** |
|---|---|---|---|---|
| SIR | sandy | 494 | *Colophospermum mopane*<br>*Combretum apiculatum*<br>*Terminalia sericea* | *Digitaria eriantha*<br>*Eragrostis rigidior*<br>*Urochloa mosambicensis* |
| SHI | clayey | 477 | *Colophospermum mopane*<br>*Flueggea virosa*<br>*Terminalia prunioides* | *Bothriochloa insculpta*<br>*Enneapogon cenchroides* |
| MOO | sandy | 472 | *Colophospermum mopane*<br>*Combretum apiculatum*<br>*Terminalia sericea* | *Eragrostis rigidior*<br>*Pogonarthria squarrosa* |
| CAP | clayey | 472 | *Colophospermum mopane*<br>*Combretum hereroense*<br>*Combretum imberbe* | *Aristida congesta*<br>*Panicum coloratum*<br>*Themeda triandra* |
| MUZ | sandy | 515 | *Senegalia nigrescens*<br>*Combretum apiculatum*<br>*Terminalia sericea* | *Panicum maximum*<br>*Pogonarthria squarrosa*<br>*Urochloa mosambicensis* |
| SAT | clayey | 508 | *Combretum apiculatum*<br>*Dichrostachys cinerea*<br>*Lannea schweinfurthii* | *Bothriochloa insculpta*<br>*Panicum maximum*<br>*Urochloa mosambicensis* |
| SKU | sandy | 597 | *Combretum apiculatum*<br>*Euclea divinorum*<br>*Peltophorum africanum*<br>*Terminalia sericea* | *Aristida congesta*<br>*Eragrostis rigidior*<br>*Sporobolus panicoides* |
| OSA | clayey | 600 | *Dichrostachys cinerea*<br>*Grewia bicolor*<br>*Grewia virosa*<br>*Ziziphus mucronata* | *Bothriochloa insculpta*<br>*Panicum maximum*<br>*Urochloa mosambicensis* |

**Table 2.** Estimated model coefficients for the top-ranked linear model describing functional rooting depths across sites (Table S1). Grass vs. tree, and sandy vs. clayey soil, were included as categorical predictors. Mean annual rainfall (MAR), a continuous variable, is in units of mm and was mean-centered to improve interpretability of interaction terms. Responses are in units of centimeters below the surface, where more *negative* numbers denote shallower functional rooting depths, and more *positive* numbers are deeper. For overall model, $R^2 = 0.51$, $F_{7,113} = 18.78$, $p < 0.001$.

| **Variable** | **Coefficient** | **Standard dev.** | ***t*** | ***p*** |
|---|---|---|---|---|
| Intercept | 20.51 | 2.19 | 9.353 | <0.001 |
| *Main effects* | | | | |
| Type=grass | -14.18 | 3.54 | -4.002 | <0.001 |
| Soil=sandy | 14.52 | 3.07 | 4.734 | <0.001 |
| MAR | -0.09 | 0.04 | -2.179 | 0.031 |
| *Interactions* | | | | |
| Grass x sandy soil | -11.43 | 5.03 | -2.273 | 0.025 |
| Grass x MAR | 0.06 | 0.07 | 0.953 | 0.342 |
| Sandy x MAR | 0.32 | 0.06 | 5.474 | <0.001 |
| Grass x sandy x MAR | -0.25 | 0.10 | -2.481 | 0.015 |

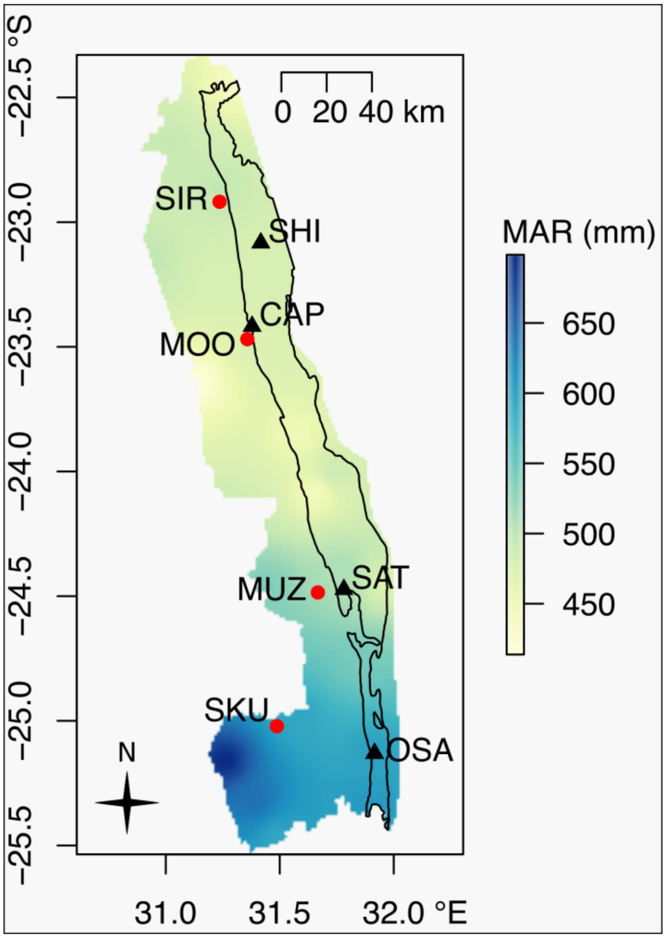

**Figure 1.** Map of study sites within Kruger National Park. Samples were collected at four sites on sandy granitic soils (red circles) and four sites on clayey basaltic soils (black triangles), paired along the rainfall gradient, which are labeled here with three-letter location codes (Table 1). The black outline denotes areas with clayey basaltic soils within the park. Yellow-to-blue shading shows mean annual rainfall, from an inverse-distance-weighted interpolation of long-term rain gauge records (Staver *et al.* 2019).

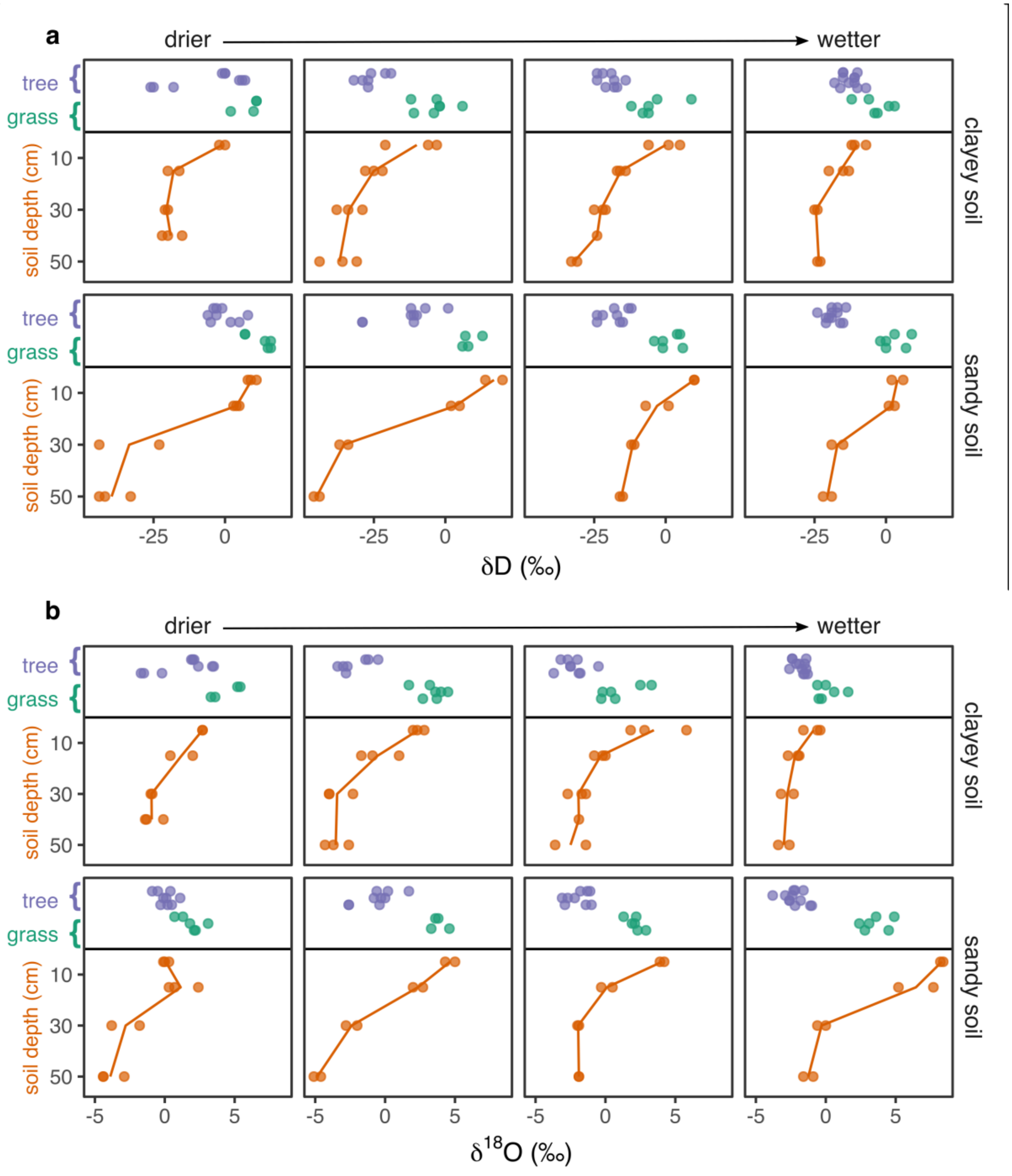

**Figure 2.** Hydrogen (a) and oxygen (b) stable isotopic composition of soil and xylem water samples. Each panel represents one site, with sites arranged by mean annual rainfall (increasing from left to right) and soil texture (clayey basaltic soils on top, sandy granitic soils below). Points correspond to tree species (purple), grass species (green), and soil layers by depth (orange), with tree and grass points horizontally aligned by species.

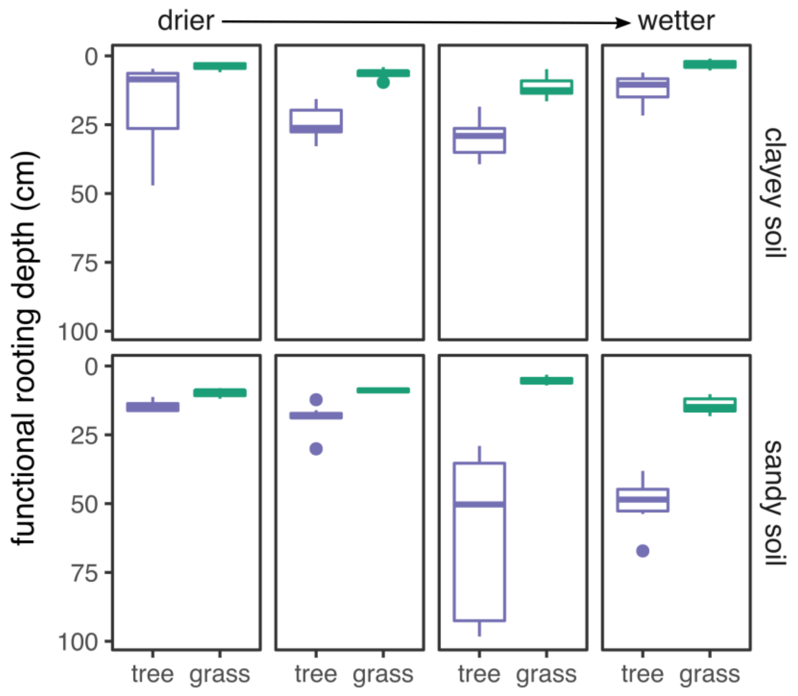

**Figure 3.** Inferred functional rooting depth values for trees and grasses across sites. Each panel represents one site, with sites arranged by mean annual rainfall (increasing from left to right) and soil texture (clayey basaltic soils on top, sandy granitic soils below).

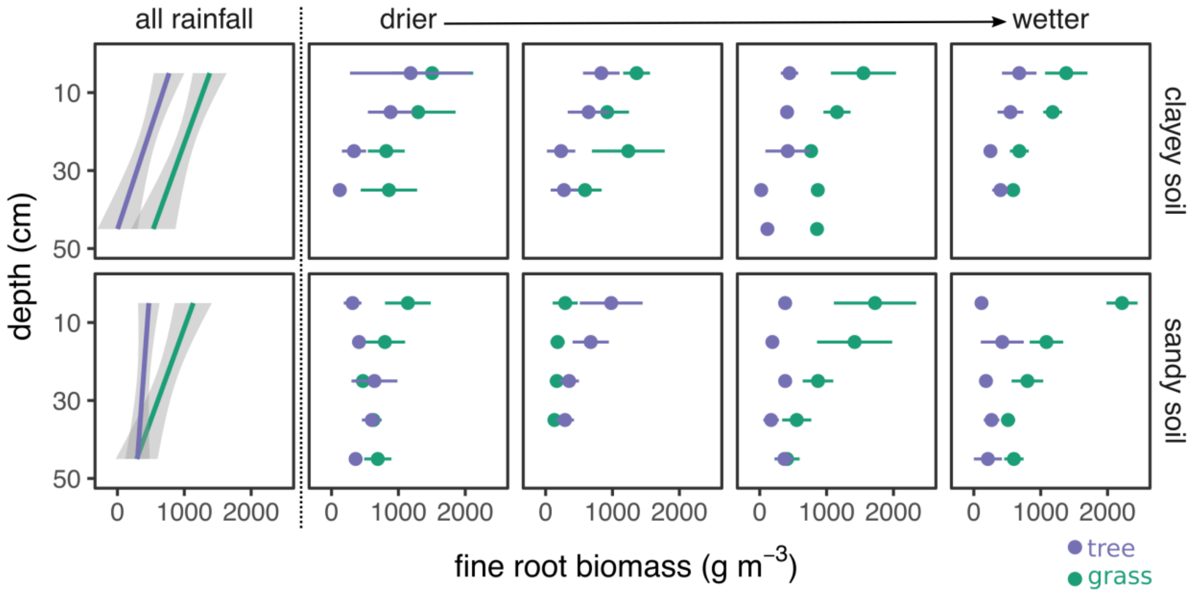

**Figure 4.** Vertical distribution of grass (green) and tree (purple) fine root biomass across sites, as estimated from fine root biomass measurements and $\delta^{13}$C mixing-model analysis. Panels on the left summarize the overall relationship between depth and fine root biomass, across all rainfall, as predicted by a separate linear model for each plant type; for grass, the best model (selected by AIC$_c$) is one where the biomass vs. depth relationship differs in the intercept but not the slope when compared across soil textures ($R^2 = 0.2231$, $F_{2,98} = 15.36$, $p < 0.001$), while for trees the intercept and slope both vary between clayey soils (top) and sandy soils (bottom) ($R^2 = 0.1268$, $F_{3,97} = 5.841$, $p = 0.001$). To the right, site-level data are shown, where each panel corresponds to a study site, arranged by soil texture (clayey soils on top, sandy soils below) and mean annual rainfall (increasing from left to right). Dots and lines depict mean and standard error of fine root biomass at each soil depth.

## References

Adler, P.B., HilleRisLambers, J. & Levine, J.M. (2007) A niche for neutrality. *Ecology Letters*, **10**, 95–104.

Berry, R.S. & Kulmatiski, A. (2017) A savanna response to precipitation intensity. *PLoS ONE*, **12**, e0175402–18.

Bhattachan, A., Tatlhego, M., Dintwe, K., O'Donnell, F., Caylor, K.K., Okin, G.S., Perrot, D.O., Ringrose, S. & D'Odorico, P. (2012) Evaluating Ecohydrological Theories of Woody Root Distribution in the Kalahari (ed HYH Chen). *PLoS ONE*, **7**, e33996.

Bond, W.J. (2008) What limits trees in C4 grasslands and savannas? *Annual Review of Ecology, Evolution and Systematics*, **39**, 641–659.

Britz, M.-L. & Ward, D. (2007) Dynamics of woody vegetation in a semi-arid savanna, with a focus on bush encroachment. *African Journal of Range & Forage Science*, **24**, 131–140.

Buitenwerf, R., Kulmatiski, A. & Higgins, S.I. (2014) Soil water retention curves for the major soil types of the Kruger National Park. *Koedoe*, **56**, 1–9.

Case, M.F. & Staver, A.C. (2017) Fire prevents woody encroachment only at higher-than-historical frequencies in a South African savanna. *Journal of Applied Ecology*, **54**, 955–962.

Case, M.F. & Staver, A.C. (2018) Soil texture mediates tree responses to rainfall intensity in African savannas. *New Phytologist*, **219**, 1363–1372.

Chesson, P. (2000) Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, **31**, 343–366.

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.

February, E.C., Cook, G.D. & Richards, A.E. (2013a) Root dynamics influence tree-grass coexistence in an Australian savanna. *Austral Ecology*, **38**, 66–75.

February, E.C., Higgins, S.I., Bond, W.J. & Swemmer, L. (2013b) Influence of competition and rainfall manipulation on the growth responses of savanna trees and grasses. *Ecology*, **94**, 1155–1164.

Fensham, R.J., Butler, D.W. & Foley, J. (2015) How does clay constrain woody biomass in drylands? *Global Ecology and Biogeography*, **24**, 950–958.

Fischer, E.M., Beyerle, U. & Knutti, R. (2013) Robust spatially aggregated projections of climate extremes. *Nature Climate Change*, **3**, 1033–1038.

Govender, N., Trollope, W.S.W. & van Wilgen, B.W. (2006) The effect of fire season, fire frequency, rainfall and management on fire intensity in savanna vegetation in South Africa.

*Journal of Applied Ecology*, **43**, 748–758.

Hipondoka, M.H.T., Aranibar, J.N., Chirara, C., Lihavha, M. & Macko, S.A. (2003) Vertical distribution of grass and tree roots in arid ecosystems of Southern Africa: niche differentiation or competition? *Journal of Arid Environments*, **54**, 319–325.

Holdo, R.M. (2013) Revisiting the Two-Layer Hypothesis: Coexistence of Alternative Functional Rooting Strategies in Savannas. *PLoS ONE*, **8**, e69625.

Holdo, R.M. & Nippert, J.B. (2015) Transpiration dynamics support resource partitioning in African savanna trees and grasses. *Ecology*, **96**, 1466–1472.

Holdo, R.M., Nippert, J.B. & Mack, M.C. (2018) Rooting depth varies differentially in trees and grasses as a function of mean annual rainfall in an African savanna. *Oecologia*, **186**, 269–280.

Kambatuku, J.R., Cramer, M.D. & Ward, D. (2013) Overlap in soil water sources of savanna woody seedlings and grasses. *Ecohydrology*, **6**, 464–473.

Knapp, A.K., Beier, C., Briske, D.D., Classen, A.T., Luo, Y., Reichstein, M., Smith, M.D., Smith, S.D., Bell, J.E., Fay, P.A., Heisler, J.L., Leavitt, S.W., Sherry, R., Smith, B. & Weng, E. (2008) Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience*, **58**, 811–821.

Knoop, W.T. & Walker, B.H. (1985) Interactions of woody and herbaceous vegetation in a Southern African savanna. *Journal of Ecology*, **73**, 235.

Kraaij, T. & Ward, D. (2006) Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. *Plant Ecology*, **186**, 235–246.

Kulmatiski, A. & Beard, K.H. (2013a) Root niche partitioning among grasses, saplings, and trees measured using a tracer technique. *Oecologia*, **171**, 25–37.

Kulmatiski, A. & Beard, K.H. (2013b) Woody plant encroachment facilitated by increased precipitation intensity. *Nature Climate Change*, **3**, 833–837.

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.

Kulmatiski, A., Sprouse, S.R.C. & Beard, K.H. (2017) Soil type more than precipitation determines fine-root abundance in savannas of Kruger National Park, South Africa. 1–11.

Laio, F., D'Odorico, P. & Ridolfi, L. (2006) An analytical model to relate the vertical root distribution to climate and soil properties. *Geophysical Research Letters*, **33**.

Le Roux, X. & Bariac, T. (1998) Seasonal variations in soil, grass and shrub water status in a West African humid savanna. *Oecologia*, **113**, 456–466.

Mazzacavallo, M.G. & Kulmatiski, A. (2015) Modelling Water Uptake Provides a New Perspective on Grass and Tree Coexistence (ed M Germino). *PLoS ONE*, **10**, e0144300–16.

McCormack, M.L. & Iversen, C.M. (2019) Physical and Functional Constraints on Viable Belowground Acquisition Strategies. *Frontiers in Plant Science*, **10**, 940–12.

Mordelet, P., Menaut, J.C. & Mariotti, A. (1997) Tree and grass rooting patterns in an African humid savanna. *Journal of Vegetation Science*, **8**, 65–70.

Nippert, J.B. & Holdo, R.M. (2015) Challenging the maximum rooting depth paradigm in grasslands and savannas. *Functional Ecology*, **29**, 739–745.

Nippert, J.B. & Knapp, A.K. (2007) Linking water uptake with rooting patterns in grassland species. *Oecologia*, **153**, 261–272.

O'Connor, T.G., Puttick, J.R. & Hoffman, M.T. (2014) Bush encroachment in southern Africa: changes and causes. *African Journal of Range & Forage Science*, **31**, 67–88.

Ogle, K., Wolpert, R.L. & Reynolds, J.F. (2004) Reconstructing plant root area and water uptake profiles. *Ecology*, **85**, 1967–1978.

O'Keefe, K. & Nippert, J.B. (2018) Drivers of nocturnal water flux in a tallgrass prairie. *Functional Ecology*, **32**, 1155–1167.

Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A.L. (2010) Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE*, **5**, e9672.

R Core Team. (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Riginos, C. (2009) Grass competition suppresses savanna tree growth across multiple demographic stages. *Ecology*, **90**, 335–340.

Sankaran, M., Hanan, N.P., Scholes, R.J., Ratnam, J., Augustine, D.J., Cade, B.S., Gignoux, J., Higgins, S.I., Le Roux, X., Ludwig, F., Ardo, J., Banyikwa, F., Bronn, A., Bucini, G., Caylor, K.K., Coughenour, M.B., Diouf, A., Ekaya, W., Feral, C.J., February, E.C., Frost, P.G.H., Hiernaux, P., Hrabar, H., Metzger, K.L., Prins, H.H.T., Ringrose, S., Sea, W., Tews, J., Worden, J. & Zambatis, N. (2005) Determinants of woody cover in African savannas. *Nature*, **438**, 846–849.

Sankaran, M., Ratnam, J. & Hanan, N. (2008) Woody cover in African savannas: the role of resources, fire and herbivory. *Global Ecology and Biogeography*, **17**, 236–245.

Schenk, H.J. & Jackson, R.B. (2002a) Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. *Journal of Ecology*, **90**, 480–494.

Schenk, H.J. & Jackson, R.B. (2002b) The global biogeography of roots. *Ecological*

*Monographs*, **72**, 311–328.

Staver, A.C., Archibald, S. & Levin, S. (2011) Tree cover in sub-Saharan Africa: Rainfall and fire constrain forest and savanna as alternative stable states. *Ecology*, **92**, 1063–1072.

Staver, A.C., Botha, J. & Hedin, L. (2017) Soils and fire jointly determine vegetation structure in an African savanna. *New Phytologist*, **15**, 613–10.

Staver, A.C., Wigley-Coetsee, C. & Botha, J. (2019) Grazer movements exacerbate grass declines during drought in an African savanna. *Journal of Ecology*, **107**, 1482–1491.

Tomlinson, K.W., Sterck, F.J., Bongers, F., da Silva, D.A., Barbosa, E.R.M., Ward, D., Bakker, F.T., van Kaauwen, M., Prins, H.H.T., de Bie, S. & van Langevelde, F. (2012) Biomass partitioning and root morphology of savanna trees across a water gradient. *Journal of Ecology*, **100**, 1113–1121.

Vadigi, S. & Ward, D. (2014) Herbivory effects on saplings are influenced by nutrients and grass competition in a humid South African savanna. *Perspectives in Plant Ecology, Evolution and Systematics*, **16**, 11–20.

Valverde Barrantes, O.J., Freschet, G.T., Roumet, C. & Blackwood, C.B. (2017) A worldview of root traits: the influence of ancestry, growth form, climate and mycorrhizal association on the functional trait variation of fine-root tissues in seed plants. *New Phytologist*, **215**, 1562–1573.

Verweij, R.J.T., Higgins, S.I., Bond, W.J. & February, E.C. (2011) Water sourcing by trees in a mesic savanna: Responses to severing deep and shallow roots. *Environmental and Experimental Botany*, **74**, 229–236.

Walker, B.H. & Noy-Meir, I. (1982) Aspects of the stability and resilience of savanna ecosystems. *Ecology of Tropical Savannas*, Ecological Studies, pp. 556–590. Springer Berlin Heidelberg, Berlin, Heidelberg.

Walter, H. (1939) Grasland, Savanne und Busch der arideren Teile Afrikas in ihrer o¨ kologischen Bedingtheit. *Jahrb Wiss Bot*, **87**, 750–860.

Walter, H. (1971) Ecology of Tropical and Subtropical Vegetation. *Soil Science*, **121**, 317.

Ward, D. & Esler, K.J. (2011) What are the effects of substrate and grass removal on recruitment of Acacia mellifera seedlings in a semi-arid environment? *Plant Ecology*, **212**, 245–250.

Ward, D., Wiegand, K. & Getzin, S. (2013) Walter's two-layer hypothesis revisited: Back to the roots! *Oecologia*, **172**, 617–630.

Westra, S., Fowler, H.J., Evans, J.P., Alexander, L.V., Berg, P., Johnson, F., Kendon, E.J., Lenderink, G. & Roberts, N.M. (2014) Future changes to the intensity and frequency of short-duration extreme rainfall. *Reviews of Geophysics*, **52**, 522–555.

Wigley, B.J., Staver, A.C., Zytkowiak, R., Jagodzinski, A.M. & Wigley-Coetsee, C. (2019) Root trait variation in African savannas. *Plant and Soil*, **441**, 555–565.

Williams, R.J., Duff, G.A., Bowman, D.M.J.S. & Cook, G.D. (1996) Variation in the composition and structure of tropical savannas as a function of rainfall and soil texture along a large-scale climatic gradient in the Northern Territory, Australia. *Journal of Biogeography*, **23**, 747–756.

Xu, X., Medvigy, D. & Rodriguez-Iturbe, I. (2015) Relation between rainfall intensity and savanna tree abundance explained by water use strategies. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 201517382–5.

Zhou, Y., Wigley, B.J., Case, M.F., Coetsee, C. & Staver, A.C. (2020) Rooting depth as a key woody functional trait in savannas. *New Phytologist*, nph.16613–13.