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Habitat-specific AMF symbioses enhance drought tolerance of a native Kenyan grass

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ABSTRACT

The role of arbuscular mycorrhizal fungi (AMF) in enhancing plant tolerance to drought is well known. However, the degree to which AMF-plant symbioses are locally adapted has been suggested but is less well understood, especially at small spatial scales. Here, we examined the effects of two arbuscular mycorrhizal fungal communities on drought tolerance of *Themeda triandra*, a native African perennial bunchgrass. In our study area, mound building activities of *Odontotermes* sp. termites produce heterogeneous habitat, particularly with respect to water availability, and do so over small spatial scales (<50 m). Thus, plants and their AMF symbionts may experience identical climatic conditions but very different edaphic conditions. We hypothesized that AMF from off-mound areas, where plants experience drought more intensely than on termite mounds, would confer greater protection from drought conditions than AMF from termite mound soils. To test this, we conducted a greenhouse experiment in which we grew plants in soils that we inoculated with fungi from on or off termite mounds, or with a sterilized control inoculum. Our results reveal habitat-specific AMF effects on host stomatal functioning and growth. Contrary to our expectations, drought stressed grasses inoculated with AMF from termite mounds closed stomata less, and produced 60% more leaves than those inoculated with off-mound AMF, thus exhibiting higher levels of tolerance. Mound-inoculated plants that were drought stressed also produced more than twice as many leaves as non-inoculated plants. Longer-term productivity measurements indicate both on- and off-mound inoculated plants were able to recover to a greater extent than non-inoculated plants, indicating that AMF associations in general help plants recover from drought. These findings highlight the important role that AMF play in mitigating drought stress and indicate that AMF affect how plants experience drought in a small scale, habitat-specific manner.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the majority of land plants (Brundrett, 2009). AMF colonize plant roots and provide plants with nutrients in exchange for organic carbon (Smith and Read, 2008). AMF can also help plants resist or tolerate a number of stressors, including pathogens (Newsham et al., 1995), herbivory (Bennett and Bever, 2007), heavy metal toxicity (Turnau et al., 2010), and drought (Augé, 2001). Recent work suggests both AMF and other fungal endophytes can confer stress tolerance to their hosts in a habitat-specific manner, a phenomenon described as habitat-adapted symbiosis (Rodriguez et al., 2008). For example, Johnson et al. (2010) found that AMF communities native to environments low in phosphorus or nitrogen were better able to provide these limiting nutrients to their host plants than those from more nutrient rich environments. Likewise, Rodriguez et al. (2008) demonstrated that fungal endophytes native to areas with high salt, heat, or pathogen loads were better able to protect plants against these stressors.

Microbially-mediated response to stress may be particularly important to plants when environmental conditions are heterogeneous over small spatial scales because gene flow among plants from different habitat-types may hinder local adaptation (Leimu

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and Fischer, 2008). Although their hosts may be genetically panmictic, the arbuscular mycorrhizal community can show strong patterns of differentiation and respond to environmental heterogeneity through changes in community composition (Schechter and Bruns, 2008), or through the evolution of locally adapted ecotypes (Marulanda et al., 2007; Stahl and Smith, 1984). Shifts in community composition and ecotypic differentiation can occur over small spatial scales (Koch et al., 2004; Mummey and Rillig, 2008) and evolutionary changes in AMF populations may happen rapidly (Johnson et al., 2013). Thus, AMF may facilitate stress tolerance, even at small spatial scales. Local environmental conditions can impact both plant and fungi independently (Singh et al., 2010) but, ultimately, AMF community members that prove more mutualistic may be favored, as in the case of preferential exchange of nutrients and photosynthates (Bever et al., 2009; Kiers et al., 2011).

Here, we asked if habitat-specific symbiosis is important to plants for withstanding heterogeneity in water availability at small spatial scales. To explore this question we inoculated plants with AMF from termite mounds (hereafter referred to as "on-mound") or with AMF from the surrounding habitat (hereafter referred to as "off-mound") and then exposed them to drought. We compared stomatal functioning and growth responses to those that were grown in soils without AMF. Our study sites are in an area of "black-cotton soil" in central Kenya where *Odontotermes* sp. termite mounds are a common and regularly spaced feature of the landscape (Pringle et al., 2010). The presence of these mounds creates dramatic heterogeneity in water availability on highly localized scales (<50 m). Mound soils are lower in clay and higher in soil moisture than surrounding off-mound areas (Darlington, 1985; Jouquet et al., 2006, 2011). In contrast, off-mound soils, with higher clay content, quickly become saturated during rains and dry out more quickly (Deckers et al., 2001). Because of the prolonged drought that occurs off-mound, we hypothesized that off-mound AMF would confer greater drought tolerance to their plant partners than AMF from on-mound soils. In this study drought tolerance was characterized as the maintenance of growth and normal stomatal functioning, relative to control plants, under low water conditions.

2. Materials and methods

2.1. Study site

Soil and root samples were collected from plants growing on and off termite mounds (Order Isoptera: *Odontotermes* sp.) on heavy clay "black cotton" soil at the Mpala Research Center (MRC), located in central Kenya on the Laikipia plateau (37E, 08N: 1800m elevation). These black cotton savannas are semi-arid, with an understory of perennial grasses, and a canopy of the mono-dominant tree, *Acacia drepanolobium*. Average annual rainfall for a ten-year period (1999–2009) was 594 ± 53 mm (mean ± SE; Maclean et al., 2011). The large, low-lying lenticular mounds of *Odontotermes* sp. termites are widespread and over-dispersed across these savanna landscapes (Pringle et al., 2010), where they may persist for a century or more (Darlington, 1985). The underground activities of *Odontotermes* significantly alter the chemical, physical, and hydrological characteristics of soils. Relative to off-mound areas, termite mounds have significantly higher soil nitrogen and phosphorus (Petipas and Brody, 2014) and lower levels of clay (Brody and Palmer unpublished data). Air humidified by fungus growing deep within the termite mound circulates up through the soil via convection, thus maintaining more constant soil moisture (Turner, 1994). In addition, termites may actively maintain soil moisture by translocating moist soil from lower down in the soil profile (Turner et al., 2006). In contrast, off-mound soils experience periods of drought interspersed with periods of rapid soil wetting (Deckers et al., 2001).

2.2. Soil collection and culturing

To examine if AMF from on and off termite mounds differed in their ability to protect plants from drought, we collected root and soil samples from the five most common grasses that occur both on and off *Odontotermes* mounds: *Pennisetum stramineum*, *Pennisetum mezianum*, *Brachiaria lachnantha*, *Themeda triandra*, and *Lintonia nutans*. Co-occurring grass species can associate with different AMF (Vandenkoornhuyse et al., 2003), thus to maximize the diversity of fungi included in our experiment, we collected inoculum under these common C4 grasses that comprise almost 90% of the understory cover (Young et al., 1998). At each of nine paired on- and off-mound locations (18 locations), we collected root and soil samples from two randomly selected grass species chosen from the five most common grasses. We defined "on-mound" as the center of a termite mound and "off-mound" by measuring double the distance of the mound diameter from the mound edge. For example, if the mound was 10 m in diameter, we measured 20 m from the mound edge into the surrounding matrix. Mounds are generally 10–20 m in diameter and no greater than 0.5 m high (Pringle et al., 2010). Roots and soil were air dried and then shipped to the University of Vermont and stored at 4 °C. All root and soil samples were collected in late June and early July of 2009. We collected seeds from a common C4 grass species, *Themeda triandra*, in January 2009 to be used in our experiment. Seeds were collected haphazardly both on and off termite mounds. We used *T. triandra* because it is commonly found both on and off termite mounds and it is amenable to cultivation in the lab and greenhouse.

To maximize the inoculum potential and encourage sporulation by additional species of AMF present in rhizosphere, we cultured AMF in the greenhouse using a highly mycotrophic host plant, sorghum-Sudan grass (*Sorghum bicolor* var. *sudanense*), following the International Culture Collection of Arbuscular Mycorrhizal Fungi protocols (http://invam.caf.wvu.edu/index.html). Because we had no prior knowledge of the AMF community members that would associate with or impart optimum benefits to *Themeda* seedlings, we utilized the trap pot method to provide seedlings with access to viable AMF propagules representing the diversity of AMF present in the field. Sorghum is commonly used in trap pot cultures because it promotes sporulation by many AMF species (Morton et al., 1993) and the resulting AMF communities are often similar in composition to that of starting inoculum (Bever et al., 1996; Eom et al., 2000). Details of trap pot methods can be found in Appendix A.1: Supplementary Methods.

2.3. Experimental design

We grew seedlings (Appendix A.1: Supplementary Methods) in 15 cm pots with a 2:3 mixture of sterilized (autoclaved for 1 h at 121 °C) calcined clay (Industrial Materials Corp., Deerfield, IL, USA) and sand. A layer of on-mound or off-mound inoculum (100 mL) was added to each pot and covered by a few centimeters of autoclaved sand/clay mixture. A layer of sterilized (autoclaved for 1 h at 121 °C) inoculum was added to control pots. To control for non-AMF microbial effects, we reintroduced a portion of the non-AMF microbial community to the control pots (Koide and Li, 1989) by applying 50 mL of on- or off-mound-specific microbial washes to on- and off-mound control pots respectively (Appendix A.1: Supplementary Methods).

Plants were grown in 12:12 L:D with temperatures between 20 °–24 °C in daytime and 17 °–19.5 °C at night. Plants were given

six weeks to establish, during which time they checked daily and watered as needed. Low phosphorus fertilizer (17:4:17, Jack's Pure Water LX, J.R. Peters, Inc. Allentown, PA USA) was added in the first week and at the conclusion of the experimental recovery period. At the end of six weeks, plants were watered to saturation and each of the four AMF treatment groups (on-AMF+, off-AMF+, on-AMF-, and off-AMF-) were randomly divided into two drought treatments (wet or dry, n = 10 for AMF + groups and n = 5 for AMF- groups −see experimental design schematic Appendix A.2, Fig. S1). The drought treatment lasted four weeks, during which time water was completely withheld from plants assigned to the dry treatment, while plants assigned to the wet treatment were watered as described above. Subsequent to drought, all plants were returned to the original watering schedule for a four-week "recovery period". After the four-week recovery, plants were grown for an additional eight weeks to examine the effects of the treatments on overall plant productivity. We used three categories of measurement to evaluate drought tolerance: 1) plant growth response and recovery from drought, 2) stomatal response during and after exposure to drought, and 3) overall plant productivity. We also evaluated the extent of mycorrhizal colonization in plants inoculated with on- and off-mound AMF.

We assessed plant growth using plant height and leaf numbers. We measured plant height one week prior to drought, and weekly thereafter until the end of the four-week recovery period. Leaf numbers were also recorded from the second week of drought onward. After the eight-week growth period that followed the recovery period, plants were harvested and separated into above- and below-ground components and dried at 80 °C to a constant weight.

Plant stomata regulate influx of CO_2 and release of H_2O in transpiration (aperture; Taiz and Zeiger, 2010). Thus, stomatal aperture is indicative of changes to plant physiology due to water stress. We measured stomatal aperture one week before the onset of the drought period, and again on the final day of the drought period. We then assessed recovery from water stress by measuring stomatal aperture at two time points (1hr and 3hr) after watering. We measured stomatal aperture by taking stomatal peels from all plants between 1300 and 1400 EST. Xantopren® silicone-based dental putty was applied to abaxial leaf surfaces to make a cast (Bayer Dental, Leverkusen, Germany), and commercially available nail polish was used to make observable impressions of stomata from the silicone cast. These nail polish impressions were mounted on slides and viewed under a compound microscope. Photos and measurements of stomata were taken using SPOT imaging software (Diagnostic Instruments, Michigan, USA). Stomatal aperture was measured as the average ratio of width to length from five different stomata on one plant.

We randomly chose five plants per treatment group for assessment of AMF colonization. We haphazardly took 0.1 g of wet root mass from these plants, cleared them with 10% potassium hydroxide (KOH) and stained them with 0.05% Trypan Blue in lactoglycerol (Phillips and Hayman, 1970). Two samples from the on-mound wet treatment were over-cleared and therefore lost, leaving us three replicates for that treatment group. Colonization was estimated using the magnified intersection method (McGonigle et al., 1990) and is reported here as the percentage of roots colonized with AMF structures (hyphae, vesicles, or arbuscules).

2.4. Data analysis

We found no difference between the AMF- control groups (on-AMF- and off-AMF-) in any analyses; therefore, we combined them into a single AMF- treatment (all p-values > 0.46). To determine whether differences existed between treatment groups prior to the drought treatment, we evaluated pre-drought stomatal aperture and plant height by two-way analysis of variance (ANOVA), with AMF (on-AMF+, off-AMF+, and AMF-) and drought treatment (wet, dry) as fixed effects. We found no significant pre-drought differences in height (p-values>0.70) or stomatal aperture (p-values>0.40). Assumptions of ANOVA were tested for each response variable using graphical approaches (Q-Q plots) and statistical tests (Shapiro-Wilk and Levene's Tests). We transformed stomatal apertures (arcsine square root), hyphal colonization (arcsine square root), and leaf number (square root) to better fit the assumptions of ANOVA.

To assess changes in plant height and leaf number over the course of the drought period, we used linear mixed effects models with treatment (on-AMF+, off-AMF+, or AMF-), drought (wet/dry), and week as fixed effects, and plant ID as a random effect. Plant identity was specified as a random effect to account for repeated censusing of the same plant. We also separately evaluated stomatal aperture, plant height, and leaf number on the last day of the drought period by two-way ANOVA, with AMF treatment (on-AMF+, off-AMF+, or AMF-) and drought (wet, dry) as main effects. In the analyses of plant height, plant height prior to drought was included as a covariate.

Stomatal recovery was assessed using the stomatal peel measurements made on the final day of drought, and the peel measurements made 1 h and 3 h s after watering. We evaluated stomatal recovery using a linear mixed effect model that incorporated treatment (on-AMF+, off-AMF+, or AMF-), drought (wet, dry), and hour (0, 1, and 3) as fixed effects and plant ID as a random effect, with stomatal aperture as the response.

Plant growth recovery from drought, was assessed using plant height and leaf number measurements taken on the last day of drought through the last day of the four-week recovery period. We evaluated plant growth recovery using linear mixed effects models with treatment (on-AMF+, off-AMF+, or AMF-), drought (wet, dry), and week as fixed effects and plant ID as a random effect, with plant height and leaf numbers as responses. On the last day of the recovery period we evaluated plant height and leaf number by two-way ANOVA, with AMF treatment (on-AMF+, off-AMF+, or AMF-) and drought (wet, dry) as main effects. In the analyses of plant height, plant height prior to drought was included as a covariate.

To examine the effects of AMF treatments on overall plant productivity, we evaluated above- and below-ground biomass by two-way ANOVA with AMF treatment group (on-AMF+, off-AMF+, AMF-) and drought (wet, dry) as main effects. We evaluated mycorrhizal colonization by two-way ANOVA with AMF treatment group (on-AMF+, off-AMF+, AMF-) and drought (wet, dry) as main effects.

In all models we included the main effects of AMF and drought treatments and the interaction between AMF and drought. We tested several pre-determined hypotheses within the ANOVA framework using planned contrasts by performing Student's T-tests on relevant comparisons and then adjusting p-values with Bonferroni corrections. We evaluated differences within each AMF treatment group between wet and dry plants, differences between AMF treatment groups for all dry plants, and differences between AMF treatment groups for all wet plants for a total of nine comparisons. We were primarily interested in differences between AMF + treatment groups, which we analyzed with separate two-way ANOVAs. This never qualitatively changed the outcome of the analysis, but helped us to understand the interaction between AMF + groups and drought better. Mixed effects linear models were performed in R version 3.0.2 (R Development Core Team, 2013) and all other analyses were performed using JMP® Pro 10.0.0 (SAS Institute Inc., Cary, NC).

3. Results

Over the course of the drought period plant height (time × drought interaction: $F_{(3,141)} = 19.44$, $P < 0.0001$) and leaf number (time × drought interaction: $F_{(2,89)} = 50.44$, $P < 0.0001$) were affected by drought, but not AMF treatment (P > 0.27 for main effects and interactions). At the end of the drought period, drought stressed plants inoculated with off-mound AMF almost completely closed stomata compared with only marginally altered stomatal functioning of the other treatment groups (AMF treatment × drought interaction: $F_{(2,41)} = 5.13$, $P = 0.01$; Fig. 1). Planned contrasts revealed highly significant differences between off-AMF + wet and dry plants ($P < 0.0009$). This interaction was especially evident when the AMF + treatment groups were analyzed alone (AMF treatment × drought interaction for the AMF + treatment groups only: $F_{(1, 27)} = 6.51$, $P = 0.02$; inset Fig. 1). On the last day of the four week drought, plants that were drought treated were shorter (drought treatment effect: $F_{(1,45)} = 27.58$, $P < 0.0001$), and had fewer leaves (drought treatment effect: $F_{(1,43)} = 9.30$, $P = 0.004$), but were unaffected by AMF treatment (P > 0.41 for main effects and interactions).

Stomatal apertures recovered to pre-drought size hours after watering equally among all treatment groups (time x AMF × drought interaction $F_{(4,84)} = 1.51$, $P = 0.21$). Over the course of the recovery period, plant height was affected by drought (time × drought interaction: $F_{(4,166)} = 3.48$, $P = 0.009$), but not AMF treatment ($P > 0.43$ for main effects and interactions). In contrast, the number of leaves added over the four week recovery period was highly dependent on AMF treatment. On-AMF + grasses, regardless of watering regime, produced nearly identical amounts of leaves (Fig. 2A), whereas off-AMF+ and AMF- plants showed reductions in leaf production over time after being exposed to drought (time x AMF × drought interaction: $F_{(8,168)} = 2.38$, $P = 0.02$; Fig. 2B and C).

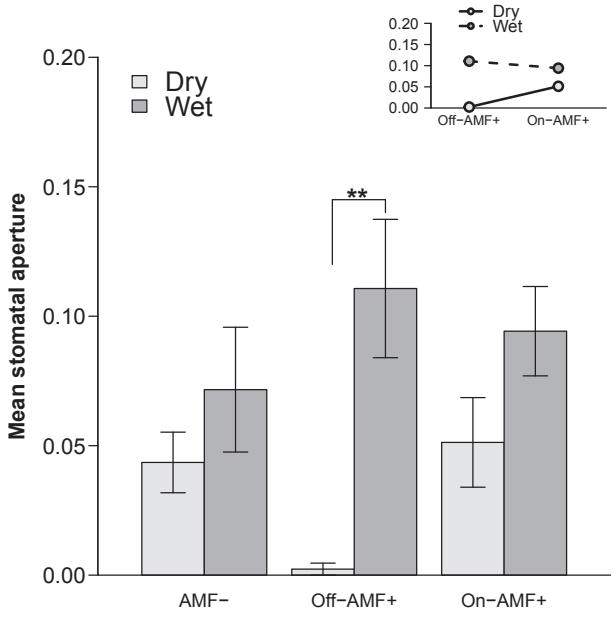

Fig. 1. Effect of mycorrhizal infection and drought on stomatal opening (aperture) size of *Themeda triandra*. Stomatal aperture was measured using stomatal peels taken on the last day of drought, and is reported as the average ratio of the width and length for five stomata per plant. Groups were compared by Bonferroni corrected planned contrasts. We found significant differences between Off-AMF + wet and dry plants ($P < 0.0009$). The inset in the top right corner shows the interaction of the AMF + treatments only. All values are means ± SE. Significant differences are denoted by asterisk symbol (*$P < 0.05$, **$P < 0.01$).

On the final day of the four-week recovery period, plants that were drought treated were still shorter than plants that were watered (drought treatment effect: $F_{(1,40)} = 18.15$, $P = 0.0001$). Plant height was not influenced by AMF treatment ($P > 0.72$ for main effect and interaction). Leaf number was influenced by AMF treatment ($F_{(2,42)} = 5.00$, $P = 0.01$) and drought treatment ($F_{(1,42)} = 9.34$, $P = 0.004$), an interaction between drought and AMF treatment was not detectable in the ANOVA ($F_{(2,42)} = 2.68$, $P = 0.08$). However, planned comparisons between treatment groups indicate that drought treated AMF- grasses produced 67% less leaves than drought treated plants inoculated with on-mound AMF ($P = 0.03$; Fig. 3), and Off-AMF + dry plants produced 56% less leaves than Off-AMF + wet plants on average ($P = 0.04$; Fig. 3). The effects are especially evident when comparing the AMF + groups alone; drought treated plants inoculated with on-mound AMF had the same number of leaves as plants inoculated with on-mound AMF that had been watered. In contrast, drought treated plants inoculated with off-mound AMF had significantly fewer leaves than plants inoculated with off-mound AMF that had been watered (AMF treatment × drought interaction for the AMF + treatment group only: $F_{(1,28)} = 5.60$, $P = 0.03$; inset Fig. 3).

Overall plant productivity was significantly reduced in drought treated plants (drought treatment effect: aboveground biomass, $F_{(1,42)} = 8.25$, $P = 0.01$, and belowground biomass, $F_{(1,42)} = 5.84$, $P = 0.02$). The effect of drought was significantly greater for AMF- plants, as non-inoculated, drought-stressed plants were significantly smaller than all other treatment plants (AMF treatment effect: aboveground biomass, $F_{(2,42)} = 9.62$, $P = 0.0004$, Fig. 4a; and belowground biomass, $F_{(2,42)} = 8.17$, $P = 0.001$, Fig. 4b). Biomass measurements were not different between the AMF + treatment groups (AMF treatment × drought interaction, $P > 0.5$). However, planned contrasts indicate that overall, non-AMF drought stressed plants produced significantly less above ($P = 0.005$) and belowground ($P = 0.02$) biomass than on-mound AMF drought stressed plants. Non-AMF drought stressed plants produced 50% less above and 56% less belowground biomass than on-mound AMF drought stressed plants.

The colonization of inoculated plants (AMF+) was significantly higher than that of non-inoculated plants (AMF treatment effect: $F_{(2,22)} = 34.16$, $P < 0.0001$). Colonization levels in AMF + plants ranged from 44% to 66%. Three of the ten non-inoculated plants had some extent of AMF colonization with one extreme outlier that had nearly 20% colonization (3%, 5%, and 19%). The extent of colonization was not influenced by drought treatment or inoculum source.

4. Discussion

Our work confirms that AMF enhance drought tolerance (Augé, 2001; Jayne and Quigley, 2013) and supports the idea that plant benefits are dependent on the local AMF community. On- and off-mound AMF influenced plant response to drought differently for some response variables in our experiment. Plants inoculated with fungi from off-termite mounds closed stomata more completely than plants inoculated with fungi from on-termite mounds. In contrast, plants inoculated with fungi from on-termite mounds were able to produce more new leaves subsequent to drought. These results indicate microbe-mediated changes to a plant's phenotype can occur over very small spatial scales (<50 m) and impact plant tolerance to drought.

We hypothesized that the backdrop of rapid dry-downs and prolonged droughts, common to off-mound areas, would select for AMF communities that would be most suited to helping plants withstand drought conditions. Contrary to our expectations, plants inoculated with on-mound AMF were more tolerant to drought and able to recover more fully post-drought. We are unable to

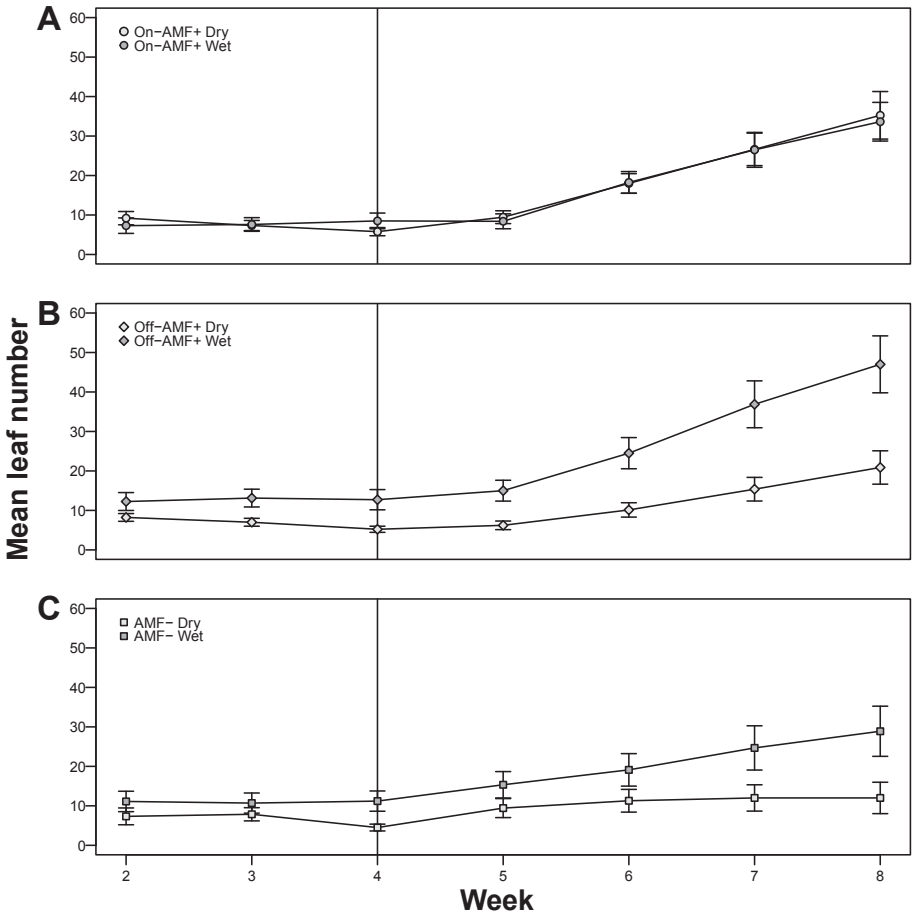

Fig. 2. The impact of drought and AMF inoculation on leaf production by *Themeda triandra* over the course of drought and recovery period. The x-axis corresponds to the number of weeks since the initiation of drought treatment. In all panels, the vertical line at week four marks the end of the drought period, when plants were re-watered, and the beginning of the four-week recovery period. **Panel A**: Leaf number was very similar for On-AMF + plants regardless of watering treatment over the course of the drought treatment and into the recovery period. **Panel B**: Leaf number was affected by watering treatment for Off-AMF + plants especially during the recovery period. **Panel C**: Leaf number was similarly affected by watering treatment for AMF- plants. For all panels, points are means for treatment groups ± SE.

distinguish if off-mound fungi are inferior in enhancing drought tolerance, or if they confer a set of benefits that is specialized for off-mound edaphic conditions. Under periods of prolonged drought, drought avoidance (*e.g.*-shutting stomata and suspending growth) can help plants persist until the next rainfall (Chaves, 2004) and may help off-mound plants cope with the rapid drying that occurs in high clay, off-mound soils during prolonged dry seasons common in East Africa. A drought avoidance strategy (rather than tolerance) may explain why plants inoculated with off-mound fungi closed stomata more quickly when drought stressed. On-mound plants and their associated microbes face very different conditions; the activities of termites contribute to a more homeostatic environment, buffered against extreme drought (Jouquet et al., 2011; Turner et al., 2006). Plants growing on-mounds may experience prolonged periods of low-level water stress but rarely do they experience the extended droughts of the off-mound areas, this is especially evident in infrared photos of the research area showing higher primary productivity on-mounds (Bonachela et al., 2015). This work poses the intriguing possibility that plants growing on-mounds are able to maintain their productivity (Bonachela et al., 2015) through a combination of symbiosis with AMF and the activities of termites. Our initial sample sizes were limited by seed germination and seedling establishment, and then further reduced by plant mortality during the experiment. Thus, at the experiment's end, we had limited statistical power to detect treatment effects (15–20% power to detect differences in biomass). Low power precludes our ability to make definitive conclusions, and we suggest future experiments with more replicates to fully assess AMF community specific effects.

We tested whole community inoculum and, therefore, cannot isolate whether our results were from the synergy of multiple community members, locally adapted ecotypes, or the effect of one or a few drought specialized fungi. Prior studies have found AMF species-specific differences in drought tolerance. In a comparison of seven species of mycorrhizal fungi, plants harboring the most efficient species only experienced a 9% reduction in growth under drought conditions, while those harboring the least efficient suffered a nearly 70% reduction in growth (Ruiz-Lozano and Gómez, 1995). We previously documented different communities of AMF found on and off-termite mounds, with one species, *Funneliformis constrictum (*formerly *Glomus constrictum)*, being unique to on-mound areas (Petipas and Brody, 2014).

Several studies have focused on the benefits of *F. constrictum* under drought conditions. *F. constrictum* inoculated marigold (*Tagetes erecta*) had higher dry weights after drought periods relative to non-mycorrhizal controls (Asrar and Elhindi, 2011). In another study, *F. constrictum* enhanced pagoda shrub (*Sophora davidii)* growth and physiological parameters including instantaneous water use efficiency and net photosynthetic rate relative to *Funneliformis mosseae* (Gong et al., 2012). However, focusing solely

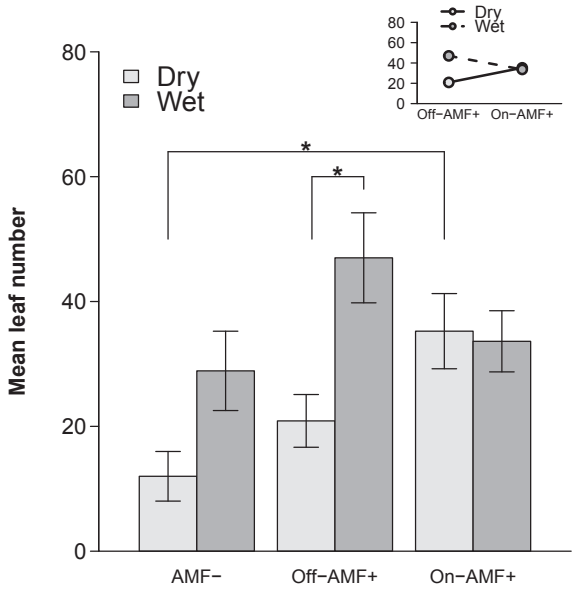

Fig. 3. The effect of mycorrhizal inoculum and drought on leaf production of *Themeda triandra* on the final day of a four-week recovery period from drought. Differences in treatment groups were evaluated by Bonferroni corrected planned contrasts. Off-AMF + wet plants produced more leaves than dry plants ($P = 0.04$), and drought stressed On-AMF + plants produced more than drought stressed AMF- ($P = 0.03$). The inset in the top right corner shows the interaction of the AMF + treatments only. All values are means ± SE. Significant differences are denoted by asterisk symbol (*$P < 0.05$, **$P < 0.01$).

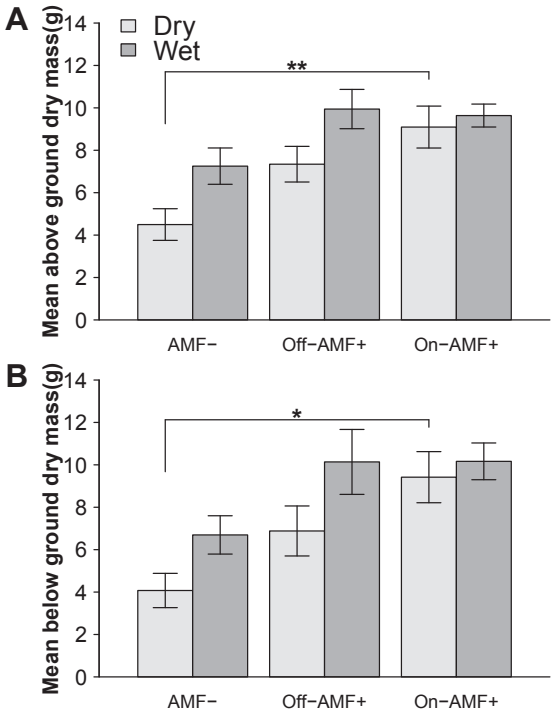

Fig. 4. The impact of AMF inoculation and drought on above (A) and belowground (B) dry mass at the conclusion of the experiment. Differences in treatment groups were evaluated by Bonferroni corrected planned contrasts. **Panel A**: AMF- dry plants produced significantly less aboveground biomass than On-AMF + dry plants ($P = 0.0045$). **Panel B**: AMF- dry plants also produced significantly less belowground biomass compared with On-AMF + dry plants ($P = 0.02$). All values are means ± SE. Significant differences are denoted by asterisk symbol (*$P < 0.05$, **$P < 0.01$).

on *F. constrictum* is likely overly simplistic for three primary (and non-exclusive) reasons. First, differences in drought recovery may be driven by AMF species abundance rather than species richness. Second, entire AMF communities may work in concert to help plants during stressful events (Jansa et al., 2008; Klironomos et al., 2004). And, third, strains of fungi may be locally adapted to tolerate local water availability (Marulanda et al., 2007; Stahl and Smith, 1984), a plausible scenario considering the mounds can be many centuries old (Darlington, 1985). Follow-up work will be needed to determine the exact mechanism(s) operating in this system. It is premature to conclude definitively that differences in community composition drive the patterns we found because of the aforementioned reasons, and because we did not sample AMF community composition in the roots of our experimental plants.

Our results lend support to the increasingly substantiated idea of microbe-mediated local adaptation (Friesen et al., 2011). However, there are several caveats of our experimental design: 1) although trap pots ensure high quality AMF propagules they can also introduce species specific culturing artifacts. For example, sorghum, a highly mycotrophic species may encourage sporulation by AMF species usually dormant in the field. However, previous investigations have found trap pot communities to be similar in composition to field AMF communities (Bever et al., 1996; Eom et al., 2000). In addition, 2) we did not monitor AMF colonization throughout the course of the experiment, so we cannot determine to what extent our results are because of differential colonization (i.e. higher inoculum potential) from our two inoculum sources. On-mound inoculum could be more infective (creating higher levels of colonization) and, therefore, more beneficial. However our previous work does not support this hypothesis; infectivity assays done in 30-day-old corn seedlings reveal higher infectivity from off-mound inoculum (Petipas and Brody, 2014). Alternatively, higher levels of colonization could compromise the host plant during drought stress. However, not only would this contradict prior studies (Augé, 2001 and references therein), we found uncolonized plants (AMF-) added the fewest leaves in the recovery period.

Despite differences during the post-drought recovery between the two AMF + treatments, off-mound AMF + plants recovered substantially by the end of the experiment (final productivity measurements). This suggests that, in general, mycorrhizal plants are better able to recover from drought than AMF- plants. The ability of AMF to ameliorate drought is well established and has been described qualitatively and quantitatively in several reviews (Augé, 2001; Jayne and Quigley, 2013). In many cases the ability of mycorrhizal plants to withstand drought is attributed to enhanced nutrient acquisition, in that larger better-nourished plants are better able to withstand the hardship of drought (Augé, 2004). However, we found no difference in plant size leading up to or during drought; therefore, differences in plant size prior to the drought treatment are unlikely to be involved in the plant response. Nonetheless, It is possible that AMF + plants in our study were better able to acquire nutrients and hence recover more efficiently once watering was resumed. AMF are specialized to acquire nutrients, especially phosphorus and some micronutrients (Smith and Read, 2008), potentially allowing mycorrhizal plants a better chance to recover. Although changes to plant nutrient status are often invoked, AMF also affect a suite of other characteristics that can impact plant water relations, including changes to root architecture, enhanced water acquisition by extra-radical hyphae, improved osmotic adjustment, and AMF-mediated changes to soil structure (Wu et al., 2013).

Although we aimed to test the role of arbuscular mycorrhizal fungi, we cannot necessarily rule out the role of other fungal

endophytes. Dark septate endophytes (DSE) are root-associated fungi that are commonly found co-occurring with AMF worldwide (Jumpponen and Trappe, 1998; Mandyam and Jumpponen, 2014). DSE can also improve drought tolerance of associated plants (Kivlin et al., 2013). We know that DSE occur in this system, albeit at much lower levels than AMF (R. Petipas, pers. obs.); however, we did not quantify the extent of DSE colonization of roots in this study and so we cannot speculate on their importance.

We have provided evidence that a common rangeland grass species can recover from drought more completely because of mycorrhizal fungi and the nature of this response is dependent on the local AMF community-context. Furthermore, our results indicate that association with habitat-adapted AMF may optimize plant response to small-scale environmental heterogeneity. These results suggest a new frontier in understanding plant tolerance to environmental conditions. Elucidating the role of symbionts in stress tolerance of plants enhances fundamental ecological knowledge, and also improves our ability to predict the future of plant adaptation to a changing environment.

Author contributions

R.H. Petipas and A.K. Brody designed the experiment. R.H. Petipas conducted experiments with assistance from J.B. González. R.H. Petipas and J.B. González analyzed the data. R.H. Petipas and A.K. Brody wrote the article with contributions from J.B. González and T.M. Palmer. All authors approved the final article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.actao.2016.12.005.

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