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Native soil microbes buffer savanna trees against nutrient limitation but are drought sensitive

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Summary

1. Belowground microbial communities are vital to ecosystem nutrient cycling, plant health, and resource acquisition, yet belowground plant-soil interactions in savannas remain understudied, especially in their responses to environmental stressors like drought and nutrient limitation.
2. Here, we evaluate if native soil microbiomes have positive or negative effects on tree growth and if these effects are dependent on the level of resource availability. We grew 6 tree species from Kruger National Park, South Africa, for 8 weeks under factorial soil inoculant, water stress, and nitrogen limitation treatments (i.e., sterile/inoculated soils, droughted/non-droughted water supply, and low/high rate of nitrogen supply).

3. In all resource treatments, inoculated plants grew significantly more than sterile plants. Under low nitrogen, trees increased investment in nitrogen-fixing nodules and mycorrhizal associations, leading to increased mass gain. Soil inoculant was most beneficial in non-droughted water conditions, indicating that microbial symbiont effects decreased under drought.
4. *Synthesis:* Belowground microbial symbionts increased savanna tree growth in limited resource environments and could be critical for plant growth in the field. However, drought substantially affected both tree growth and the effects of native soil microbes on tree growth, indicating that extreme droughts could create lasting consequences for both aboveground tree growth and belowground beneficial microbial communities.

Key words: soil microbes, nitrogen, drought, nitrogen fixation, mycorrhizae, savannas, inoculation

Introduction

Savannas are critically important ecosystems, covering 25% of the earth's surface (Strömberg & Staver 2022) and accounting for 25% of global carbon uptake (Moore et al. 2016). Savannas are defined by the coexistence of trees and grasses, with plant community composition strongly shaped by resource limitation (Davies et al. 2013, Ludwig et al. 2004, February et al. 2013b). However, while it has long been known that root symbioses and plant-soil interactions can help savanna vegetation overcome nutrient limitation (Högberg 1986), the influence of plant-soil interactions in savanna ecosystems is relatively unknown, especially in response to environmental factors like drought or nutrient limitation. As global change accelerates shifts in nitrogen availability (Lamarque et al. 2013, Zhu et al. 2016, Kicklighter et al. 2019), hydrological cycles (Dai 2013, Kaisermann et al. 2017), and tree growth (Bond & Midgley 2000), understanding how plant-soil feedbacks interact with the environment could be critical to maintaining diverse, productive savanna ecosystems.

Plant-microbial interactions can range from positive to negative depending on contributions from symbionts vs. pathogens. Beneficial microbial symbionts like nitrogen-fixing bacteria can provide access to nitrogen while arbuscular mycorrhizal (AM) fungi can increase drought resistance and access to nitrogen and phosphorus (Vitousek et al. 2013, Bowles et al. 2022), mitigating resource limitation in high-stress growth conditions (Kulmatiski et al. 2008). Alternatively, plants can be negatively impacted by microbial communities through pathogens (Comita et al. 2014, Rutten et al. 2016) or microbial competition for resources (Kaye and Hart 1997, Reynolds et al. 2003). Additionally, resource stress may make plants even more susceptible to pathogens (Reynolds et al. 2003, Sinha et al. 2019) or may make mutualists more parasitic (Bennett and Groten 2022), with more carbon costs than benefits to the plant (Walder

and Heijden 2015). Inoculation experiments highlight the variability of microbial effects, ranging from positive to neutral to negative (Hoeksema et al. 2010, Wolf et al. 2017, Taylor and Menge 2021) and demonstrate the variability of microbial effects on plant growth. Although nitrogen fixation, mycorrhizal fungi, and plant pathogens influence vegetation dynamics, the importance of these belowground interactions in savannas remains understudied.

The net effects of these symbiotic vs. pathogenic plant-microbe interactions can depend on resource availability (Fahey et al. 2020) and environmental context (Fuchslueger et al. 2014). On the one hand, nitrogen fixation might be more advantageous under low nitrogen availability (Batterman et al. 2013) or drought (Adams et al. 2016, Gei et al. 2018, Batterman 2018), sometimes referred to as the "stress-gradient hypothesis" (Minucci et al. 2017, Hernandez et al. 2021). This hypothesis has been invoked to suggest that the abundance of nitrogen-fixing Fabaceae species in African savannas compared to other ecosystems might reflect widespread nitrogen-limitation under frequent burning (Pellegrini et al. 2016) or competition from grass (Cramer et al. 2007, 2012). Both trees and grasses also associate with mycorrhizal fungi, whose degree of colonization can vary with fire frequency and herbivory intensity (Hartnett et al. 2004, Petipas and Brody 2014, Gonzalez et al. 2018), again perhaps reflecting some degree of resource limitation. Pathogen effects could exacerbate this, since pathogens (especially fungal pathogens) have systematically more negative impacts on plants in warm and wet conditions (Reynolds et al. 2003), resulting in larger net positive effects of microbes in drier, harsher conditions.

On the other hand, limited microbial activity due to decreased resource availability could result in less beneficial plant-microbe interactions under stressful conditions. For example, while fungi are generally considered more drought-tolerant than bacteria (Vries et al. 2018), plants highly dependent on mycorrhizal fungi during drought may have increased vulnerability because

of declining fungal activity that reduces the plant's ability to obtain water (Kaisermann et al. 2017, Johnson et al. 2010). Additionally, plants can reduce the transfer of carbon to certain symbionts in stressful conditions, resulting in less investment in, *e.g.*, nitrogen acquisition under water limitation (Fuchslueger et al. 2014, Dovrat and Sheffer 2019). How these potential effects influence and interact with savanna trees and their microbial communities remains unclear.

Here, we ask how soil microbial communities influence savanna tree responses to nitrogen and water stress via a greenhouse experiment with six tree species from Kruger National Park, South Africa under factorial nutrient, water, and soil inoculant additions. We specifically aim to understand if native soil microbes provide positive or negative effects to trees and if effects change with varying levels of resource stress. If microbial communities provide a net positive effect, we expect (1a) that trees in inoculated treatments should grow larger than sterile treatments with benefits from nitrogen-fixing bacteria and mycorrhizal fungi (Reynolds et al. 2003, Yang et al. 2009, Bever et al. 2010, Johnson et al. 2010, Ulrich et al. 2019), even in resource-limited conditions. By contrast, if native microbiomes provide a net negative effect, we expect (1b) that trees in inoculated treatments should grow smaller than in sterile treatments due to pathogenic effects in resource-rich environments and microbial competition in resource-limited environments (Kaye and Hart 1997, Reynolds et al. 2003, Rutten et al. 2016, Wolf et al. 2017). If microbial effects change depending on different levels of resource limitation, (2a) resource scarcity could increase facilitative interactions between plants and microbes because plants and microbes rely more heavily on their symbiotic partners in stressful environmental conditions (Bertness and Callaway 1994, Thrall et al. 2007, Hernandez et al. 2021). Alternatively, (2b) the benefits of microbial symbionts might instead decrease in more resource-limited contexts, if resource limitation increases antagonistic interactions between plants and

microbes competing for the same resources (Kaisermann et al. 2017, Vries et al. 2018). Combined, our findings should elucidate important interactions between savanna trees and their microbial symbionts in the context of resource limitation.

Materials and Methods [Please included any details of Fieldwork permits used during this study, if applicable]

Experimental Design

Seedlings of six tree species native to Kruger National Park, South Africa (see Supplementary Table 1), were grown in a fully factorial microbial inoculation (sterile vs. inoculated), nitrogen availability (N_{Low} vs. N_{High}), and water availability (W_{Low} vs. W_{High}) greenhouse experiment, for a total of eight treatments. Each treatment was replicated five times for each species, for a total of 240 pots. The experiment was run in batches by species in a specialized quarantine growth suite at Yale University from September-January 2023, with each batch receiving treatment for eight weeks before harvest. Plants were grown at 28°C and 500 μmol/m^2/s of light (full spectrum UV) for 12 hours a day for the duration of the experiment to replicate a sub-tropical growing season and continuously monitored for compliance. Sterile and inoculated pots were isolated on opposite sides of the chamber to minimize cross-contamination, but pots were rotated once a week within a side to minimize light variability.

All treatments were initiated from sterile conditions. Tree seeds (purchased from Silverhill Seed Company, South Africa, in August 2022 and stored until September-October 2022) were sterilized either with sulfuric acid (98% sulfuric acid) or bleach (3.5% sodium hypochlorite) (Jordaan et al. 2006, Oyebanji et al. 2009, Nasr et al. 2013) depending on seed coat durability (see Supplementary Table 1). Seeds were then soaked in sterile water for 24 hours to

encourage germination and rinsed with a 1% bleach solution followed by sterile water again before sowing. All pots and landscaping fabric were soaked in a 50% bleach solution for at least 30 seconds, then rinsed with a 10% and then a 1% bleach solution. All growth chamber surfaces were wiped down with a 50% bleach solution followed by a 70% ethanol solution. Pots and equipment were pre-sterilized and air-dried for 2 hours before transplant to ensure optimal sterility. All growing media were autoclaved (275°F for a minimum of 30 minutes), including potting soil for germination, Turface, and perlite. Seed germination and transplanting was staggered to ensure small quantities of seeds were handled to avoid potential contamination. After germination, seedlings were transplanted into 4-liter tree pots (Stuewe & Sons, Inc., 0.10 m diameter × 0.35 m height) filled with Turface (All-Season Turface, SiteOne Landscaping, New Haven CT) for treatment. Once transplanted, soil surfaces were covered with an autoclaved perlite layer (~ 4 cm thick) to provide a barrier to minimize cross-contamination risk from flies, airflow, or water splashback. To maintain a sterile environment and ensure minimal cross-contamination from inoculated treatments, all surfaces in the growth chamber were sterilized at least once a week, first with a 50% bleach solution and then 70% ethanol.

Soils used as microbial inoculant were collected from Kruger National Park and Wits Rural Facility, South Africa, and were kept on ice during travel/shipment and frozen at –20°C during storage. Soils were collected in triplicate (~30 g in each replicate, ~90-100 g per tree) from the base of 10 individuals of 5 tree species (*Colophospermum mopane, Senegalia burkei, Dichrostachys cinerea, Combretum apiculatum,* and *Vachellia exuvialis*). Before the start of the experiment, soil samples were sequenced with Illumina MiSeq for both 16S bacterial genes and ITS fungal genes to determine the microbial community compositions of the native soils, following the Earth Microbiome Protocol (Thompson et al. 2017). Most of the bacterial and

fungal sequences were classified to the genus level. Before inoculant soils were mixed in growing media, they were homogenized and sieved with a 1-cm mesh to remove coarse fragments and avoid potential nutrient gains from large rocks (Hu et al. 2018, Segnitz et al. 2020). Our growing media, Turface (calcined, non-swelling illite clay), was mixed with 15 g of either sterile potting soil (for 'sterile' control treatments) or native South African soils (for 'inoculated' treatments; the pooled homogenized samples), to minimize soil moisture and nutrient differences across treatments.

To calculate water treatments, the average monthly rainfall in Kruger National Park was estimated during the wet season months of November to March either (1) from average monthly rainfall across six sites in Kruger (Punda Maria, Shingwedzi, Letaba, Satara, Skukuza, Pretoriuskop) across a 92-year period (1910-2002) (Zambatis 2003) or (2) from the 1991/1992 drought across the same six sites (Zambatis and Biggs 1995). The average monthly rainfall during the 1991/1992 wet season drought was 40 mm per month from November-March (Zambatis and Biggs 1995), while the average monthly rainfall during a non-drought wet-season from November to March was 100 mm rainfall per month (Zambatis 2003). Monthly rainfall values were scaled to the pot size to obtain weekly water estimates per pot: (1) 250 mL per pot per week in W_{Low} treatments and (2) 625 mL per pot per week in W_{High} treatments. To obtain rainfall estimates that were scaled to the pot size, we calculated the volume per pot (0.0243 m^2/pot) and multiplied pot volume by monthly rainfall estimates in mm converted to L/m^2. For example, for the drought treatment, 40 mm rainfall equals 40 L/m^2 which was then multiplied by pot volume 0.0243 m^2/pot to get 0.972 L of water per pot per month; this value was rounded to 1 L/pot per month, or 250 mL per pot per week. Watering was spread throughout the week: (1) W_{Low} treatments were watered only twice a week; 100 mL on Tuesdays, and 150 mL (including

fertilizer) on Fridays. (2) W_{High} treatments were watered every day of the week with 125 mL per day from Monday-Thursdays and then 150 mL (including fertilizer) on Fridays.

Nitrogen treatments were designed to mimic the range of lowest and highest nitrogen availability in field settings observed by Ludwig *et al.* (2004) (N_{Low}: 15 mg N and N_{High}: 50 mg N per pot per week). Each treatment received the same stoichiometrically scaled phosphorus concentration of 5 mg P and the same concentrations of N- and P-free Hoagland's nutrient solution, which included potassium, calcium, sulfur, magnesium and all essential plant micronutrients: chloride, sodium, manganese, zinc, copper, molybdenum, boron, and iron.

Response Variables

At the time of transplanting, 10 individuals of each tree species were harvested for initial size and biomass estimates. We recorded starting biomass, stem height, basal diameter, diameter at 10 cm height, root diameter, root length, wet and dry leaf stem mass, and fine root (0-2 mm) and coarse root (> 2 mm) dry mass. We also recorded stem height and basal diameter of all transplanted trees. Throughout the experiment, soil moisture was measured at three depths (2 cm, 5 cm, 10 cm) for all trees every two weeks, both before and after watering, to ensure that soil moisture was consistent across the same watering treatments (with probes sterilized with 70% ethanol after every pot). Soil moisture remained consistent within W_{Low} or W_{High} treatments with slight variation across the three depths measured over the course of the experiment, ensuring there were no substantial water availability differences across species or treatment, with the W_{Low} treatment receiving 40% less water than the W_{High} treatment (Supp. Fig. 1). Seedlings were harvested after 8 weeks, when we recorded stem height, basal diameter, diameter at 10 cm, root diameter, root length, and wet and dry leaf, stem, coarse root, fine root, and nodule biomass.

To calculate mass gain, we subtracted final total dry weights from the averaged total dry weight of the 10 seedlings harvested at the start of the experiment. Allometric equations were not used since all seedlings were small and similar in size at transplant, immediately after germination.

Additionally, fine root samples were collected from each seedling and stored at 4°C in 95% ethanol for mycorrhizal colonization analyses. Ten intersections of mycorrhizal colonization were taken per species per treatment (2 roots per individual tree). Roots were pooled by species and treatment, soaked in deionized water, and rinsed three times to remove ethanol. Roots were cut into 1-cm sections, cooked at 70°C in 10% KOH for 2-6 hours, acidified with 1% HCl, and stained with a 0.05% trypan blue solution for 15 minutes. Roots were de-stained in a lactic acid glycerol solution overnight (Wurzburger and Wright 2015). Root sections were mounted on microscope slides and the number of mycorrhizal structures (arbuscules, vesicles, and hyphae) were quantified with a compound microscope using a random-intercept method (McGonigle et al. 1990). Mycorrhizal colonization was calculated as the percentage of mycorrhizal density colonized with arbuscules, vesicles, and hyphae by fine-root length (Wurzburger and Wright 2015). Neither nodulation nor mycorrhizal colonization was observed in 'sterile' treatments, whereas both were abundant in 'inoculation' treatments, which confirms that treatments worked as intended.

Statistical Analyses

All statistical analyses were conducted in R (R Core Team 2021) using linear mixed-effects models (LMMs) to test the effects of soil and resource treatments. Variables of interest were tree mass gain, root-to-shoot ratio, mass fraction of stem, leaf, coarse root, fine root, nodule biomass, and mycorrhizal colonization in interaction with soil treatment, nitrogen treatment,

water treatment, and nodulation status with tree species as random effects. Model selection was done via AIC, with the best model selected as the simplest model within $\Delta AIC < 2$ of the overall minimum AIC. Linear mixed-effects models were run in *lme4* (Bates *et al.* 2015) and figures in *ggplot2* (Wickham 2016). All pairwise comparisons were calculated using the *emmeans* and *cld* functions from *emmeans* and *multcomp* packages, respectively (Lenth et al. 2018, Hothorn et al. 2008).

Species nodulation status and plant family were not included in the best fit models for plant growth and allocation (Supplementary Table 2), either because of confounding factors or because of low replication. The response variables included in best fit models for plant growth and allocation included inoculation treatment, nitrogen treatment, and water treatment. *Colophospermum mopane* may be a nitrogen fixer although it is not capable of forming nodules (Jordaan et al. 2000, Burbano et al. 2015), potentially confounding the significance of nodulation status. Alternatively, because 5 out of the 6 species in the experiment were Fabaceae species (Fig. 4), our study may not have enough replicates to determine if there were significant phylogenetic differences between families. Additionally, although non-Fabaceae savanna tree species may not fix nitrogen, they may gain substantial benefits from mycorrhizal fungi.

Results

Soil inoculation consistently increased mass gain (Fig. 1a, $p < 0.001$, $n = 240$, $df = 227$) across all species and resource treatments. The effect size of native soil inoculant was larger than nitrogen or water addition (Supplementary Table 2), indicating that soil microbes improved growth more than increased nitrogen or water availability. Soil inoculation also interacted with nitrogen and water treatments, such that trees grew faster when inoculated under high nitrogen

and water (Fig. 1a-d, $p < 0.001$, $n = 30$, $df = 227$). In sum, soil microbes were beneficial in all resource environments but especially so when resources were abundant.

While soil inoculation was the strongest predictor of mass gain, mass gain also increased in response to added nitrogen (Fig. 1b, 1c, $p < 0.001$, $n = 120$, $df = 227$) and to a lesser extent with added water (Fig. 1c, $p < 0.001$, $n = 120$, $df = 227$). Trees increased their root-to-shoot ratios, root mass fractions, and fine root mass fractions in drought treatments (Fig. 2a-c, $p < 0.001$, $n = 120$, $df = 233$). Nitrogen addition and soil inoculation had limited effects on biomass allocation except that stem mass fraction increased with increased nitrogen (Supplementary Table 2, $p < 0.001$, $n = 120$, $df = 233$) and coarse root mass fraction increased when trees were grown without their microbes (Supplementary Table 2, $p < 0.001$, $n = 120$, $df = 233$).

Investment in microbial symbionts – measured by nodule biomass and mycorrhizal colonization – varied with nitrogen and water treatments (Fig. 3a-b, $p < 0.001$, $n = 120$, $df = 112$). Nodule biomass increased in N_{Low} treatments (Fig. 3a, $p < 0.001$, $n = 120$, $df = 122$), consistent with the hypothesis that nitrogen fixation is most beneficial when nitrogen is limiting, although trees still produced nodules even in N_{High} treatments (Fig. 3a, $p < 0.001$, $n = 60$, $df = 112$). Nodulation increased with water addition (Fig. 3a, $p < 0.001$, $n = 60$, $df = 112$).

Mycorrhizal colonization followed similar patterns. Rates of colonization increased in N_{Low} treatments (Fig. 3b, $p = 0.01$, $n = 60$, $df = 112$) and also increased in W_{High} treatments (Fig. 3b, $p = 0.001$, $n = 60$, $df = 112$). Interestingly, both nodulation and mycorrhizal colonization rates were lowest in the N_{High}, W_{Low} treatment (Fig. 3a-b). Mass gain was significantly correlated with both nodule biomass and mycorrhizal colonization (Supp. Fig. 2a-b, $p < 0.001$, $n = 240$, $df = 227$), suggesting that soil microbes helped buffer against drought and nutrient limitation.

Soils contained a diversity of bacteria that may influence plant growth. DNA analysis showed that nitrogen-fixing bacteria, arbuscular mycorrhizal fungi, ectomycorrhizal fungi, pathogenic fungi, and pathogenic bacteria were all present in the native soils used for inoculum (Supplementary Table 5). Interestingly, nitrogen-fixing bacteria from the genus *Frankia* (which forms associations with actinorhizal plants) (Chaia et al. 2010) and *Herbaspirillum* (which forms associations in rice and maize stems, leaves, and roots) (Elbeltagy et al. 2001, Alves et al. 2015) were also present in native soils.

Discussion

We conducted a greenhouse experiment to test if native soil microbial communities provided (1a) net positive or (1b) net negative effects on savanna tree growth in response to nitrogen and water stress and to determine (2a) if beneficial symbiotic effects increased because microbial symbionts are more beneficial in more resource-limited treatments (Bertness and Callaway 1994, Hernandez et al. 2021) or (2b) if lower nitrogen and water treatments increased antagonistic effects of microbial symbionts because of resource stress and limitation (Kaisermann et al. 2017, Vries et al. 2018). Soil microbes increased tree growth and buffered resource limitation in all treatments, consistent with hypothesis 1a. Trees invested more in nitrogen-fixing and mycorrhizal associations in low nitrogen treatments (hypothesis 2a) but less in dry than in wet treatments (hypothesis 2b).

Soil microbes increased plant mass gain, even in stressful conditions, highlighting their importance in buffering plants against resource limitation. Our results are consistent with a variety of studies showing that native microbial mutualists support plants in drought and nutrient-limited conditions (Kumar and Verma 2018, Averill et al. 2019, Ulrich et al. 2019,

David et al. 2020). Additionally, we saw no indication of negative effects of microbial inoculants in our experiment, though other experiments have reported negative plant-soil feedbacks in savanna trees (Rutten et al. 2016). Compared to sterile soil, soil with both mutualists and potential pathogens could still confer an overall competitive advantage in savanna trees.

Surprisingly, given the aridity of many savanna systems, trees increased growth more with added nitrogen than with water addition. While water is vital for overall plant function and strongly structures savanna plant communities (Case & Staver 2018), our results are consistent with other studies that suggest that biomass accumulation is heavily dependent on nitrogen availability because of its vital role in the formation of chlorophyll, proteins, nucleic acids, and cell walls (Krapp 2015, Lambers and Oliveira 2019). The importance of nitrogen is underscored by the fact that all tree species capable of nitrogen fixation in our experiment nodulated when grown with their microbial symbionts, irrespective of nitrogen and water treatment. However, nodule biomass was higher in low-nitrogen and high-water treatments. This suggests that nitrogen acquisition via fixation may be generally important for savanna trees but that the amount of nitrogen fixed may depend on environmental context at the seedling-to-sapling stage. Nitrogen-fixing Fabaceae species comprise a large percentage of tree species in African savannas (Midgley and Bond 2001, Pellegrini et al. 2016), and global studies suggest rates of fixation in savannas are high (Cleveland et al. 1999, Houlton et al. 2008). Despite this, nodulation and nitrogen fixation rates in savannas remain largely unknown due to a lack of empirical studies, especially in African savannas. Therefore, our results suggest fixed nitrogen may be a vital component of nutrient acquisition and microbial investment for savanna trees, with downstream impacts on plant growth and resource competition between trees and grasses. If young saplings invested in nodules readily in our resource-limited conditions without grass competition, it is

likely that young trees invest in nitrogen-fixing bacteria nodules to fix nitrogen in the field to minimize grass competition for resources (Cramer et al. 2007, 2010), at a life stage where tree-grass rooting zones overlap substantially and resource competition is more severe for saplings (February and Higgins 2010, February et al. 2013a).

Although nodulation status and plant family did not influence mass gain, it is possible Fabaceae trees are abundant in savannas because of their ability to utilize their microbial symbionts and minimize resource limitation (Midgley and Bond 2001, Cramer et al. 2007). While there were minimal species differences in the effects of inoculation, it was notable that *Dichrostachys cinerea* significantly increased mass gain when grown with inoculants, as *D. cinerea* is a problematic woody encroacher (Utaile et al. 2021, Zhou et al. 2021). *D. cinerea's* improved growth with microbial inoculants is consistent with the observation that many invasive species are nitrogen fixers (Stinca et al. 2015, Raghurama and Sankaran 2022). Lastly, two nitrogen-fixing bacteria that are known to form associations with non-Fabaceae species were found in sequenced native soils (*Frankia* and *Herbaspirillum)*. Many of these microbial symbionts have not been studied in African savannas but require further study (Burbano et al. 2015), especially as our results suggest soil microbes are important to savanna tree growth.

Water limitation strongly influenced tree rooting strategies and symbiotic relationships, increasing tree root investment but limiting nodulation and mycorrhizal colonization in droughted conditions. Trees varied their allocation responses to water more than to nitrogen regardless of inoculation status, suggesting that they may rely less on their microbial symbionts to mitigate the effects of drought stress. In field conditions, trees may mitigate water stress by allocating roots to deeper soil layers. Therefore, trees can dynamically respond to water limitation without their microbial symbionts but are more reliant on microbial symbionts for

nutrient acquisition. However, microbial investment was water-limited, likely because trees allocated more carbon to their symbionts when water was plentiful (Bardgett et al. 2008, Fuchslueger et al. 2014).

Generally, we found symbiotic microbial investment increased in low-nitrogen treatments and as water availability increased, indicating microbes were water-limited. We saw no increase in antagonistic or pathogenic interactions as resources became more available, a result generally consistent with the Stress Gradient Hypothesis (David et al. 2020). In fact, microbes continued to become more beneficial as resources – especially water – became more available, suggesting belowground facilitation is common but restricted by limited resources. While microbial symbionts were most important for nitrogen acquisition, it is also possible mycorrhizal fungi could provide increased drought resistance in field settings through extensive hyphal networks, which are challenging to establish in a greenhouse setting (Worchel et al. 2013).

Although trees consistently benefitted from growing with native microbes, soil inoculants were least beneficial in dry, high nitrogen treatments (Fig. 1d, 3a-b), likely because microbial investment was less vital due to already available nutrients and because either water limitation prevented tree investment in microbes or constrained microbial activity (Schimel 2018). These dry, high nitrogen conditions may become more prevalent in the future as both drought and nitrogen deposition are predicted to increase (Lamarque et al. 2013), potentially making savanna tree microbial symbionts less beneficial. Other studies have shown that climate extremes like drought can have substantial impacts on soil microbial communities with consequences for ecosystem processes and plant community dynamics (Kaisermann et al. 2017, Vries et al. 2018). Drought induces microbial community compositional changes and decreases the abundance and activity of beneficial microbes (Cavagnaro et al. 2016). Our findings suggest that water

limitation can decrease symbiotic associations, potentially decreasing the abundance of beneficial microbes due to increased plant stress and less available carbon to allocate towards their beneficial symbionts (Cavagnaro et al. 2016, Vries et al. 2018). Therefore, extreme droughts may negatively impact microbial symbionts either directly or through their plant symbionts, decreasing their ability to relieve drought stress for trees and resulting in lasting consequences of extreme drought events for savanna trees (Fensham et al. 2009, 2015, Case et al. 2019).

In conclusion, our study shows that plant-soil interactions support savanna tree growth. Soil microbes like nitrogen-fixing bacteria and mycorrhizal fungi provided substantial belowground facilitation by providing trees increased access to nitrogen and buffering against both water and nitrogen limitation. However, savanna trees and microbial symbionts experienced water limitation in drought conditions, suggesting trees and their microbial symbionts are sensitive to climate extremes, which could alter microbial community composition and create lasting impacts on savanna vegetation in the future.

Data Availability Statement [Please update with Dryad DOI and citation]

All data is published and made freely available and is archived on Dryad (upon acceptance of the manuscript).

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Author contributions

Arielle Biro and A. Carla Staver designed this study in conversation with Sarah A. Batterman. Arielle Biro conducted greenhouse set up, germination, fertilization, and plant care over the course of the experiment and greenhouse harvesting and data collection. Jane M. Lucas assisted Arielle Biro in DNA sequencing and Michelle Y. Wong assisted Arielle Biro with mycorrhizal staining. Arielle Biro and A. Carla Staver performed statistical analyses and wrote the manuscript with extensive feedback from all authors.

Conflict of Interest Statement

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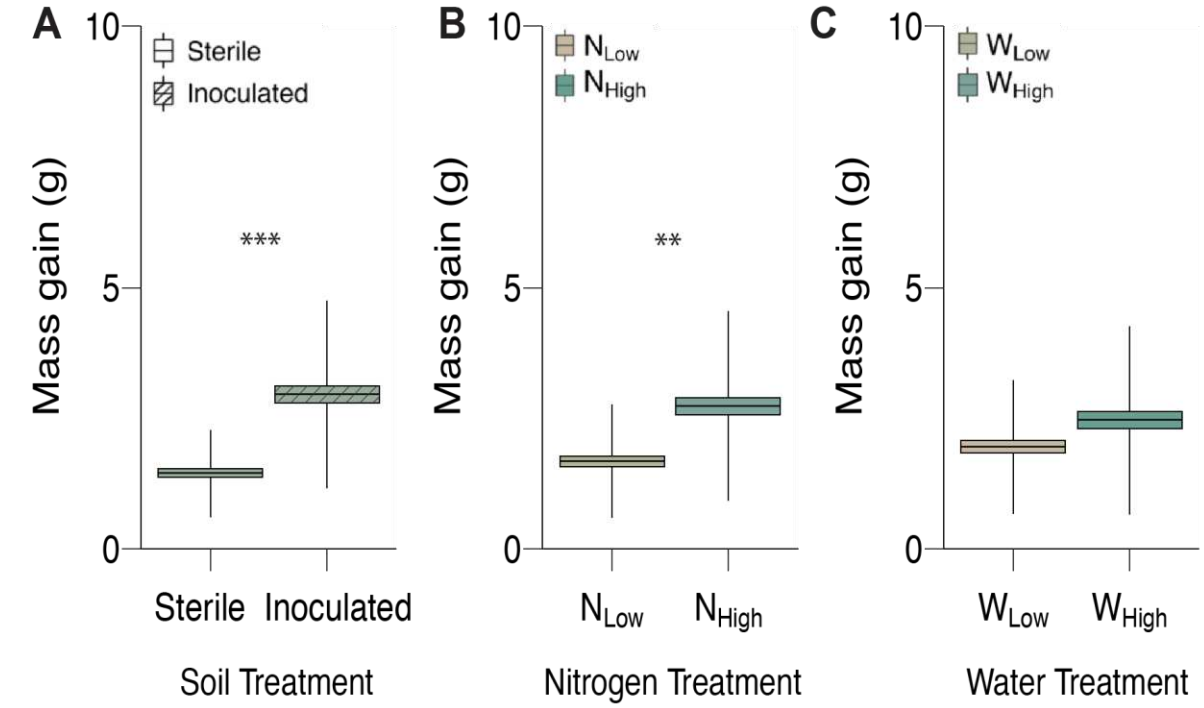
Figures [Please provide your figures as individual, high-quality image files]



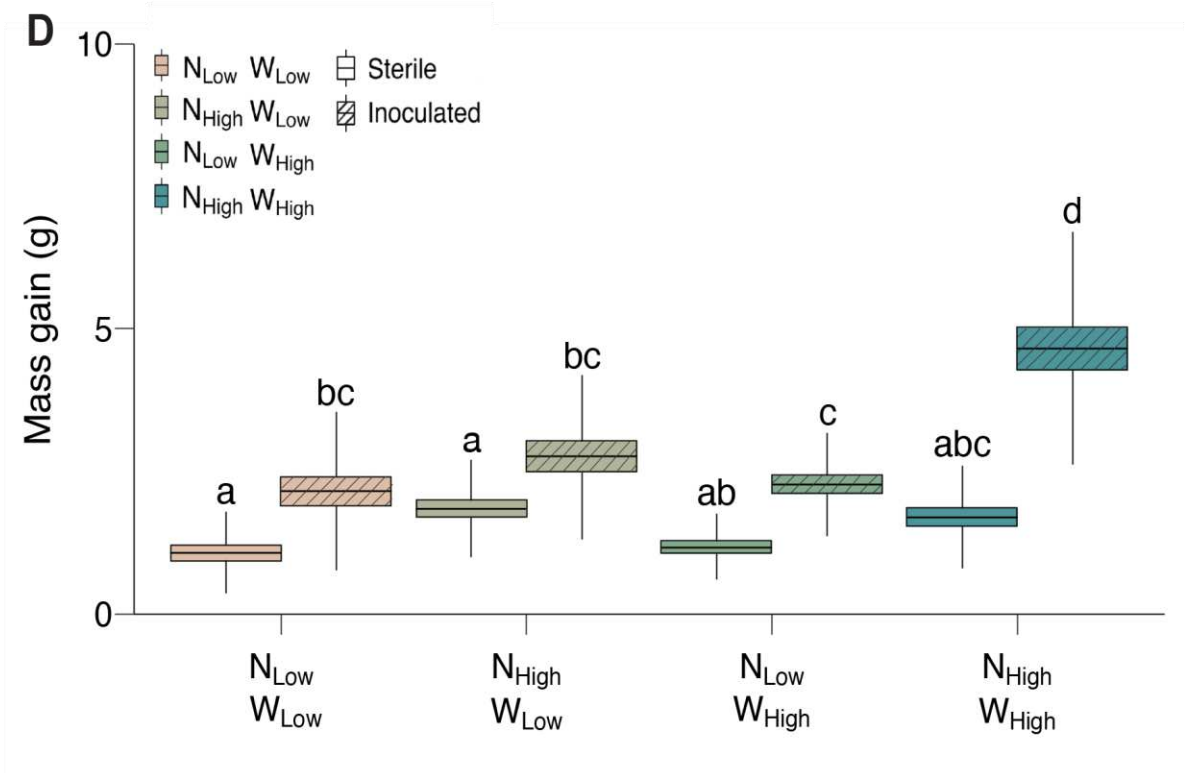


Figure 1. Tree sapling mass gain by inoculation treatment (A), by nitrogen fertilization treatment (B), by watering treatment (C), and by resource treatment and inoculation treatment (D). Values were rescaled to control for plotting by subtracting the species random effect values from mass gain (g). Boxplots show standard errors and means, and whiskers represent standard deviations (n = 30 trees per treatment group in panel A, n = 120 trees per treatment group in panels B-C). Letters or asterisks denote significant differences in pairwise comparisons among treatments, where bars that share letters are not significantly different at p = 0.05.

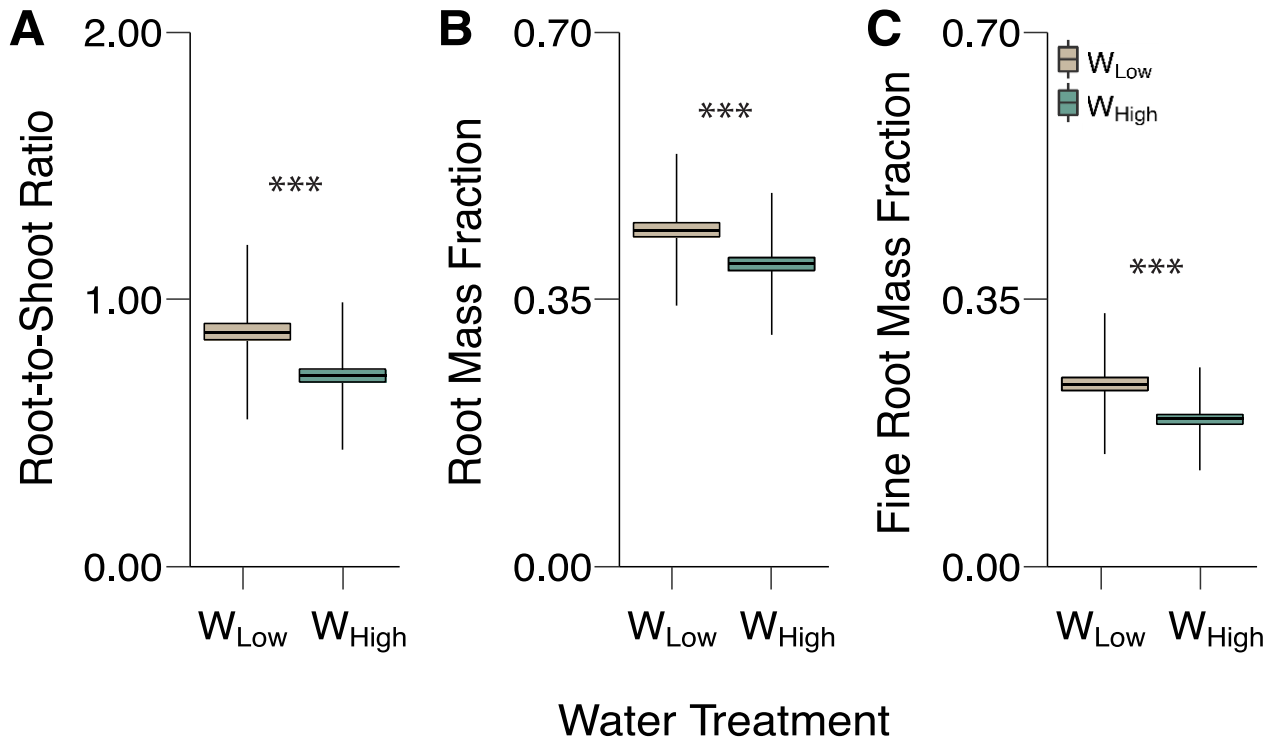


Figure 2. Tree sapling root-to-shoot ratio (A), root mass fraction (B), and fine root mass fraction (C), by watering treatment. Root-to-shoot ratios were calculated by dividing total dry belowground tree biomass by total dry aboveground tree biomass. Root mass fraction was calculated by dividing root dry biomass by total dry biomass. Fine root mass fraction was calculated by dividing fine root dry biomass by total dry biomass. Values were rescaled to control for platting by subtracting the species random effect values from the variables of interest. Boxplots show standard errors and means, and whiskers represent standard deviations (n = 120 trees per treatment group). Asterisks denote significant differences in pairwise comparisons among treatments.

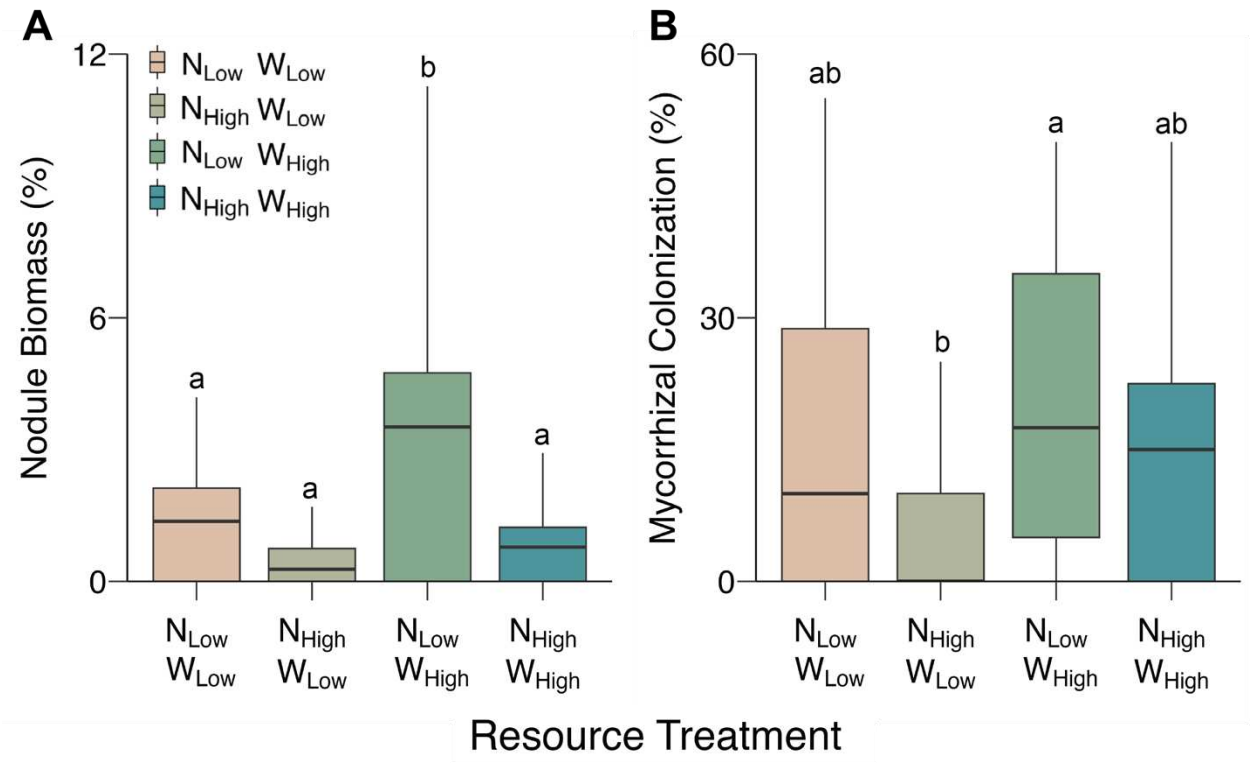


Figure 3. Tree sapling nodule biomass (%) (A), and mycorrhizal colonization (%) (B), by resource treatment. Nodule biomass (%) was calculated by dividing the nodule biomass by total biomass and multiplying by 100. Mycorrhizal colonization was calculated as the percentage of mycorrhizal density colonized with arbuscules, vesicles, and hyphae by fine-root length (Wurzburger and Wright 2015). Boxplots show 25%, median, and 75% of values and whiskers represent lowest and highest values ((A) n = 20 trees per treatment group, (B) n = 30 trees per treatment group). Letters denote significant differences in pairwise comparisons among treatments, where bars that share letters are not significantly different at p = 0.05.

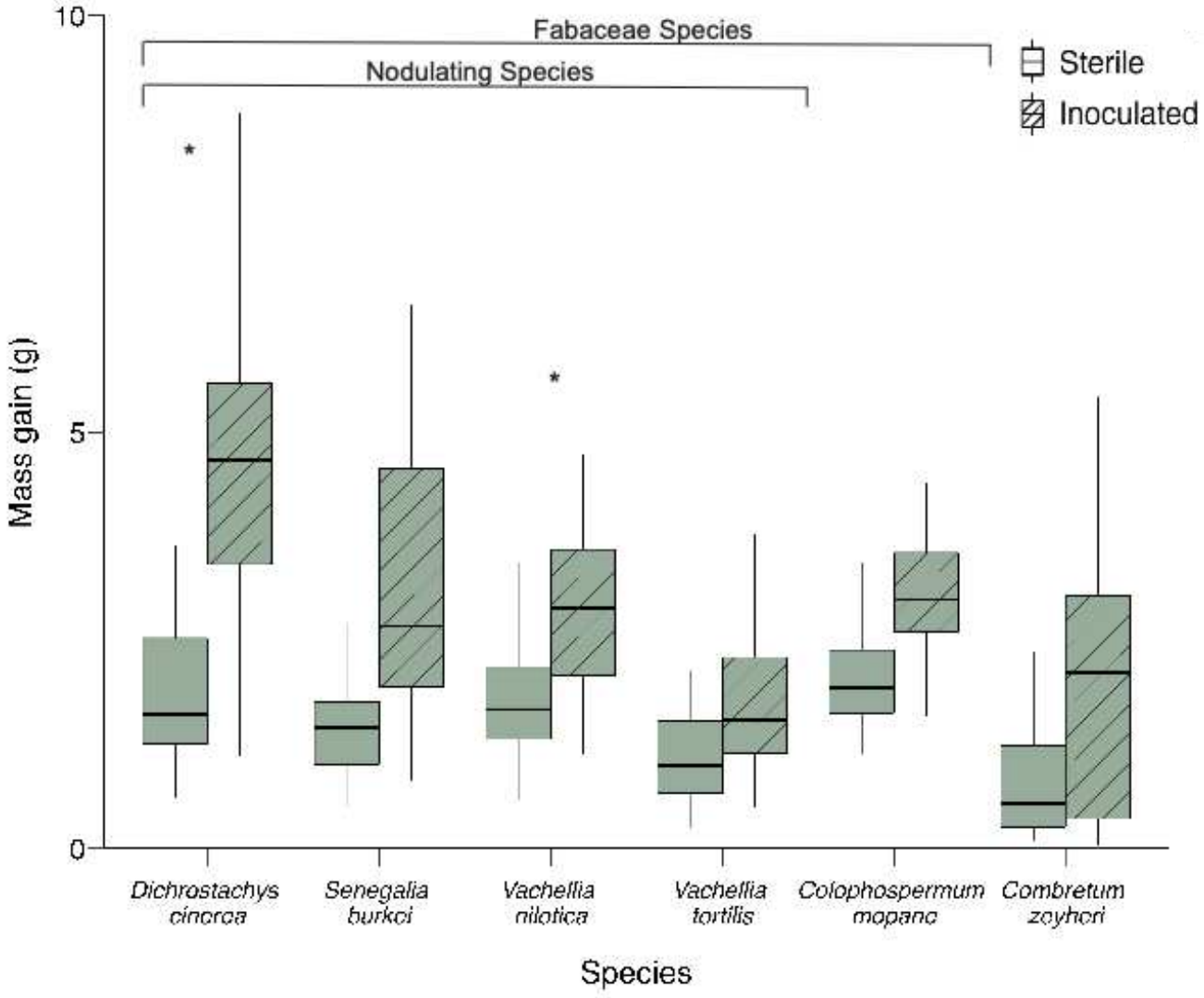


Figure 4. Tree sapling mass gain by inoculation treatment. Nodulating nitrogen fixers and Fabaceae species are denoted with brackets. Boxplots show 25%, median, and 75% of values and whiskers represent lowest and highest values (n = 5 trees per treatment group). Asterisks denote significant differences in pairwise comparisons among treatments.