Above- and below-ground allocation and functional trait response to soil water inputs and drying rates of two common savanna grasses

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1. Introduction

Semiarid biomes are characterized by complex vegetation patterns that regionally generate a mosaic landscape of grassland, shrubland and woodland patches (Whittaker, 1975). Particularly, extensive C4 permanent grasslands in semiarid systems have attracted the attention of ecologists (Bond, 2008). Complex grass-tree patterns in savanna systems are a problem that has been addressed from bottom-up processes, particularly at broad scales like the biome (Breckle, 1999; Woodward et al., 2004). However, at regional to local scales most work focus on top-down approaches to explain the permanence of grassland patches, focusing on the effects of disturbances (Bucini and Hanan, 2007), namely fire (see Louppe et al., 1995; Keeley and Rundel, 2005; Sankaran et al., 2005; Staver et al., 2011; Schertz et al., 2015), herbivory (see Owen-Smith, 1987; Van de Koppel and Prins, 1998; Kartzin et al., 2014) and human activity (see Young et al., 1995; Bugalho et al., 2011; Boughton et al., 2013). Some theoretical work has connected semi-arid plant community spatial patterns to plant-plant water competition (see for instance Pärtel and Wilson, 2002; Rietkerk et al., 2004; Martínez-García et al., 2013) but we need more empirical work devoted to disentangling belowground water competition between C4 grasses and trees, especially when the latter are at the seeding stage and the competition for water may asymmetrically favor grasses (Campbell and Holdo, 2017).

The study of plant functional traits is a growing branch of functional ecology, and belowground traits, despite being more cryptic and poorly understood, may contribute valuable information about plant resource foraging (Zemunik et al., 2015). Disentangling the patterns of variation of plants’ belowground responses to different soil resource availability can contribute to our understanding of resource acquisition strategies and may forecast species survival (Li et al., 2017). The examination of both belowground and aboveground plant functional traits may not only help understand species coexistence (Mueller et al., 2013; Zuppinger-Dingley et al., 2014, Ravenek et al., 2014), but can also provide useful information for explaining complex patterns of vegetation physiognomy driven by competitive exclusion of completely different functional groups, such as tree thickets and C4 grasses in savannas. In this study, assessing the architectural response of roots, we examine variation in four belowground functional traits in response to different water availability conditions: Root to shoot biomass ratio (R:S), superficial (< 6 cm) to deep root biomass ratio (S:D), root diameter (D), and root estimated surface area to shoot biomass (R:S). By assessing these four key functional traits, we explore strategies that grasses may use to compete with neighbors for water, may these neighbors be other grasses, forbs, or woody plant seedlings and saplings.
Long-lasting glades are a characteristic vegetation feature in central Kenyan ecosystems, occurring alongside woodland communities. Medium sized (around 5 ha) glade patches are a common element in the vegetation mosaic dominated by savanna woodlands that have been associated with settlements of pastoralists abandoned decades ago (Young et al., 1995). As discussed above, the persistence of these patches could be facilitated by herbivory and fire, but few empirical studies have investigated whether competition between C4 grasses and tree seedlings could contribute to the permanence of these patches. We conducted a comparative study in the Mpala Research Center (MRC), in Laikipia County (Republic of Kenya). We assessed the phenotypical response of two species typically present at our study site, Cynodon plectostachyus (K.Schum.) Pilg., and Pennisetum stramineum Peter., that were commonly found on glades and in the neighboring grass-tree ecotones. Different water availability conditions were tested using a water input factor driven by average annual precipitations, and a soil drying rate factor driven by changes in insolation and soil temperature due to canopy cover. Our empirical work was focused on testing two main sets of hypotheses:

Firstly, we tested whether plants benefit from concentrating their roots in shallow soil depths under water stress (shallow roots hypothesis). This hypothesis is conceptually based on the “two-layer hypothesis” and the idea that differences in root vertical distributions may explain tree-grass codominance in dry savannas, each functional group being the superior competitor in its soil layer (Ward et al., 2013). A recently published model suggested that grasses may outcompete trees when rainfall is intermittent but frequent (D’Onofrio et al., 2015), predicated on the assumption that small burst of precipitation may evaporating from the soil surface. Alternative hypotheses state that grasses in drier environments should develop deeper root systems, but this would only be true when a large enough portion of rainfall is able to infiltrate before evaporating from the soil surface.

Secondly, we hypothesize that root diameter may interact with root:shoot ratios, thus necessitating a different index that considers the overall geometry of roots in order to capture a deeper understanding on root to shoot variations (Root diameter hypothesis). Plants are generally assumed to follow optimization allocation strategies putting more resources into root growth (high R:S ratios) when growing under soil water limiting conditions, to increase their ability to uptake water or nutrients (Chapin et al., 1987; Crick and Grime, 1987; Gedroc et al., 1996; Schenk and Jackson, 2002). However, in species with high Ø variability, like C. plectostachyus, alternative indexes may provide supplementary information about plant biomass allocation strategies. Fundamental geometry demonstrates that a wider cylinder must have higher absolute surface area but less surface to volume ratio. For a given volume of root (root assumed cylindrical), increasing root diameter would result in less total surface. More allocation into roots may be driven by causes other than foraging nutrients: for instance as storage organs. Only by controlling for root geometry can we disentangle these causes. To control for Ø in our C. plectostachyus data, we created an alternative ratio Rs:S using an estimated root surface area Rs (as a function of R and Ø) and the shoot biomass, and we tested its results and compared them with R:S ratios in different environments.

In addition to the shallow roots hypothesis and the root diameter hypothesis, we also evaluated two additional hypotheses. First, we hypothesized that plants investing in non-palatable aboveground organs may benefit from self-shading the soil, by means of reducing evaporation and facilitating the survival of rhizomes in high water stress conditions. This hypothesis is important to consider given the role that facilitation may play in self-organized spatial patterns in semi-arid grasslands according to the scale-dependent feedback hypothesis (Rietkerk et al., 2002). Second, we tested whether the net effect of thicket canopy on C4 grasses growth was negative (interference) or positive (facilitation). The net interaction between C4 grasses and savanna thickets results from complex interactions between antagonistic forces related to the effects of shade (Valladares et al., 2016). On the one hand, the presence of a canopy may reduce water stress (Brooker...
2. Material and methods

2.1. Study site

The study was carried out on the Mpala Ranch at MRC (0°20' N, 36°53' E, 1650–1800 masl), on the Laikipia Plateau in north central Kenya (Fig. 1) during the dry season of 2017. Two long-term glades were selected for the study, one in the north of the ranch (0°28'19"N 36°51'31"E) and a second one in the south (0°16'53"N 36°53'30"E). Both glades were located on red soil, i.e. sandy friable loams of metamorphic origin (Ahn and Geiger, 1987). The vegetation in the area is an open *Acacia* thicket with a biodiverse understory (Taiti, 1992). Both the north and the south glades are dominated by three grass species, *C. plectostachyus*, *Sporobolus fimbriatus* (Nees ex Trin.) Nees and *P. stramineum*. The grasses *C. plectostachyus* and *S. fimbriatus* cover the glade with few competitors forming a non-densely populated prairie where bare soil is present in the interspaces between individuals. *C. plectostachyus* is a geophyte plant that produces stolons for vegetative reproduction and is intensively grazed by wild animals and cattle, who spend high amounts of time in these glades, and whose excretions represent an important source of nutrients. Individuals of *P. stramineum* are often found in the glades, especially at the edges, and also in the acacia woodlands around the glades. *P. stramineum* is a caespitose plant that creates dense monospecific patches often of a single individual that expand with dense elongated rhizomes. This grass species has stems of low palatability, especially in the basal parts that are avoided by grazers.

The average rainfall in the study area is ~500 mm y⁻¹, with the highest peak in mean rainfall occurring in March–May and the longest and most pronounced dry season typically from December through March. There is a marked precipitation gradient due to nearby Mount Kenya's effect on winds, such that the overall rainfall is markedly lower in the north (294.2 mm annual 2014–2016 average, data supplied by Mpalta Tower meteorological station) than in the south (597.60 mm annual 2014–2016 average, data supplied by MRC meteorological station). The two glades selected were located in different latitudes, in order to represent different rainfall regimes. We used a latitude (LAT, North v. South) factor to prospect for differences caused by annual water input levels due to the precipitation gradient found in MRC (Fig. 2).

To assess the microclimatic differences due to water evaporation, we used different grass locations within each glade. Location differences were characterized by the presence or absence of *Acacia etbaica* Schweinf thicket canopy. Evaporation has been shown to be increased in inter-canopy patches as compared to under-canopy in warm climatic conditions in a semi-arid ecosystem very similar to our study system (Breshears et al., 1998). The temperature and the illumination at the surface of the soil are, along with wind, humidity and soil hydraulic properties, the main drivers of soil water evaporation (Smits et al., 2012). In this context, soil hydraulic properties being equal, woody plant canopy and the associated decrease in illumination and temperature play a key role in buffering evaporation. We used a location (LOC, Open vs Shade) factor to assess the shading effect of the 2–3 m tall multi-stem dense acacia thickets and its effect of superficial soil evaporation (Fig. 2).

2.2. Grass measurements

Despite the debate on the differences between what coarse and fine roots functions are and what criteria may be used for their classification (McCormack et al., 2015) – especially pertinent to the case of perennial woody plants–, in general grass roots, and in particular the roots of the individuals we sampled, did have small root diameters (Ø < 2 mm) and usually only presented two root orders. That means that these grasses may have little root differentiation, and all roots may function as both absorptive and transportation tissues (see Freschet and Roumet, 2017; McCormack et al., 2017). Here, we use the term “structural roots” to refer to the roots that may survive the dry season, potentially excluding ephemeral roots that may develop during the growing season to increase water uptake, and therefore providing data on the long temporal scale. In this study, root allocation was assessed during the dry season, and the entire root system of individuals was extracted and measured, as described below, and is referred to as structural roots.

Fifteen randomly selected individuals of *C. plectostachyus* and *P. stramineum* were collected from within each glade (North and South), and fifteen more randomly selected individuals of *P. stramineum* were collected under the shade of *A. etbaica* in the surrounding woodlands of each glade. Each grass individual was extracted from the soil by digging a hole of 50 cm diameter and 36 cm depth, carefully crumbling the soil to maintain the root system as intact as possible. Structural roots were differentiated into superficial roots—all the roots located between 0 and 6 cm from the ground level, and deep roots—below 6 cm deep from the ground level. The 6 cm threshold is arbitrary, but it allowed us to differentiate between surface roots and deep roots (all sampled plants did have between 95% and 100% of their observed root biomass within the first 30 cm of soil depth). However, structural roots are perennial and they indicate how the overall grass root structure develops. Shoots (including rhizomes and all aboveground biomass) were also collected for each individual. Shoots and leaves, superficial roots, and deep roots were oven dried at 70 °C to a constant weight with the final weight recorded. Superficial (< 6 cm) to deep root biomass ratio (S) was calculated as dry weight of roots in the superficial layer divided by dry weight of roots in the deep layer. The root to shoot biomass ratio (R:S) was calculated as the dry weight of the total root biomass (R, g) divided by the dry weight of the aboveground biomass (S, g).

*C. plectostachyus* showed a high variability in root diameter (Ø, mm) between glades (personal observations), and Ø was measured in all the individuals only of this species to see whether these differences correlated with rainfall regimes, and also to control for the different root diameters in the R:S analysis. Photographs of 10 randomly selected main roots—adventitious roots born directly from the shoots—selecting only the first centimeter of root from its insertion point, were taken with a metric referenced ruler on the same plane, for each individual of *C. plectostachyus*. The length in pixels of the metric reference and of the width of the roots were measured by means of digital photographic processing performed using GIMP 2.8.18 (GNU), and mean Ø were then calculated for each individual in millimeters.

An alternative root surface area to shoot biomass ratio (Rs:S) was calculated for *C. plectostachyus*, using the estimated root surface area (Rs, mm²). The ratio Rs:S was calculated dividing Rs by S. The Rs equation was obtained by solving for the height h in the volume of a cylinder equation, substituting the solution for h into the equation of lateral surface area of a cylinder, and replacing the volume by Rρ, where p is the root dry weight biomass density (g.mm⁻³). We obtain the formula Rs = 4 R(Ø). This Rs estimate is based on the assumption that roots are perfect cylinders in which diameter is uniform. Changing this assumption for a truncated cone geometry or for any other more realistic taper function would only change the estimate by a negligible fraction, given the high length relation to diameter of roots. Furthermore, grass roots do not experience secondary growth, and maintain root diameter fairly constantly for a given root at different distances from the insertion. This assumption is consistent with personal observations during the laboratory work.

For our calculations, we used a constant p = 0.02 g mm⁻³ for all *C. plectostachyus* roots. We do not have direct measures for this parameter,
but an across-species variation range of grass ρ of 0.01–0.03 g mm$^{-3}$ seems realistic (Ryser, 1996). The use of a different ρ value constant across individuals would have no effects on the use of Rs:S ratios for comparative purposes, as the error would be the same across individuals. However, if the parameter ρ was not constant in the sample, and its variation was dependent on the diameter of the roots, then variations in ρ driven by Ø may affect our ratio estimation. For this reason, we conducted alternative analysis for Rs:R accounting for possible variations in ρ driven by Ø to check the robustness of our model results, showed in SM Box 1.

In order to test whether the net interference of canopy thicket on grasses was negative, we calculated the interaction intensity RII index (Armas et al., 2004) for the different biomass portions of *P. stramineum*. The index was calculated for each possible pair of individuals (shade-open individual pairs) for each glade, considering aboveground biomass and belowground biomass. In addition, the presence of canopy cover may have other indirect effects on the interference due to possible changes in soil hydraulic properties or soil nutrients. For this reason, in this study we control for microhabitat variability that may change with our latitude and location factors to disentangle the net effect of water.
Table 1
Chi-squared and p-values for the Kruskal-Wallis test and z-statistic and p-values for the Dunn’s tests performed for soil temperature and illuminance data in the north (N) and south (S) glades. Asterisk indicate significant result for a 95% significance level.

<table>
<thead>
<tr>
<th></th>
<th>Kruskal-Wallis test - df = 2</th>
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<tr>
<td></td>
<td>$\chi^2$</td>
<td>p</td>
</tr>
<tr>
<td>Temperature</td>
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</tr>
<tr>
<td>Open-Penn</td>
<td>41.66</td>
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</tr>
<tr>
<td>Penn-Shade</td>
<td>37.25</td>
<td>0.000*</td>
</tr>
<tr>
<td>Illuminance</td>
<td>36.89</td>
<td>0.000*</td>
</tr>
<tr>
<td>Open-Penn</td>
<td>40.78</td>
<td>0.000*</td>
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</table>

Fig. 4. Mean and standard error for illuminance (A) and temperature (B) measured on the surface of the soil in different locations; in the open glade dominated by C. plectostachyus (Open), within the P. stramineum patches (Penn) and under the protection of A. etbaica bushes (Shade). North and south locations were measured on two consecutive days on January 2017 at noon, and analyzed separately. Lower case letters indicate significant differences in the north and lower case letters with apostrophe indicates significant differences for each variable in the south.

Fig. 5. Quartile boxplots of the distribution of data for the four covariates used in the plants response variable models. Distributions are shown for the both factors, latitude (north and south), and locations: open glades dominated by C. plectostachyus (Open), open glades within P. stramineum patches (Penn), and under canopy (Shade). A- Soil texture (% sand); B- Bare soil (% cover); C- Soil K; D- Soil available P; E- Soil NH4; F- Soil NO3. Significant differences in the two-way ANOVA analysis are reported using (***) for $p < 0.001$, (**) for $0.001 < p < 0.01$ and (*) for $0.01 < p < 0.05$. When differences in the LOC factor were found, the groups yielded by post-hoc analysis are indicated by lower case letters.
**Fig. 6.** Mean (black circles) and 95% t-based confidence intervals for the mean (bars, red: North glade; blue: South glade) for the RII index assessing the interaction between adult *Acacia* trees and *P. stramineum* individuals. Negative values in the South indicate predominance of interference in the wetter glade, whereas the values being non-significantly different from zero in the North indicates null interactions in the dryer glade. A: Aboveground biomass; B: Belowground biomass. North A 0.014 95%CI (−0.023, 0.052), B 0.026 95%CI (−0.006, 0.058), South A −0.234 95%CI (−0.266, −0.202), B −0.096 95%CI (−0.127, −0.064). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 2**

Average values of estimates and 95% confidence intervals (95% CI) for the similar best fitting linear models for the superficial (0–6 cm) roots to deep (6 + cm) roots dry biomass ratio (S:D) and for the root to shoot ratio (R:S) in *P. stramineum*. Estimate values in bold and marked with asterisk indicate significant result within the 95% confidence interval. A description of the independent variables used can be found in SM Tables 2–3.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>95% CI</th>
<th>Estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>S:D</td>
<td>4.907*</td>
<td>2.287; 6.906</td>
<td>0.144*</td>
<td>0.097; 0.191</td>
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<tr>
<td>R:S</td>
<td>−1.791*</td>
<td>−2.638; −0.942</td>
<td>3.11e−2</td>
<td>−0.014; 0.076</td>
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<tr>
<td>LOC (Shade)</td>
<td>−1.245</td>
<td>−4.566; 2.077</td>
<td>−3.37e−2*</td>
<td>−0.066; −1.2e−3</td>
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<tr>
<td>LAT (South)</td>
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<td>−7.1e−3; 1.9e−3</td>
<td>−1.06e−3</td>
<td>−1.3e−4; 1.1e−4</td>
</tr>
<tr>
<td>%Sand</td>
<td>3.51e−2</td>
<td>−0.053; 0.123</td>
<td>−1.64e−3</td>
<td>−4.7e−3; 1.4e−3</td>
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<tr>
<td>%Bare</td>
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<td>−0.019; 0.035</td>
<td>2.55e−5</td>
<td>−1.1e−3; 1.1e−3</td>
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<tr>
<td>K</td>
<td>−1.91e+2*</td>
<td>−0.036; −2.4e−3</td>
<td>4.28e−4</td>
<td>2.9e−4; 1.1e−3</td>
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<tr>
<td>NH₄</td>
<td>−2.53e−2</td>
<td>−0.086; 0.035</td>
<td>1.28e−3</td>
<td>1.3e−3; 3.8e−3</td>
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<tr>
<td>NO₃</td>
<td>−1.35e−2</td>
<td>−0.106; 0.079</td>
<td>2.91e+3*</td>
<td>3.6e−4; 5.7e−3</td>
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<tr>
<td>LAT-NH₄</td>
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<td>0.198; 0.276</td>
<td>4.9e−3e; −0.013; 3.4e−3</td>
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<td>LAT-K</td>
<td>4.24e−3</td>
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<td>1.8e−5</td>
<td>−2.1e−4; 2.5e−4</td>
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<tr>
<td>LAT-%Sand</td>
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<td>2.8e−3</td>
<td>3.6e−3; 9.2e−3</td>
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<td>LOC-P</td>
<td>3.17e−3</td>
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<td>−1.35e−3</td>
<td>−2.9e−3; 1.9e−3</td>
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<td>LOC-%Bare</td>
<td>−1.77e−2</td>
<td>−0.062; 0.026</td>
<td>2.83e−4</td>
<td>−1.4e−3; 2.0e−3</td>
</tr>
</tbody>
</table>

2.3. Microhabitat variability

As all the phenotypical responses of the studied grasses may be affected by factors other than water availability, in this study we gathered measures of variables that allowed us to control for habitat variability. The % bare soil and herbaceous cover in a quadrat of 50 × 50 cm around each individual was visually estimated as an index of competition intensity in the individual neighborhood area. Additionally, soil measures were collected in the surrounding of each individual in order to control for soil nutrient availability and its potential effect on the plants' belowground response. For each individual grass collected, the soil that was extracted, crumbled and pooled, and a sample of 200 g was collected. The soil sample was sifted using a 2 mm sieve to discard stones and organic matter particles, and then sifted again using a 0.5 mm sieve to separate the sand (0.5–2 mm) from the fraction composed of silt and clay (< 0.5 mm). Both granulometric fractions were individually weighed to estimate the % sand as sand weight divided by total soil weight. Nitrate (NO₃) and ammonium (NH₄) soil content in the samples were measured by extraction with potassium chloride (Keeney and Nelson, 1987). Extractable phosphorus (P) soil content was measured in the samples by extraction with sodium bicarbonate (Olsen et al., 1954). Potassium (K) available in soil samples was measured by extraction by means of Mehlich 3 extraction (Mehlich, 1984).

To test the magnitude of the effect of thicket on soil evaporation, and the effect of the shade shed by non-palatable grass aerial organs on the potential soil drying rates in glades, we collected data of illumination and temperature at the soil surface. Twenty measures of illumination taken using a Lutron Lx-101a Light Meter and of temperature taken in the exact same locations using an Extech Dual Laser Infrared Thermometer were gathered in each glade, in three different locations: under the *A. ethica* canopy (shade measures); inside the *P. stramineus* patches in the glades (P-patches measures); and in *C. plectostachyus* patches in the glades (bare ground measures, as *C. plectostachyus* aboveground organs are intensively grazed). Measures in both glades were taken on two consecutive days at noon, and alternating locations to randomize atmospheric absorption variations. All measures were taken at the level of the soil surface, after cleaning the ground of litter. ‘Shade’ measures were taken inside the acacia thicket canopy at a distance of 30–40 cm of the trunk insertion. ‘Penn’ measures were taken within the *P. stramineus* patches in the glades, after cleaning the ground of litter. ‘Open’ measures from patches of *C. plectostachyus* were taken in the soil 5–10 cm around the insertion point of a *C. plectostachyus*, majorly surrounded by bare ground.

2.4. Statistical analysis

For the analysis of the temperature and illumination datasets, the assumption of normality was tested using Shapiro-Wilk normality test (Royston, 1982), and the assumption of homoscedasticity was tested using the Bartlett (1937) test for homogeneity of variance. Soil temperature and soil illumination did not meet the assumptions for parametric tests, and were analyzed by means of a one-way Kruskal-Wallis analysis of variance (Kruskal and Wallis, 1952) and Dunn (1964) test for post-hoc multiple pairwise comparisons.

The plant response variables S:D, R:S, Ø, and Rs:S for *C. plectostachyus*, and S:D and R:S for *P. stramineus* (Ø and Rs:D were not measured for this species) were tested by means of lineal models setting LAT and LOC factors to assess differences in water input and soil drying rates respectively (see Suppl Mat Table 1 for more information about the factors and covariates included in the models). The ANCOVA assumption of no collinearity was tested using one-way ANOVA models between factors and covariates for each species datasets. Some covariates were not independent of the factors (For *P. stramineus* LOC:P F = 27.68 p = 2.16e-06; LOC-%Bare F = 10.43 p = 0.00214; LAT-NH₄ F = 7.218 p = 0.0094; LAT-K F = 27.2 p = 2.56e-06; LAT-%Sand F = 5.627; p = 0.021; and for *C. plectostachyus* LAT-%Sand F = 5.943 p = 0.0214.) For this reason, grass responses were analyzed by mean of lineal models that included the factors, the covariates, and the factor-covariate interactions for all non-independent pairs.

For each grass response variable, the averaged model was created using the Akaike Information Criterion for small samples (AICc), a likelihood-based measure to rank alternative models in terms of complexity and performance (Burnham and Anderson, 2002). Model selection was carried out by testing a model and deleting the covariate...
with higher p-value result at each step, and all models were averaged using AICc weights (see details of the model selections carried out in Suppl Mat Tables 2–7). The best fitting models were validated by checking the homoscedasticity and normality by visualizing residual Q-Q plots, fitted residual plots, and Leverage and Cook's distances plots. All statistical analyses were performed using R version 3.2.2 (R Development Core Team, 2016) and Rstudio version 0.99.879.

For the microclimatic variables %Sand, %Bare, K, P, NH3, and NO4, two-way ANOVA were conducted, using the factors latitude (North grade vs. South glade) and location (‘Open’ referring to Cynodon/bare ground-dominated sites; ‘Penn’ referring to *Pennisetum* dense patches in the glades; and ‘Shade’ referring to patches covered by *Acacia etbaica* canopy). When the ANOVA reported significant differences by locations, Tukey tests were conducted to see where the differences lied. The normality and homocedasticity of the models was tested. For the analysis of the RII index dataset, the assumption of normality was tested using Shapiro-Wilk normality test. For each RII data group (calculated for *P. stramineum* based on both aboveground and belowground biomass in the North glade and the South glade), we tested the hypothesis that the mean of the population was different from zero, using the one-sample Student t-test.

### Table 3

Average values of estimates and 95% confidence intervals (95% CI) for the similar best fitting linear models for the superficial (0–6 cm) roots to deep (6 + cm) roots dry biomass ratio (S:D), for the root to shoot ratio (R:S), for the root diameter (Ø) and for the root estimated surface to shoot mass ratio (Rs:S) in *C. plectostachyus*. Estimate values in bold and marked with and asterisk indicate significant result within the 95% confidence interval. A description of the independent variables used can be found in SM Tables 4–7.

<table>
<thead>
<tr>
<th></th>
<th>S:D</th>
<th>R:S</th>
<th>Ø</th>
<th>Rs:S</th>
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<tr>
<td>Intercept</td>
<td>2.242*</td>
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<td>0.311*</td>
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<td>LAT (South)</td>
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<td>−0.079; 0.455</td>
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<tr>
<td>%Sand</td>
<td>0.026</td>
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</tr>
<tr>
<td>%Bare</td>
<td>−0.019</td>
<td>−0.044; 6.3e-3</td>
<td>5.89e-4</td>
<td>−3.1e-3; 4.2e-3</td>
</tr>
<tr>
<td>K</td>
<td>1.06e-4</td>
<td>−2.8e-3; 3.0e-3</td>
<td>−2.15e-5</td>
<td>−4.6e-4; 4.2e-4</td>
</tr>
<tr>
<td>P</td>
<td>−6.66e-3</td>
<td>−0.013; 2.5e-6</td>
<td>1.27e-3*</td>
<td>3.7e-4; 2.2e-3</td>
</tr>
<tr>
<td>NH4</td>
<td>1.06e-3</td>
<td>−0.082; 0.084</td>
<td>1.19e-3</td>
<td>−8.6e-3; 0.011</td>
</tr>
<tr>
<td>NO3</td>
<td>0.057*</td>
<td>4.3e-3; 0.109</td>
<td>−4.65e-3</td>
<td>−0.012; 2.4e-3</td>
</tr>
<tr>
<td>LAT-%Sand</td>
<td>−0.014</td>
<td>−0.273; 0.245</td>
<td>−1.73e-2</td>
<td>−0.051; 0.017</td>
</tr>
</tbody>
</table>

**Fig. 7.** Mean and standard error for the response variables measured in each latitude and location for A- *P. stramineum* (1- root to shoot ratio by location, 2- root to shoot ratio by latitude, 3- superficial to deep roots ratio by location, and 4- superficial to deep roots by latitude) and B- *C. plectostachyus* (1- root to shoot ratio by latitude, 2- root estimated surface to shoot biomass ratio by latitude, 3- superficial to deep roots ratio by latitude, and 4- Root diameter by latitude). Asterisks (and darker plant symbols) indicate significant differences for the factor in the averaged model.
3. Results

3.1. Microhabitat variability

The differences in rainfall for a period of three years prior to sampling, a time considered adequate to represent the effects of rains on the sampled plants growth, depicts clear differences in water inputs between north and south sites. In the three-year period before our study, the average rainfall was \( \sim 103\% \) higher in the south glade (wetter) than in the north glade (drier) (Fig. 3). The comparison between inter- and under-canopy showed an illuminance and soil temperature diminishment driven by acacia cover. Both the illuminance and the temperature data analysis showed significant differences between locations in both latitudes, and the post-hoc analysis yielded significant differences between all the different sites (Table 1). Both temperature and illuminance were higher in bare ground, intermediate within P-patches and lower under the acacia thickets. The temperature changed drastically both soil surface temperatures from \( \sim 46^\circ C \) in exposed areas to \( \sim 26^\circ C \) in the Acacia shade (Fig. 4A) and illuminance from \( \sim 9e4 \) to \( \sim 1e4 \) lux (Fig. 4B), buffering superficial evaporation.

Cover and soil measures were used as covariates for explaining the grass response. However, some observations are drawn from the visual analysis of a descriptive boxplot of these covariates and the statistical analysis (Fig. 5). In general, no big differences were observed between North and South, despite soil samples from the South showed higher K concentration, and less sand percentage. The differences between inter-canopy measures –where grazers provide repeated nutrient input from their excretions– and under canopy measures –where thorns are likely to reduce ungulate access but where litter production is likely to enrich soil content– indicate severe differences in P. This nutrient had higher
concentration in open glades, being more limiting for plants under canopy. Herbaceous plant cover was lower under thicket canopy, where both P and light availability are reduced, than in the glade, indicating less competition between herbaceous plants in under-canopy patches.

3.2. Tree-grass interaction

Even though herbaceous competition was reduced under the canopy, we observed that tree-grass interactions (for fully grown A. etbaica individuals) were either neutral or negative, pointing out that C4 grasses tended to be equally or more successful in open environments that under canopy. The RII index mean values were null (non-different from zero according to Student’s t-test) in the north glade where rainfall is scarcer, indicating that interference was neutral under these conditions, yet it acquired significant negative values in the wetter conditions of the south glade (Fig. 6). In benign environments, competition for light seemed to override the positive effects of canopy on water availability. Under more stressful conditions, however, facilitative forces gained strength in accordance with the stress gradient hypothesis (Callaway and King, 1996).

3.3. Grass response to water availability

Controlling for the covariates, we were able to draw belowground phenotypic responses of grass roots to the two water factors. The S:D ratios showed no significant response to the water input assessed by LAT (Tables 2 and 3). When the S:D response was tested in different potential evaporation conditions, however, the factor LOC did have a significant effect (Table 2). The individuals of the species P. stramineum showed ~ twice as many superficial roots than deeper roots under canopy, but this ratio increased by ~50% (~three times as many) in the drier open sites (Fig. 7a).

We detected a significant response to the water input tested using LAT, but not LOC. In P. stramineum, the biomass allocation to roots decreased by ~16.7% (from R:S ~ 0.18 to ~0.15) with increasing water inputs, while it did not change with soil drying rates, nor for the other species with increasing water inputs (Fig. 7b). For C. plectostachyus, the mean R:S ratio increased by ~37.5% (from R:S ~ 0.4 to ~0.55) in wetter environments, a result that was marginally significant (95% CI = [−0.079; 0.455]). However, for this species, a significant change in Ø was detected at different levels of the LAT factor (Table 3), roots showing a striking ~50% root diameter increase (from ~1 to ~1.5 mm) when moving from dry conditions in the north to wet conditions in the south (Fig. 7c). The covariate %Sand and its interaction with the factor were both significant, Ø increasing in soils with more sand in the north glade (drier) but decreasing with %Sand in the south glade (wetter). Nutrients had no effect in Ø, indicating that in case wider roots are indeed storing resources, the stored resource must be water. When accounting for root geometry, Rs:S was significantly higher in the north glade (drier), decreasing by ~75% in the southern plots (wetter) (from Rs:S ~ 8 to ~2) (Fig. 7d), the significance of the result being robust (SM Box 1).

3.4. Grass response to nutrient availability

Even though the response to water availability was our main interest, our data allowed us to detect some interesting changes in grass belowground functional traits in association with soil nutrient availability. We found a trend to increased root allocation when nutrient availability increased since plants were producing relatively more roots when more nutrients were present. This pattern was consistent across species, nutrients, factors and Root diameters. Indeed, Rs:S increased significantly (Tables 2 and 3) at both LAT levels with P in C. plectostachyus (Fig. 8a) and with NO3 in P. stramineum (Fig. 8e). In addition, Rs:S also increased significantly (Table 3) at both LAT levels with K in C. plectostachyus (Fig. 8c).

The S:D ratio was also affected by nutrient availability. Superficial root allocation increased significantly with NO3 in C. plectostachyus, and decreased significantly with P in P. stramineum (Table 3; Fig. 8d). Interestingly, even though the results were not significant for both nutrients and in both species, the trend for P seemed to be consistent across species (for C. plectostachyus slope = −6.64e-3, 95%CI = [−0.013; 2.5e-6], Table 3).

4. Discussion

In this study, we measured grass functional traits of two common savanna C4 grasses that were more successful in inter- (higher %cover and RII index) than under-canopy patches, and analyzed variation in response to different water conditions controlling for soil nutrient availability and other factors having a potential effect on the functional traits. We found soil water availability and their dynamics to have a complex effect on the root allocation of plants. While root to shoot ratio increased with water stress in the species with homogeneous root diameter P. stramineum, it did not in C. plectostachyus. The latter showed a noticeable plasticity in Ø, and a decrease in root surface area to shoot
biomass ratio with increasing water inputs. Also, we showed that soil drying rates driven by evaporation affected the shallow root allocation, more evaporation translating into higher superficial to deep root ratio. We found that root allocation increased with nutrient availability, and shallow root allocation increased with NO_p concentration but decreased with available P. Finally, we were able to detect a significant effect of shading by P. stramineum straw patches on soil temperature and illuminance on glade soils, potentially affecting soil drying rates inside patches of this species in open sites.

Root to shoot ratios are one of the most commonly used descriptors of the relationship between root and shoot biomass, and R:S ratios are known to covary with several environmental and biological factors (Mokani et al., 2006). The ratio has been shown to increase with water limitation due to growth optimization, as the less limiting soil resources are, the more plants benefit from investing in photosynthetic tissues (Chapin et al., 1987; Schenk and Jackson, 2002). Not accounting for root geometry can lead to misleading interpretations of R:S for species with high Ø plasticity. In our case, C. plectostachyus showed higher Ø in higher water input locations. Some studies have shown that agronomical C4 grasses tend to develop wider roots when more water is available due to a restriction in apical root growth (Sharp et al., 1988; Liang et al., 1997), increasing water transport (Varney et al., 1991). However, these observations would only be sound under the growth optimization hypothesis when plants are also developing more vigorous aboveground systems. This was not the case in our study, since C. plectostachyus is also proportionally allocating more to root growth in wetter environments. In this case, we suggest that increased root allocation may not be a response to water limitation, but a strategy to use these roots as water storage organs. It is common for plants growing in dry environments to develop water storage organs, and the wide roots of C. plectostachyus seem to serve that function. However, our results indicate a potential shift in life history strategy under changing environmental conditions that merits further attention. When enough water is available, the species may survive the dry season as a rhizome geophyte (sensu Raunkiær, 1904), storing water in wider, longer lived roots (van der Krift and Berendse, 2002). Nonetheless, in drier environments in which the drought is extreme during the dry season, C. plectostachyus may not store water but use it to grow and reproduce during the growing season, acting as an annual (narrower roots and little rhizome lignification, personal observations).

Increased root depth is often associated with dryer conditions (Huang and Eisenstat, 2000; Schenk and Jackson, 2002). The vertical distribution of roots provides further understanding on plant root structure as compared to maximum rooting depth, and many agrophysiological studies have reported relatively low root densities in shallow soil layers in water stressed C4 grasses (Bolaños and Edmeades, 1993; Campos et al., 2004; Hund et al., 2009). Most of these studies assessed only differences due to changing water input dynamics. The S:D ratio allowed us to assess the vertical profile of allocation of roots in wild populations of C4 grasses. We found no response to water stress driven by water inputs, but shallower and more densely compacted root systems when water stress was driven by drying rates (Fig. 9). Water table depth is, along with rainfall, an important ecological factor that is energetically demanding and likely hindered by shade. Therefore savanna C4 grassland species may outcompete taproot species at the seedlings stage, reducing thicket encroachment and eliminating negative feedbacks that would reduce their competitive advantage. Our results suggest that each grass species in our study used a different strategy to efficiently use water from rainfall, and compete with woody plants. Species may benefit from having palatable aerial organs, leaving the soil exposed during the dry season to conditions that make sup-pporting self-organized spatial patterns in grasslands is a mechanism that should be further investigated as a possible driver of self-organized spatial patterns along with triggering water infiltration. This effect may not only benefit the grass, but potentially also facilitate the development of other individuals, including tree seedlings and saplings (Dios et al., 2014). Nevertheless, we observed that the same grass species that was creating these “oasis” patches within the glades was also densely concentrating its roots in a very shallow soil layers, potentially hindering the recruitment of enemies.

5. Conclusions

C4 grasses benefit from direct exposition to sunlight as their metabolism is efficient at high temperatures but this type of photosynthesis is energetically demanding and likely hindered by shade. Therefore savanna C4 grassland species may outcompete taproot species at the seedlings stage, reducing thicket encroachment and eliminating negative feedbacks that would reduce their competitive advantage. Our results suggest that each grass species in our study used a different strategy to efficiently use water from rainfall, and compete with woody plants. Species may benefit from having palatable aerial organs, leaving the soil exposed during the dry season to conditions that make sup-pporting life difficult. These grasses may either adopt an annual life cycle or, if water is sufficiently abundant during rain seasons, survive as rhizome geophytes developing water storing roots. Alternatively, spe-cies may benefit from self-shading that reduces evaporation in glades. Such facilitation could also benefit taproot competitors, but we found which this model should also be highly sensitive.

Nutrients were used as covariates in our study in order to control for their influence on root structure and functional traits, and to separate their effects from those associated with water acquisition. Root allo-cation is generally expected to decrease when more nutrients are available in the soil (Chapin et al., 1987; Crick and Grime, 1987; Gedroc et al., 1996), but we found that R:S increased with nutrient concentration, and this result was consistent across species and nutrients (K, P, NO_p). One explanation for this response, especially for K, is that deficiencies of nutrients that are not fundamental for organic matter but that are closely associated with carbon fixation may lead to a sub-optimal allocation to light acquisition (Ericsson, 1995). A recent model of competition under water and nitrogen co-limitation predicted that the increase of R:S in response to water and nitrogen addition may be met if only one resource is limiting, whereas when there is water-nutrient co-limitation and one of these resources is added, plants may allocate more in roots in order to increase their ability to uptake the other resource (Farrior et al., 2013). If macronutrients such as N or P are added to a co-limited individual, the individual may allocate more of this resource into roots to collect more water. This may explain the R:S response observed in our study, assuming that nutrients are co-limiting plant growth along with water.

Facilitation mechanisms have been considered key factors in explaining the emergence of self-organized spatial patterns in grasslands in the arid-semiarid climatic transition (Rietkerk et al., 2002). The ef-fect of plant roots on soil compactness and the consequent soil hy-draulic properties have been the main facilitative force considered by these models (HilleRisLambers et al., 2001). In our study we observed that P. stramineum invests carbon in creating unpalatable fibrous tissues in the basal parts of the aerial stems, creating dense packages of straws when growing in open glades: These ~20 cm vertical straws remain ungrazed due to the behavior of wild grazers and livestock present in the area (Odadi et al., 2011), giving a characteristic physiognomy to the P. stramineum patches which are visible from far away (Fig. 10). It is unlikely that this protection directly affects plant survival under her-bivory since these aerial organs die during the dry season. Plants, however, can benefit from shade decreasing soil surface temperature by ~6 °C and illuminance by ~40% in exposed areas, potentially reducing water evaporation and air water demand. The facilitative effect of un-palatable aboveground shading organs in open grasslands is a me-chanism that should be further investigated as a possible driver of self-organized spatial patterns along with triggering water infiltration. This effect may not only benefit the grass, but potentially also facilitate the development of other individuals, including tree seedlings and saplings (Dios et al., 2014). Nevertheless, we observed that the same grass species that was creating these “oasis” patches within the glades was also densely concentrating its roots in a very shallow soil layers, potentially hindering the recruitment of enemies.
that the species creating shade patches densely concentrated most of its roots in a very shallow soil layer, probably depleting soil moisture after most intermittent rain events, thus impeding the water infiltration to deeper layers where taproots develop.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1101/j.jarendenv.2018.06.008.

References
