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# Rooting depth varies differentially in trees and grasses as a function of mean annual rainfall in an African savanna

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## Abstract

A significant fraction of the terrestrial biosphere comprises biomes containing tree–grass mixtures. Forecasting vegetation dynamics in these environments requires a thorough understanding of how trees and grasses use and compete for key belowground resources. There is disagreement about the extent to which tree–grass vertical root separation occurs in these ecosystems, how this overlap varies across large-scale environmental gradients, and what these rooting differences imply for water resource availability and tree–grass competition and coexistence. To assess the extent of tree–grass rooting overlap and how tree and grass rooting patterns vary across resource gradients, we examined landscape-level patterns of tree and grass functional rooting depth along a mean annual precipitation (MAP) gradient extending from ~ 450 to ~ 750 mm year$^{-1}$ in Kruger National Park, South Africa. We used stable isotopes from soil and stem water to make inferences about relative differences in rooting depth between these two functional groups. We found clear differences in rooting depth between grasses and trees across the MAP gradient, with grasses generally exhibiting shallower rooting profiles than trees. We also found that trees tended to become more shallow-rooted as a function of MAP, to the point that trees and grasses largely overlapped in terms of rooting depth at the wettest sites. Our results reconcile previously conflicting evidence for rooting overlap in this system, and have important implications for understanding tree–grass dynamics under altered precipitation scenarios.

**Keywords** African savanna · Environmental gradients · Tree–grass coexistence · Two-layer model · Stable isotopes

## Introduction

Resolving where in the soil profile plant roots acquire water is important for understanding plant performance and for predicting vegetation dynamics in plant communities with diverse rooting strategies (Nippert and Holdo 2015; Ogle et al. 2004; Schenk and Jackson 2002). The distribution of

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soil moisture as a function of depth is strongly influenced by soil texture and precipitation patterns (Guswa et al. 2002). Both of these factors vary across space, but precipitation regimes can also shift over time, e.g., as a result of climate change (Kulmatiski and Beard 2013b). Changes in precipitation regimes may thus have important consequences for plant communities, which could follow different trajectories (in terms of community composition) depending on the relative dominance of shallow vs. deep-rooted species (Kulmatiski and Beard 2013b). The importance of rooting depth is particularly relevant in the savanna biome, where two functional groups (trees and C$_4$ grasses) that differ in terms of their water relations (Scholes and Walker 1993) coexist within a broad precipitation envelope. Much of the past research on tree–grass water use in savannas has focused on rooting separation between these two functional groups and its role in enabling coexistence between them (Kambatuku et al. 2013; Walter 1971; Ward et al. 2013). To the best of our knowledge, however, no systematic studies of tree and grass rooting depth have been undertaken across the savanna precipitation gradient.

The 'two-layer' niche-partitioning hypothesis (Walter 1971) proposes that while grasses access soil moisture most efficiently from shallow soil layers, trees primarily rely on deeper water (Walker and Noy-Meir 1982). Since it was originally articulated, the two-layer hypothesis has been the focus of a large number of studies (reviewed in Ward et al. 2013), but the evidence for differential rooting between trees and grasses has been mixed. While many studies have found little or no evidence to support differences in rooting depth between trees and grasses (February and Higgins 2010; Hipondoka et al. 2003; Mordelet et al. 1997; Roux et al. 1995), others have found the opposite, particularly in temperate ecosystems (Brown and Archer 1990; Dodd et al. 1998; Nippert and Knapp 2007b; Sala et al. 1989; Weltzin and McPherson 1997). Potential reasons for the mixed evidence for vertical root separation include: the fact that the environmental conditions that promote or enable separation may vary from site to site (Ward et al. 2013); that studies vary in terms of methodology; and that they tend to be limited to single sites. In terms of the first issue, of particular interest is the role of precipitation as a driver of vertical root separation (Holdo 2013), especially in tropical savannas. This is because in the tropical savanna biome, mean annual precipitation (MAP) imposes an upper limit on woody cover (Bucini and Hanan 2007; Sankaran et al. 2005), and therefore emerges as a fundamental factor driving tree–grass competitive dynamics. In a continental-scale study of woody cover in the African savanna biome, Sankaran et al. (2005) argued that below an MAP threshold of approximately 650 mm year$^{-1}$, savannas are inherently stable and "climatically determined", with water limitation being the main driver of tree–grass ratios. Above this threshold, savannas are hypothesized to be bistable and limited by disturbance (Sankaran et al. 2005). Implicit in this hypothesis is the notion that important changes in tree–grass interactions and competition for water occur across this threshold. Some of these changes could be driven by systematic variation in tree–grass rooting depth. For example, if differences in rooting depth between trees and grasses diminish as a function of MAP, this may lead to a reduction in the extent of niche partitioning and competitive exclusion of grasses—in the absence of disturbance—above the Sankaran threshold.

A second challenge is that different methods have been used for quantifying rooting depth, but these do not necessarily provide suitable quantitative metrics for cross-site comparisons. Many studies have used structural metrics of root density or mass as a function of depth (Hipondoka et al. 2003; Holdo and Timberlake 2008; Mordelet et al. 1997), and focused on maximum rooting depth (MRD) as a key variable. A global meta-analysis of studies of MRD by Schenk and Jackson (2002) concluded that MRD increases as a function of MAP for grasses, but is insensitive to MAP for trees.

This supports the idea that vertical root separation is greater at the dry than the wet end of the spectrum (Schenk and Jackson 2002). It is unclear, however, to what extent MRD alone reflects integrated patterns of uptake across the entire root profile, given that root profiles are highly variable in terms of their depth distribution of biomass (Nippert and Holdo 2015). Furthermore, purely structural metrics such as root mass distributions may not necessarily reflect functional (with respect to water uptake) rooting profiles (Kulmatiski et al. 2010; Nippert et al. 2012). An alternative to structural metrics is provided by isotope-based methods, which exploit depth-related variation in the natural abundance of O and H stable isotopes in soil water to provide inference on patterns of plant water uptake (Nippert and Knapp 2007a; Ogle et al. 2004; Weltzin and McPherson 1997). The stable isotope approach allows the reconstruction of functional (as opposed to structural) rooting profiles (Ogle et al. 2004), which are bound to be more ecologically relevant for understanding niche partitioning than structural profiles. In the present study we use the term "rooting profile" specifically in the context of water uptake and the relative contribution of shallow and deep water sources to the plant isotopic signature.

To gain a more comprehensive understanding of the extent of tree–grass vertical root separation over large spatial scales, we conducted a standardized study of tree and grass relative rooting depth that extended along a 340-km N–S transect across nine sites in a tropical/subtropical savanna in Kruger National Park, South Africa. As is the case at a continental or global scale, evidence for vertical root separation in the Kruger savanna ecosystem has been mixed, with evidence both for (Kulmatiski and Beard 2013a, b; Nippert and Holdo 2015) and against (February and Higgins 2010; Verweij et al. 2011) separation. It is difficult to draw any clear conclusions about the relationship between differences in tree vs. grass rooting depth and MAP, however, because studies conducted to date have tended to concentrate in the wettest portion of the park. In this study, we used stable isotopes from soil and plant water collected from grasses and three species of savanna trees along the Kruger MAP gradient to investigate tree–grass vertical root separation in two different years. We tested two main hypotheses: (1) grasses have consistently shallower mean rooting depths than trees; and (2) trees and grasses exhibit divergent rooting depth patterns as a function of MAP.

## Materials and methods

### Study site

Soil and plant samples were collected in Kruger National Park (KNP hereafter), South Africa. KNP extends over ~ 20,000 km$^2$ within the tropical and subtropical
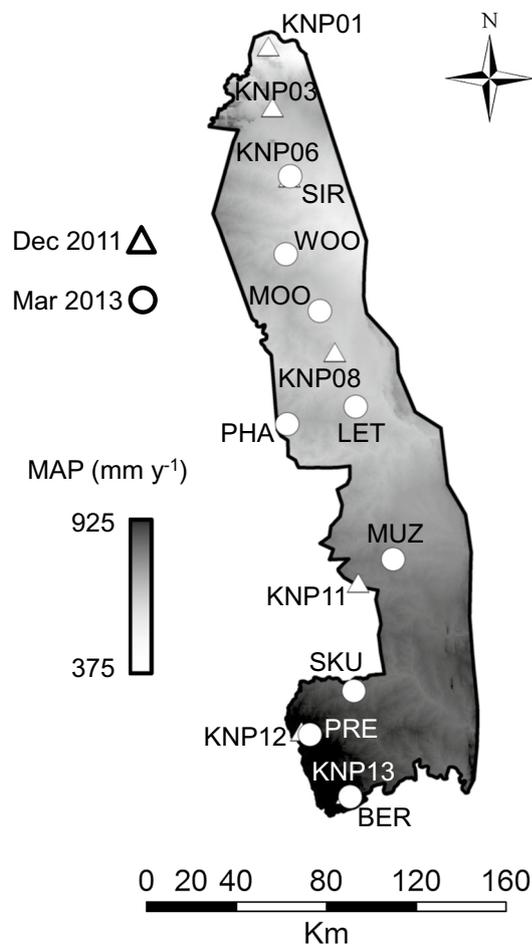
lowveld (low-altitude) savanna biome in Eastern South Africa (Fig. 1). Rainfall is highly seasonal (the wet season spans the period October through May), with mean annual precipitation (MAP) ranging between < 400 mm in the northern sector of the park and > 750 mm in the Pretoriuskop area in southern KNP (Holdo and Mack 2014; Venter et al. 2003). This rainfall gradient is bisected perpendicularly by a geomorphological transition, with granitic-derived soils in the West and basalt dominating in the East (Venter et al. 2003). Our study took place on the crests of catenas on granite, where soils tend to be nutrient-poor (total C < 1%, total N < 0.1%; Holdo and Mack, unpublished data) and sandy, with a clay content < 10% (Venter et al. 2003). The woody component of the vegetation is dominated by broad-leaved tree species in the Combretaceae and Caesalpiniaceae, whereas the herbaceous layer consists of a relatively



**Fig. 1** Map of Kruger National Park, South Africa, showing sampling location sites for stable isotope study of functional root separation in trees and grasses in Dec 2011 and Mar 2013. The Mean Annual Precipitation (MAP) scale is based on long-term Bioclim data. The 2011 sites (triangles) are labeled with the prefix 'KNP' and two-digit numbers; the 2013 sites (circles) were given three-letter codes corresponding to nearby rain gauges

continuous cover of mostly perennial $C_4$ grasses such as *Eragrostis* spp., *Aristida* spp. and *Themeda triandra*. The tree layer in the Northern section of the park is dominated by *Colophospermum mopane* (Online Resource 1). Although quantifying tree biomass across KNP was beyond the scope of this study, we note that there is no clear evidence of an upper limit to tree cover below a MAP of 650 mm in this system, as suggested by the Sankaran curve (Sankaran et al. 2005), and no relationship between tree basal area and MAP within our sample (Online Resource 1).

## Field sampling and laboratory analyses

To test for variation in rooting differences across the KNP rainfall gradient, we sampled seven sites in 2011 and nine in 2013 along the N–S axis of the park (Fig. 1, Online Resource 1). To identify potential sites, we stratified the park into MAP bands in ArcMap 10.0 using WorldClim data (http://www.worldclim.org), which captures global long-term (50-year) global precipitation and temperature means at a global scale. We used site-specific MAP values from the same dataset in subsequent analyses. Once in the field, we used these potential locations as starting points and drove along existing roads to the top of the nearest catena. We then walked perpendicularly to the road for 100–200 m until we found a site containing at least two of our focal tree species, which were *Terminalia sericea* and *Combretum apiculatum* in the Combretaceae and *C. mopane* in the Caesalpiniaceae. We chose these species a priori because they are widespread and highly representative of the woody layer in lowveld savanna, although we note that their contribution to total tree basal area across the ecosystem is highly variable (Online Resource 1). We restricted our sampling to the crests of topographic catenas to minimize variation in vegetation, soil moisture distribution and soils introduced by topographic factors.

Our sampling took place in December 2011 and March 2013, during the first and second halves of the wet season, respectively. Our site and tree selection processes differed slightly in 2011 and 2013. In 2011 we sampled trees of our target species haphazardly within an area of about 100 m$^2$. In 2013, we identified sites that were within 5 km of established rain gauges. Some of these sites overlapped broadly (i.e., they were within a few hundred m of each other) with 2011 sites in terms of general catena position (Fig. 1), but different sets of individual trees were sampled in each period. Whereas 2011 sites were given numeric codes, the 2013 sites were given three-letter codes corresponding to their respective rain gauges, even though we ultimately did not use the gauge data due to significant data gaps. To select trees in 2013, we first identified and marked candidate trees and then sampled a random subset of the marked individuals. In both years we focused on trees with a basal

diameter range between 5 and 10 cm to control for potential effects on rooting depth introduced by tree size. This diameter range fell within the size range available for these species across our sites (Online Resource 1). For each tree, we collected twigs from three branches and immediately placed the samples in Exetainer® vials (Labco, Lampeter, UK) for transport to the US. We simultaneous collected grass culms (Corbin et al. 2005) from three individual grass tufts (irrespective of species) from an area comparable in extent to our tree sampling area. We sampled grass species that were representative of the community prevalent at the site, but did not collect data on grass community composition across sites. We only sampled grasses that were away from tree canopies, given that grass species composition is strongly influenced by tree canopy effects (Treydte et al. 2011). For both tree and grass tissue, we ensured that only non-photosynthetic tissue was collected to avoid biasing our samples with water that may have undergone fractionation via transpiration (Corbin et al. 2005). To sample soil, we dug two soil pits per site (separated from each other by several m), and collected ~ 10 g of soil from multiple depths. In 2011 we sampled at three depths: 5, 10, and 30 cm. Some of our sampling depths deviated from these target depths, but in all cases we measured and recorded our sampling depth. In 2013, we sampled four depths per pit, up to 40 or 50 cm depending on conditions at each site. Previous work in this system has shown that trees and grasses do not rely on ground-water (Kulmatiski and Beard 2013a), and that the bulk of water uptake occurs in the top 50 cm of the soil profile in both mesic (Kulmatiski and Beard 2013a; Kulmatiski et al. 2010) and dry (Kulmatiski and Beard 2013b) ends of the MAP gradient. Moreover, all study species are drought-deciduous during the dry season at our sampling sites, suggesting little to no reliance on groundwater. We stored all samples immediately in Exetainer vials to prevent evaporation from occurring. We kept the vials frozen prior to water extraction by cryogenic distillation, conducted at the University of Missouri. To perform the extractions, we used an open manifold system (Nippert and Knapp 2007a) that allows the removal of non-condensable gases and potential organic contaminants. We analyzed all the extracted water samples using a Picarro L1102-*i* CRDS analyzer at the Stable Isotope Mass Spectrometry Laboratory at Kansas State University for δD and δ$^{18}$O values. In addition to the isotopic analyses, we revisited the sites in 2016 to collect community composition data for the tree layer. We used a stratified design comprising a large ($50 \times 20$ m) and small ($50 \times 4$ m) plot, in which we recorded the species identity and diameter of all stems > 5 and 1 cm diameter, respectively. For some sites with a particularly high stem density, we used narrower ($50 \times 10$ m and $40 \times 2$ m) plots. We calculated the total and species-specific basal area across all sites (Online Resource 1).

## Data analysis

We used δD and δ$^{18}$O in plant stem water to test for vertical rooting differences between trees and grasses, and (for the 2013 data) to explore variation in these differences across the Kruger MAP gradient. One method of achieving this consists of using isotopic mixing models to infer the proportional contribution to a sample of a combination of putative sources (Ogle et al. 2004; Parnell et al. 2010). The use of multiple isotopes allows better source (in this case soil depth) determination among samples. Mixing models require that sample values occur within the bounds imposed by the sources, however. In our case, a large fraction of the plant water samples had δD and δ$^{18}$O values that were smaller than those of any of the soil water samples (Figs. 2, 3). A primary reason for this discrepancy is likely that deeper sources than those we sampled contributed to plant uptake in some cases. Rather than use mixing models, we took a different approach, in which we first analyzed our soil water samples to quantify the degree to which soil water isotope values varied with depth across sites. Once we had estimated site-specific relationships between isotope values and depth for soil water, we compared plant isotopic values within sites to determine whether vertical rooting differences exist, and then compared these differences across sites after controlling for site-specific evaporative enrichment rates. This method does not allow for the direct quantification of absolute magnitude of differences in rooting depth, but does provide a robust test for the existence of relative differences in the mean depth of water uptake by species or growth forms.

A visual examination of soil water isotopes suggested that changes in δD and δ$^{18}$O with depth were highly correlated (2011: $P \ll 0.0001$, $r = 0.75$; 2013: $P \ll 0.0001$, $r = 0.80$). Therefore, in order to leverage the information provided by the two isotopes into a single analysis, we combined them using a PCA conducted on the correlation matrix (Holdo and Nippert 2015), and then used the negative of the first principal component ($-$PC1) as a single isotope variable capturing variation in both δD and δ$^{18}$O. We used negative values of PC1 to reflect the fact that samples with a higher fraction of heavier isotopes have less negative values, consistent with the original variables. We conducted the PCA independently for the two sampling periods because this approach captured more of the variation in the original variables than pooling the samples. We used mixed models to test for significant evaporative enrichment within each sampling period by regressing $-$ PC1 against log-transformed depth using the *lme* function in the *nlme* package (Pinheiro and Bates 2000) in R (R Development Core Team 2011), treating pit within site and site as random effects. We conducted somewhat different analyses for the 2011 and 2013 data sets, given the differences in sampling intensity and maximum depth. In 2011
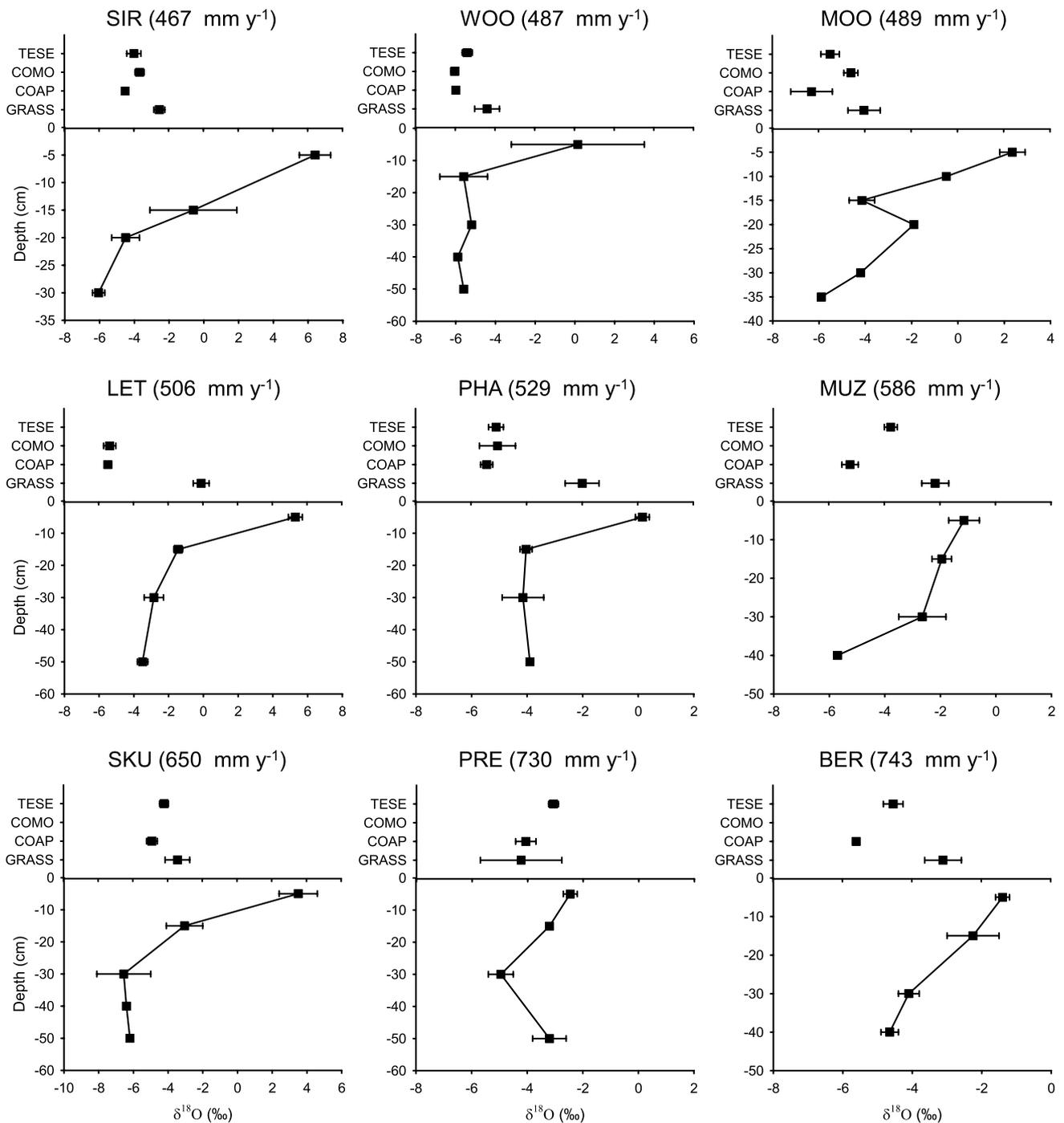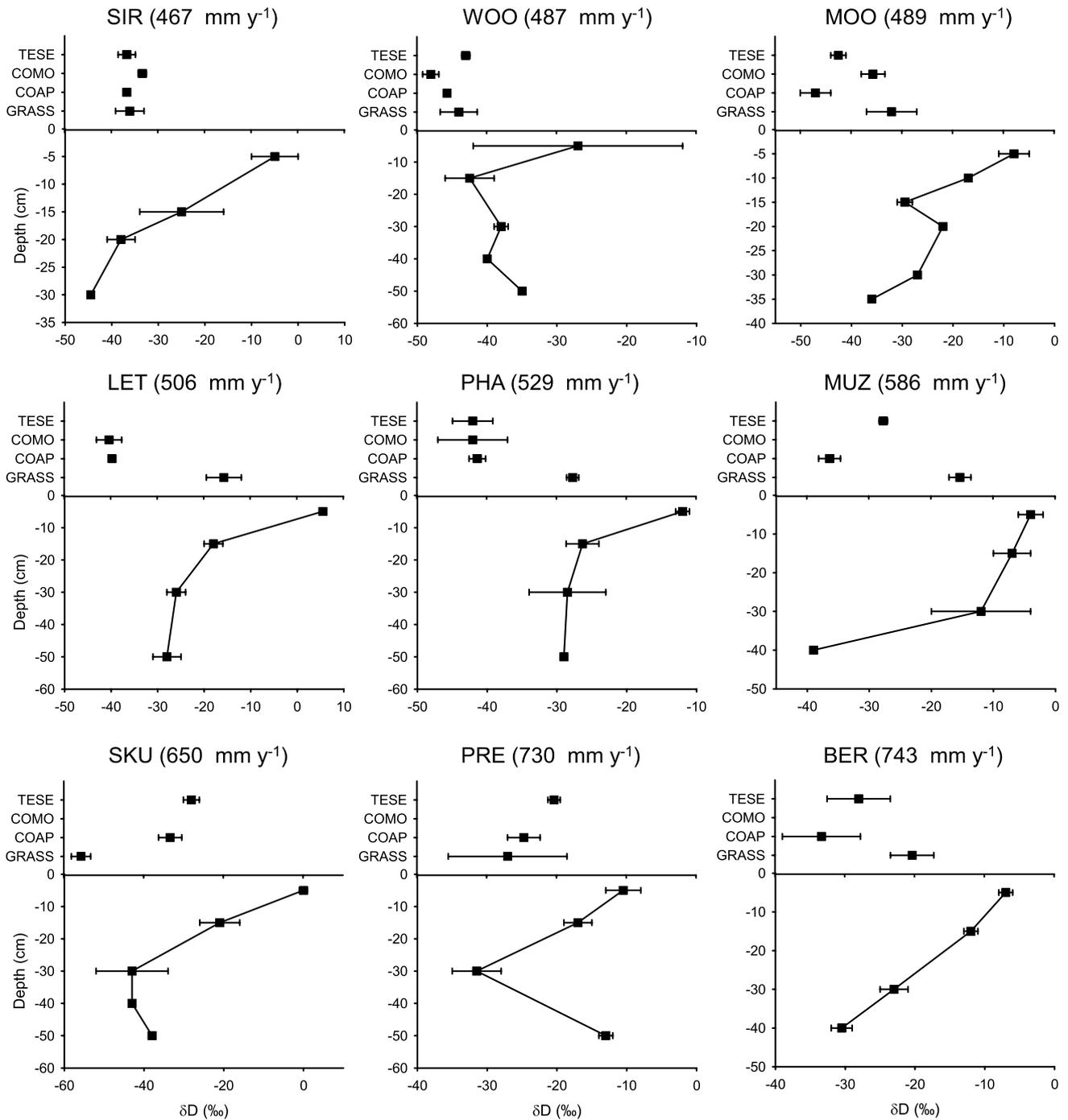
**Fig. 2** Soil (mean ± SE, for two pits) and plant (mean ± SE) water $\delta^{18}O$ values (as a function of depth across species/functional groups, respectively) across March 2013 isotope sampling sites in Kruger National Park. Sites are ordered by MAP. Tree species codes: *COAP*, *Combretum collinum*; *COMO*, *Colophospermum mopane*; *TESE*, *Terminalia sericea*

we only sampled 2–3 soil depths in the upper 20–30 cm, so we lacked the statistical power to compare evaporative enrichment rates as a function of depth across sites (i.e., by testing for variation in the slope of regressions between − PC1 and log (depth) as a function of MAP). During the 2011 sampling campaign, our main goal was to examine how widespread tree–grass rooting differences were across the KNP landscape, and our soil isotope data were collected primarily to establish that detectable evaporative enrichment occurred as a function of depth. In 2013, however, we collected more samples per pit and sampled up to 50 cm depth, and one of our additional objectives was to explicitly test

**Fig. 3** Soil (mean ± SE, for two pits) and plant (mean ± SE) water δD values (as a function of depth across species/functional groups, respectively) across March 2013 isotope sampling sites in Kruger National Park. Sites are ordered by MAP. Tree species codes: *COAP*, *Combretum collinum*; *COMO*, *Colophospermum mopane*; *TESE*, *Terminalia sericea*

for changes in the relative extent of vertical root separation across the KNP MAP gradient. For the 2011 data, we used an intercept-only random effects model and log (depth) was the only fixed effect. For the 2013 data, we used a more flexible model with both random intercept and slope components, and log (depth) and MAP (and their interaction)

as fixed effects. The log (depth) × MAP interaction effect allowed us to test for MAP effects on the rate of evaporative enrichment. The 2013 relationship between − PC1 and log (depth) suggested that the relationship between these variables was close enough to linear (Online Resource 2) to justify our use of the slopes of these regressions as indices

of the rate of evaporative enrichment as a function of depth for a given site.

After establishing that soil water isotopic signatures became more negative as a function of depth, we interpreted plant water − PC1 as an index of rooting depth. To test for differences in mean rooting depth among species and between trees and grasses, we first used *lme* with site as a random effect to test for overall species/functional group differences in − PC1 for each sampling period. We then conducted planned pairwise comparisons of − PC1 scores between grasses and each tree species using Tukey's HSD with the *glht* function in the *multcomp* package. To test for changes in rooting depth across the MAP gradient for the 2013 data, we used a mixed model with species/functional group and MAP (and their interaction) as fixed effects, plus main effects of the slope of the regressions of − PC1 on log (depth) for each site. This allowed us to test for MAP effects while controlling for variation in soil evaporative enrichment rates across sites. We found strong collinearity between the intercept and slope of the − PC1 vs. log (depth) regressions ($r = 0.97$), so only included the slope in the mixed model. There is disagreement about the appropriate sum of squares (Type I or Type III) to use in a highly unbalanced analysis of variance with categorical factors, particularly in the presence of interaction effects (Herr 1986). We tested both sums of squares, with appropriate contrasts (Pinheiro and Bates 2000). To examine the potential relationship between rooting depth and short-term rainfall (as opposed to long-term MAP patterns), we tested the relationship between − PC1 and (a) the previous year's (2012) rainfall, and (b) the 5-year mean (2008–2012) rainfall at each site. We obtained these data from the Rainfall Estimator (RFE), which integrates rain gauge data with satellite-based microwave data, and is available at a 0.1° spatial resolution and a daily temporal resolution for the African continent (Herman et al. 1997; Novella and Thiaw 2013). We note that these data only capture diurnal (6 AM–6 PM) rainfall events, and therefore underestimate total rainfall. We developed annual rainfall rasters from the daily layers and extracted raster values for the pixels corresponding to our nine 2013 sites. We conducted all GIS work in R v3.3.

## Results

### Soil water isotopes

Water was successfully extracted cryogenically and analyzed for isotopic composition from 101 soil samples ($N = 30$ and 71 for 2011 and 2013 collections, respectively) from depths ranging between 5 and 50 cm (Figs. 2, 3, Online Resources 3 and 4). A regression of δD vs. $\delta^{18}$O in soil water had a shallower slope than the closest calculated Local Meteoric Water Line (West et al. 2014), with surface soil samples being evaporatively enriched and deeper soil samples exhibiting isotopic ratios resembling the expected values for rainfall that infiltrates beyond shallow soil layers that is less impacted by evaporative fractionation (Fig. 4a, b). These patterns are comparable to those obtained for a separate study in a nearby site (Holdo and Nippert 2015). Plant samples tended to fall along this line, with grasses showing isotope ratios typical of shallow soils and trees exhibiting ratios resembling those of deeper soils in both 2011 and 2013 (Fig. 4a, b), although these aggregate patterns do not capture inter-site variation. The first component of the PCA (PC1) explained 97.7 and 90.1% of the variance in δD and $\delta^{18}$O in 2011 and 2013, respectively. − PC1 declined significantly as a function of log-transformed depth (mixed models fit by REML, with site and pit within site as random effects: 2011: $F_{1,9} = 13.9$, $P < 0.0001$; 2013: $F_{1,51} = 29.7$, $P < 0.0001$) in both collection periods (Online Resource 2), suggesting that changes in isotope ratios as a function of soil depth occurred reliably across locations sampled. The standard deviations for the site random effects (2011 intercept: 0.25, in PC1 units, vs. a residual model standard deviation of 0.56; 2013 intercept and log (depth) slope: 2.11 and 0.71, respectively, vs. a residual model standard deviation of 0.70) suggested substantial heterogeneity in evaporative enrichment across sites. Despite this, there was no evidence that this variation was related to MAP, as neither the main effect of MAP (which would reflect rainfall effects on surface-layer isotopic values) nor the MAP × log (depth) interaction (which would capture the rate of isotopic change with depth) was significant ($P = 0.53$ and 0.17, respectively).

### Plant water isotopes

The final plant water dataset for KNP consisted of 129 samples ($N = 39$ and 90 for 2011 and 2013 collections, respectively, Figs. 2, 3). There were significant species/functional group differences in both collection years (mixed models fit by REML, with SITE as a random effect: 2011: $F_{2,30} = 44.1$, $P < 0.0001$; 2013: $F_{3,78} = 11.8$, $P < 0.0001$), and in both cases, grasses had higher − PC1 scores (meaning shallower rooting depth) than all tree species in each year (Table 1, Fig. 4c, d). Grasses had the highest − PC1 value in all 7 sites in 2011 and in 7 of the 9 sites in 2013 (Fig. 4c, d). For the analysis including MAP with the 2013 dataset, we found a significant main effect of species ($F_{3,75} = 12.9$, $P < 0.0001$) and a significant interaction between species and MAP ($F_{3,75} = 3.54$, $P = 0.019$) on plant − PC1, but no main effects of MAP ($P = 0.21$) or the slope of the soil − PC1 vs. log(depth) regression ($P = 0.62$). The results were identical with both Type I and Type III sums of squares. The significant interaction effect rendered the main effects difficult to interpret, so we explored simple effects of slope and
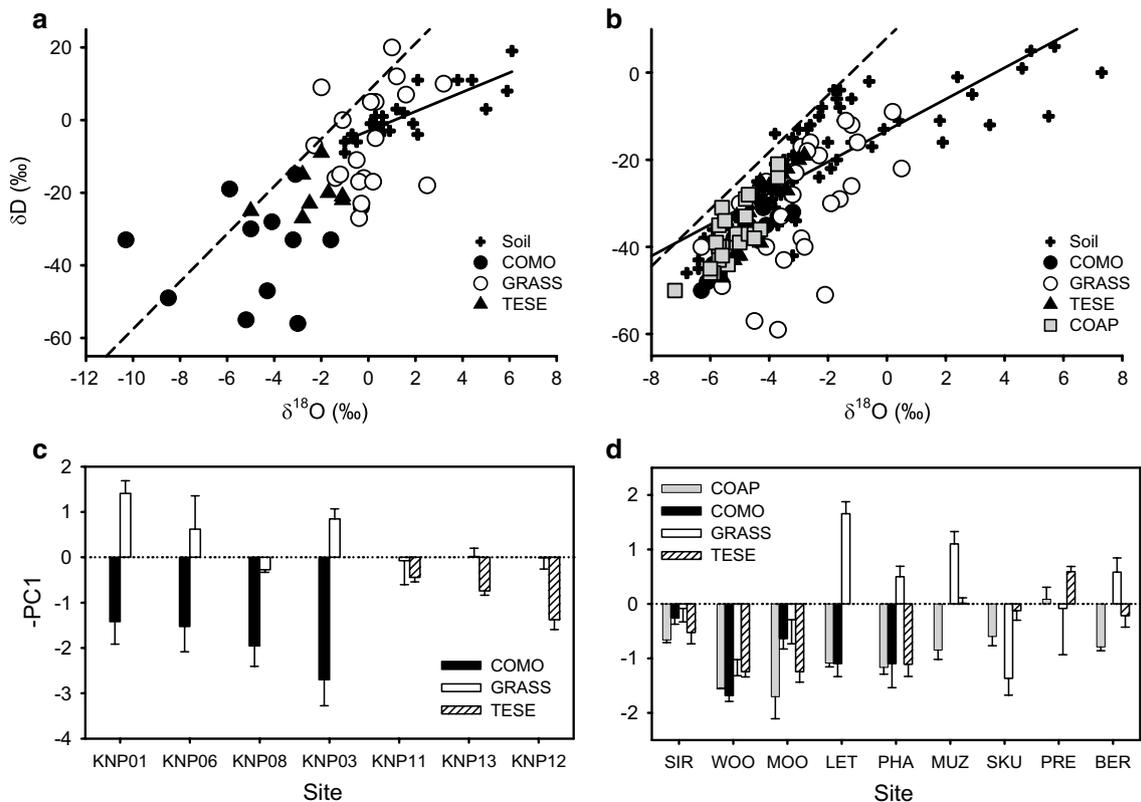
**Fig. 4** Soil and plant water δD vs. δ18O values in **a** Dec 2011 and **b** March 2013, and plant water isotope PC1 scores (mean ± 1 SE) across sites in **c** Dec 2011 and **d** Mar 2013. In **a** and **b**, the nearest calculated local meteoric water line (Pretoria) is shown as a dashed line (West et al. 2014), and the solid line represents a regression through the soil water data (crosses). In **c** and **d**, sites are ordered from left to right by MAP. Tree species codes: *COAP*, *Combretum collinum*; *COMO*, *Colophospermum mopane*; *TESE*, *Terminalia sericea*

**Table 1** Post hoc planned comparisons between grass stem water isotopic PC1 values and stem water isotopic − PC1 values for individual tree species across the Kruger National Park mean annual precipitation gradient for two sample collection periods

| Period | Contrast[a] | Difference in − PC1 | | | |
|---|---|---|---|---|---|
| | | Mean | SE | Z | P |
| Dec 2011 | Grass—*C. mopane* | 2.42 | 0.26 | 9.21 | ≪ 0.0001 |
| | Grass—*T. sericea* | 1.01 | 0.31 | 3.28 | 0.0021 |
| Mar 2013 | Grass—*C. mopane* | 0.88 | 0.22 | 3.92 | < 0.001 |
| | Grass—*C. apiculatum* | 1.02 | 0.18 | 5.64 | < 0.001 |
| | Grass—*T. sericea* | 0.54 | 0.19 | 2.87 | 0.012 |

[a]Tukey HSD comparisons, with site as a random effect

MAP for each species/functional group. We found significant negative relationships between − PC1 and MAP for *T. sericea* ($P = 0.032$, Fig. 5a) and *C. apiculatum* ($P = 0.033$, Fig. 4b), but not for *C. mopane* ($P = 0.58$, Fig. 4c) or for grasses ($P = 0.75$, Fig. 4d). The slope effect was not significant in any of the four cases. This pattern held when we tested − PC1 against both 2012 rainfall (TESE: $P = 0.0085$;

COAP: $P = 0.031$) and mean rainfall over the preceding 5 years (TESE: $P = 0.024$; COAP: $P = 0.025$). Both of these variables were strongly correlated with the MAP values obtained from WorldClim (2012 rainfall: $r = 0.95$; 2008–2012 rainfall: $r = 0.96$).

All raw data files and R code are available in Online Resource 6.

## Discussion

Our results suggest that tree and grass differences in depth of water uptake appear to occur across much of the rainfall gradient in KNP, supporting our first hypothesis. The contrasting isotopic pattern exhibited across the MAP gradient by the two dominant tree species (*T. sericea* and *C. apiculatum*) relative to the grasses suggests that the extent of root separation may be rainfall-dependent, supporting the second hypothesis. The third tree species, *C. mopane*, exhibited no clear pattern across the gradient. If anything, the trend in relation to MAP was opposite to that observed for the other two species (Fig. 5c). Given that *C. mopane* is
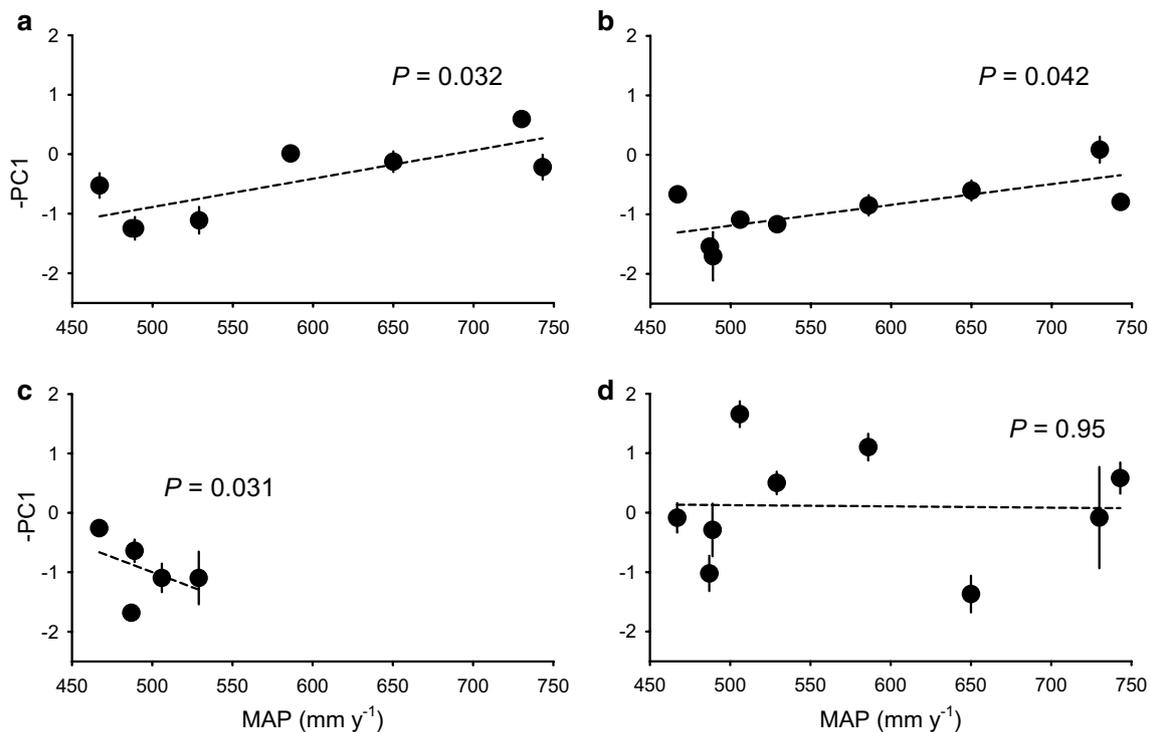
**Fig. 5** Plant water isotope − PC1 scores (mean ± 1 SE) as a function of site MAP in Mar 2013 in **a** *Terminalia sericea*, **b** *Combretum apiculatum*, **c** *Colophospermum mopane* and **d** grasses

restricted to the drier Northern portion of KNP, however, and therefore only occurred over a limited portion of the MAP gradient, the extent to which the rainfall vs. rooting depth relationship can be evaluated in this species is rather limited. Support for hypothesis 2 is mitigated somewhat by the fact that tree–grass differences in rooting depth appear less pronounced at the driest end of the gradient (MAP < 500 mm year$^{-1}$) than they do at intermediate sites (Fig. 4b), suggesting that factors other than MAP may play a role in driving rooting differences. A visual examination of grass − PC1 scores across the rainfall gradient suggested a possible nonlinear relationship. We conducted a post hoc test to determine whether a quadratic relationship would provide a better fit than an intercept model, but found no support for this, suggesting no clear trend in grass rooting depth across the MAP gradient. Our results also suggest that substantial variation in rooting depth may occur within the tree functional group, and that the relationship between rooting depth and rainfall may be species-dependent. Within sites, for example, *T. sericea* appeared to be consistently more shallow-rooted than *C. apiculatum* (Fig. 4b).

Our results also indicated a substantial amount of variation in grass isotopic signatures across sites, suggesting that the factors driving rooting depth may vary more across sites for grasses than for trees (Fig. 4). Despite the pronounced inter-site variation in grass isotope values, we note that

grasses were not notably more variable than trees within sites. A possible interpretation of this finding is that there is significant species turnover across (but not within) sites in the grass community, and that grass species differ in terms of rooting depth. We also note that our results reflect intraspecific variation within relatively common tree species, so it is not yet clear that the trend we report will be mirrored by patterns of species turnover across the rainfall gradient, i.e., with shallow-rooted species tending to dominate at the dry end of the gradient and vice versa. A clear next step is to systematically characterize weighted functional rooting depths across the savanna community by taking into account all of the following: both interspecific differences and intraspecific variation in rooting depth, as well as shifts in species abundances across the rainfall gradient. Other factors may also play a role in driving some of the variation we observe in this system, such as soil depth (Holdo and Timberlake 2008) and nutrient limitation. For example, there is evidence to suggest that N limitation may play an important role in modulating grass effects on trees in these systems (Cramer et al. 2010), but how N and other nutrients may be partitioned as a function of depth remains unknown.

This caveat notwithstanding, our results appear to support the hypothesis that the degree of tree–grass vertical root separation declines with increasing rainfall (Belsky 1994; Schenk and Jackson 2002). Tree–grass differences in mean

rooting depth were the norm across our study sites, except in two of the wettest locations (Pretoriuskop and Skukuza, in the S of KNP), where MAP exceeds 650 mm year$^{-1}$. This pattern helps reconcile some of the apparently contradictory evidence for vertical root separation in this system. In a root-severing experiment conducted at Pretoriuskop, Verweij et al. (2011) showed that trees tend to utilize relatively shallow water, suggesting little vertical root separation between trees and grasses. Conversely, in an experiment conducted at the drier (MAP = 544 mm year$^{-1}$) end of the Kruger MAP gradient, Kulmatiski and Beard (2013b) demonstrated that the tree–grass biomass ratio shifts in favor of trees when deep infiltration occurs, supporting the case for root separation. Both of these results are consistent with our findings, and with the conclusion that the extent of vertical root separation may be rainfall-dependent to a certain degree. A caveat here is that the extent of this plasticity may also be constrained given that we found consistent patterns of tree–grass root separation both at the end of the 2011 dry season and at the height of 2012–2013 wet season.

Paradoxically, our results suggest that this pattern is due to changes in tree rooting depth along the rainfall gradient, with trees becoming shallower-rooted and therefore more "grass-like" at the mesic end of the gradient, rather than being caused by grasses becoming more "tree-like" and deeper-rooted as conditions become wetter, as proposed by Schenk and Jackson (2002). Previously, Holdo (2013) proposed that functional root separation might be expected to increase with MAP, simply because under wetter conditions, deep infiltration should become more pervasive, thereby increasing the viability of deep rooting strategies and increasing the range of niches than can potentially be exploited. This was based, however, on the assumption that rooting patterns are relatively fixed within a particular species or functional group. Kulmatiski and Beard (2013a), in a previous study conducted in KNP, showed that while grasses tend to exhibit shallow rooting profiles throughout the annual cycle, trees are quite plastic, drawing on moisture from shallow layers when water is available (i.e., during the wet season) and shifting to deeper layers during the dry season. Our results may represent an analogue of this pattern writ large: the greater rooting plasticity of trees leads them to draw water from the topsoil at the mesic end of the rainfall gradient and to draw water from deeper layers at the dry end. These two extremes tend to reflect—on average—the conditions encountered during the wet and dry season, respectively. Under this interpretation, grasses are relatively constrained by their adventitious morphology to exploit topsoil layers regardless of soil moisture conditions, while trees exhibit intraspecific variation, governed by prevailing environmental conditions.

The assumption that deep infiltration should increase as a function of MAP was also based on an analysis of precipitation patterns from LTER sites in North America, where rainfall intensity (as captured by the size of daily precipitation events) is positively correlated with MAP (Holdo 2013). Here, however, we found the opposite pattern to be true. In a follow-up analysis, we extracted daily diurnal wet season precipitation data for KNP from the RFE database and calculated mean values for log daily precipitation amounts from 1983 to 2016. We found that in KNP (unlike the general pattern for the African continent; Holdo, in prep.), daily precipitation amounts increase as MAP declines (Online Resource 5), suggesting that deeper infiltration may occur in the drier North of the park, potentially explaining the deeper tree rooting patterns there.

Our results have potentially important implications for understanding how the savanna biome might respond to changes in the intensity and timing of rainfall events. It has already been shown that imposing fewer, larger rainfall events in the semi-arid portion of KNP favors trees at the expense of grasses (Kulmatiski and Beard 2013b). The reduction in rooting depth differences as MAP increases would suggest that, at least on the basis of rooting profiles, there should be smaller differences in the responses of trees and grasses in mesic sites. This has widespread implications for our ability to predict how savannas are likely to respond to climate change at large spatial scales. For example, our analysis of RFE data (Holdo, in prep.) also shows that KNP rainfall has been shifting steadily to a regime of larger daily precipitation amounts, with potentially important consequences for woody expansion, particularly in the drier Northern sections of the park. A recent modeling study (Yu et al. 2017) has suggested that increasing interannual variability in precipitation will favor trees in dry (but not wet) savannas, under the assumption that trees are deeper-rooted than grasses in these systems and that more variability equals more deep infiltration events. Our study bolsters this argument. Any increases in tree cover could have widespread ramifications, including changes in hydrology at multiple spatial scales (Le Maitre et al. 2002). In the meantime, a key next step will be the integration of rooting depth data with other functional traits governing water uptake and growth, as well as other environmental drivers of plant transpiration dynamics ignored here, such as patterns of vapor pressure deficit, leaf temperature, and potential evapotranspiration. This will allow us to develop more realistic models of savanna dynamics, and allow for better predictive tools for assessing vegetation responses to novel climate scenarios.

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