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Nitrogen and phosphorus availability alters tree-grass competition intensity in savannas

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Summary

1. Plant essential macronutrients like nitrogen (N) and phosphorus (P) can limit savanna tree growth and are important determinants of savanna vegetation dynamics, along with rainfall, fire, and herbivory. How nitrogen and phosphorus shape tree-grass competition and their coexistence remain unclear, hindering our ability to predict how savannas may respond to altered nutrient cycling.
2. Here, we evaluate (1) if trees and grasses respond differently to N vs. P availability, or (2) if grasses are more competitive in low nutrient environments while trees are more competitive in high nutrient environments. To do this, we grew saplings of 6 tree and 1 grass species from Kruger National Park, South Africa, for 16 weeks under fully factorial nutrient and competition treatments (with/without competitors, low/high rate of N supply, and low/high rate of P supply) under a watering regime designed to mimic wet season rainfall in a mesic savanna.
3. Trees and grasses foraged most aggressively for nitrogen and allocated biomass differently depending on nitrogen availability. Overall, tree growth decreased in competition with grass, even in high nutrient environments where they grew faster. Grasses were always better belowground competitors, utilizing aggressive nutrient foraging strategies, including high root phosphatase activity in response to nitrogen and large root biomass allocation.
4. *Synthesis.* In low nutrient environments (e.g., on nutrient-poor sandy soils), nutrients may limit tree growth. Nutrient rich environments enable tree growth, but grasses continue to compete effectively with trees. Understanding what this means for ecosystem responses to nutrient availability is not trivial, especially in the context of fire and herbivory. However, it is clear that soil nutrients likely affect tree and grass

growth and competition in savannas, which suggests that future changes in nutrient cycling, such as N deposition, may have important effects on savanna vegetation.

Key words: soil nutrients, nitrogen, phosphorus, tree-grass competition, phosphatase enzymes

Introduction

Savannas are dominated by two plant functional types – trees and grasses, and their abundances are maintained by disturbances such as fire and herbivory and resources like water (Scholes and Archer 1997, Sankaran et al. 2004, Staver et al. 2009, 2011, Kulmatiski and Beard 2013). Compared to fire, herbivory, and water, nutrients remain understudied, even though we know that macronutrients like nitrogen (N) and phosphorus (P) contribute to savanna vegetation structure and stability (Sankaran et al. 2005, 2008, Hoffmann et al. 2012, Pellegrini 2016, Staver et al. 2017). Nutrients can regulate plant growth, and increased access to N and P in savannas could increase a tree's chances of reaching its fire resistance threshold, potentially increasing the probability of a savanna transitioning to a forest (Kraaij & Ward 2006, Lehmann et al. 2011, Hoffmann et al. 2012, Pellegrini 2016, van der Waal et al. 2009). We lack a basic understanding of how N and P impact tree-grass competition, which hinders our ability to predict how savanna vegetation may respond to N deposition, P pollution, and changes in nutrient cycling from fire and other disturbances.

Since savannas are sometimes situated on highly weathered soils low in P (Walker and Syers 1976, Lambers et al. 2008), and since savanna fires frequently volatilize large amounts of N (Hernández-Valencia and López-Hernández 2002, Cech et al. 2008, 2010), both N and P could limit savanna productivity (Scholes 1990, Cech et al. 2008). Competition for N and P could therefore be strong (February and Higgins, 2010), especially since root-niche separation, whereby trees and grasses root in different soil layers to minimize water competition (Scholes and Archer 1997), is less effective for minimizing nutrient competition. Nutrients are most highly concentrated in the top 20 cm of soils (Zhou et al. 2018), an overlap that likely results in

direct competition between trees and grasses for N and P in shallow soil layers (Ludwig et al. 2004, February and Higgins 2010, February et al. 2013a).

To address nutrient limitation, trees and grasses could use diverse nutrient acquisition strategies to help reintroduce nutrients (*e.g.*, from N-fixation) or cycle nutrients (*e.g.*, via phosphatase activity). However, disentangling how nutrients limit grasses and trees and their competitive interactions in savannas remains challenging since nutrient availability is correlated with soil texture and hydrology (Scholes 1990, Case and Staver 2018), can be confounded by herbivores that move nutrients across the landscape through excretion (Bardgett and Wardle 2003, Roux et al. 2020), and since experiments addressing N vs. P competition by savanna trees and grasses are sparse. Therefore, the role of nutrient limitation in structuring tree-grass competition remains poorly resolved in savannas.

Ecological theory offers two hypotheses to explain how trees and grasses might coexist in the face of nutrient competition. A first hypothesis holds that trees and grasses could specialize on different soil resources (Tilman 1982, 1984, 1990), thereby minimizing competition. Exactly how this might work is not known. On the one hand, grasses may be especially good at acquiring N when N is scarce. African C_4 grasses have high N use efficiencies, via decreased photorespiration (Niu et al. 2008) thus requiring less N-rich Rubisco (Tjoelker et al. 2005, Cech et al. 2008), suggesting that they may tolerate low N and can build more biomass with less N. They are also known to be aggressive competitors for N, especially when N is scarce (Haling et al. 2013). On the other hand, N-fixing trees are abundant in savannas, and several woody encroachers are known N-fixers (Midgley and Bond 2001, Pellegrini et al. 2016, Varma et al. 2018) and can meet their N requirements through N fixation, at a carbon cost (Vitousek and Howarth 1991, Cramer et al. 2007). Experiments have shown that N fixers grew better when grown with grasses in mesocosms with lower N:P ratios (Sitters and Venterink 2021), suggesting

that they deal better with low soil N than P. Indeed, in other systems, N-fixing trees have higher P requirements (Vitousek and Howarth 1991) and increase their growth in higher P treatments (Sitters and Venterink 2021). Yet another possibility is that, since fixers are always N replete, N-fixers have strategies for obtaining P by investing in phosphatase enzymes (Houlton et al. 2008) that convert soil organic P into plant-available forms (Margalef et al. 2017) when P is limiting (Venterink 2011, Nasto et al. 2014), which may help explain why certain N-fixers are successful woody encroachers. The possibility that fixers are particularly good at acquiring P is supported by experimental and field studies that have shown *Acacia* woodlands tend to be more P-limited than N-limited (Cech et al. 2008) and that grass competition increased nodulation in savanna trees, potentially providing a competitive advantage by avoiding direct N competition with grasses (Cramer et al. 2007, 2010, 2012). If trees and grasses coexist by differentiating on either N or P, we might expect a differential response of grasses vs. trees to N vs. P addition, and that nutrient delivery might change the outcomes of tree-grass competition.

A second hypothesis suggests that trees and grasses instead specialize on aboveground vs. belowground resources. Specifically, grasses may be especially efficient belowground competitors whereas trees invest relatively more aboveground, with longer-term investments via perennial biomass allocation instead of producing annually recruited shoots as grasses do. In support of this perspective, grasses are known to compete aggressively and are usually unaffected by tree competition for water (Tjoelker et al. 2005, Haling et al. 2013), whereas trees are strongly impacted by grass competition for water (Riginos 2009, Cramer et al. 2012, Donzelli et al. 2013, February et al. 2013b, 2013a), suggesting trees are unable to compete effectively for resources with grasses in dry environments. Trees may also be more able to escape competition on nutrient-rich soils (Silva et al. 2013, van der Waal et al. 2009), indicating that trees may compete more effectively in higher nutrient environments by investing more biomass

aboveground due to decreased belowground competition (Hoffmann et al. 2012, Pellegrini 2016). While savannas are not considered classically light-limited (Hoffmann and Franco 2003, Ratnam et al. 2011), tree-grass competition for light may be substantial (Ludwig et al. 2001, Vadigi and Ward 2013, Moustakas et al. 2013) and trees also accumulate aboveground biomass as a strategy to escape the effects of fire and herbivory by growing tall (Staver et al. 2009, Hoffman et al. 2011, Wakeling et al. 2011). Under this second hypothesis, we expect (1) that grasses will experience little belowground competition from trees (Tjoelker et al. 2005, February et al. 2013b, Moustakas et al. 2013), invest biomass in roots for more aggressive nutrient acquisition, aggressively compete for both N and P in low nutrient settings, and increase their phosphatase activity (Craine 2005, Craine et al. 2008), whereas (2) trees will experience substantial competition from grasses (Riginos 2009) and invest aboveground when nutrients are readily available, as tree biomass in savannas should theoretically increase with higher nutrient availability (Hoffmann et al. 2012, Pellegrini 2016) to avoid shading or fire disturbance.

Here, we examine how various tree species from Kruger National Park, South Africa, respond to grass competition under different soil nutrient conditions in a greenhouse study in constant field water conditions designed to mimic the wet season in a mesic savanna (~750 mm MAR). If trees and grasses differentiate their use of different soil resources to minimize competition, we expect grasses and trees to have differential responses to N vs. P addition, with nutrient delivery rates influencing the outcomes of tree-grass competition (H1). If trees and grasses instead differentiate their belowground vs. aboveground biomass allocation, we expect (H2a) grasses to invest biomass belowground and outcompete trees in low N and low P treatments and (H2b) trees to invest biomass aboveground when N and P are readily available in order to overcome aboveground competition or disturbance effects.

Materials and Methods

Experimental Design

The primary goal of the experiment was to understand how trees and grasses respond to different nutrient and competition regimes. Six tree species and one grass species were selected based on phylogenetic diversity and nitrogen fixation ability for this experiment (Table 1). The grass species chosen for this experiment was *Melinis repens,* the only annual grass species common in Kruger National Park (Wigley-Coetsee and Staver 2020), one of the largest natural reserves that is situated in the low-lying savannas of South Africa.

Plants were grown in a full-factorial nutrient-addition experiment with water availability held constant across treatments. Each pot was watered three times a day for a total of 400 mL per day, designed to mimic a wet growing season in the Pretoriuskop region of Kruger National Park, which receives about 750 mm of rainfall per year. The four nutrient treatments were (1) low N and P (N_{Low}, P_{Low}), (2) high N and low P (N_{High}, P_{Low}), (3) low N and high P (N_{Low}, P_{High}), and (4) high N and P (N_{High}, P_{High}). These nutrient treatments were applied factorially to trees grown with and without grasses and to grasses grown without trees for a total of 260 pots. Each nutrient and competition treatment was replicated five times in a randomized block design (except *Schotia brachypetala*, of which we had enough seedlings for only three replicates each).

Grass and tree seeds were collected in Kruger National Park or purchased from Silverhill Seed Company in South Africa in 2019 and stored until germination. In July-August 2020, tree seeds were clipped and soaked in water for 24-72 hours before planted in potting soil. After sowing, seeds were watered and placed in growth chambers set at 28°C and 500 μmol/m^2/s of light for the duration of germination. Grass seeds were germinated on watered potting soil in the same growth chambers. In September 2020, germinated tree seedlings and grasses were

transplanted into 30-liter tree pots (Stuewe & Sons, Inc., 0.25 m diameter x 0.60 m height, sold as 8-gallon pots) filled with turface (All-Season Turface, SiteOne Landscaping, New Haven CT). One tree sapling was planted per pot without grass competition, and two grass tufts were planted per pot without tree competition. For competition treatments, one tree sapling and two grass tufts were planted per pot (tree sapling in the middle, one tuft on either side).

All pots were fertilized once a week for 16 weeks at their assigned levels from October 2020-January 2021. Low nutrient treatments were designed to replicate nutrient limitation, while high nutrient treatments were designed to alleviate nutrient limitation while avoiding nitrogen or phosphorus toxicity. To determine the appropriate concentrations of N and P for each treatment, we derived monthly estimates of fertilizer from Tomlinson *et al.* (2019) along with field nutrient estimates from Ludwig *et al.* (2004). All N_{Low} treatments received 100 mg N and P_{Low} treatments 10 mg P per pot per week to mimic field conditions via ammonium nitrate and potassium phosphate, with N_{High} treatments receiving 800 mg N and P_{High} treatments 100 mg P per pot per week. Along with N and P fertilization, each treatment received the same quantity of N- and P-free Hoagland's micronutrient solution, which included all essential micronutrients: potassium, calcium, sulfur, magnesium, chloride, sodium, manganese, zinc, copper, molybdenum, boron and iron. Pots were located in a single greenhouse bay at Yale Marsh Botanical Gardens in a block-randomized design, kept at 28°C for the duration of the experiment, and supplemented with grow lights for 12 hours a day to replicate a sub-tropical growing season. Soil moisture was measured throughout the experiment to ensure that soil moisture remained constant across pots.

Plant Responses

At the time of transplanting, 10 individuals of each tree and grass species were harvested for initial size and biomass estimates. For tree seedlings, 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 (sorted into two diameter classes: 0-1 mm and 1-2 mm) and coarse root (> 2 mm) dry mass. For grasses, we recorded basal circumference, maximum culm and sward height, root diameter, and wet and dry weight of aboveground and belowground grass biomass.

Throughout the experiment, measurements were taken every four weeks. Tree measurements consisted of stem height, basal diameter, and diameter at 10 cm. Grass measurements consisted of basal circumference and sward height. Along with tree and grass measurements, light availability was measured using an fPAR meter (fraction of incident photosynthetically active radiation, 400-700 nm) at three different heights (tree base, tree canopy height, and grass height). At the final harvest in January 2021, we repeated all routine measurements and measured wet and dry weights of each plant tissue type. Time to harvest did not differ among species or blocks nor with respect to any experimental treatment (n = 260).

To calculate mass gain, we subtracted the mean total dry weight of the 10 tree seedlings or grasses harvested at the start of the experiment from the final total dry weights at the end of the experiment. We used allometric relationships to evaluate whether competition and nutrient treatments influenced the growth form of trees. To do this, we calculated the allometric exponent β as follows (Feldpausch et al. 2011):

$$Height = Diameter^{\beta}$$

for both shoot and root systems. For shoots, we used stem height and basal diameter (mm). For roots, we used taproot length for 'height' and root diameter (mm). We refer to the traits represented by these allometric exponents as 'shoot elongation' and 'root elongation', respectively. Competition intensity was calculated by dividing the average mass gain of a tree or

grass grown with competition by the average mass gain of a tree or grass grown out of competition and subtracted by one for each nutrient treatment within one species (*i.e.*, only individuals of the same species were compared). Negative values (< 0) indicate competitive effects while positive values (> 0) indicate facilitative effects (Carlyle et al. 2010, Jabot and Pottier 2012).

$$Competition\ Intensity = \frac{Average\ mass\ gain\ of\ plant\ grown\ with\ competition}{Average\ mass\ gain\ of\ plant\ growth\ without\ competition} - 1$$

To quantify root phosphatase activity, we used a method adapted from Turner et al. (2001), using para-nitrophenyl (pNPP) as an analogue for phosphomonoesterase, a root phosphatase that hydrolyzes simple phosphate monoesters to release a phosphate ion for plant uptake (Browman and Tabatabai 1978, Tabatabai 1994). For each plant within a pot, we collected three subsamples of fine roots for measuring phosphomonoesterase activity and one for measuring color production without substrate added. For each plant, 500 mg of fresh root was added to a glass vial with 9 mL of 50 mM sodium acetate-acetic acid buffer (pH 5.0) and shaken in a water bath at 28°C for five minutes to simulate wet season surface soil temperatures at Kruger National Park. To initiate the reaction, we added 1.0 mL of 50 mM pNPP (5.0 mM final concentration). The reaction was terminated by mixing 0.5 mL of buffer/substrate solution with 4.5 mL of 0.11 M NaOH, which was then vortexed and measured for absorbance at 405 nm against paranitrophenol (pNP) standards. Two sodium acetate-acetic acid buffer and substrate blanks were run for every assay. Roots were removed from the vials and dried at 65°C for 3 days to express the activity as μmol pNP g^{-1} dry root mass h^{-1}.

Calculations and statistical analyses

Statistical models were run separately for grasses and trees. We used linear models to test the effects of nutrient and competition treatments on grass mass gain, grass root mass fraction,

and phosphatase enzyme activity. We used linear mixed-effects models (LMMs) to test the effects of nutrient and competition treatments on tree mass gain, root mass fraction, root phosphatase enzyme activity, shoot elongation, root elongation, mass fraction of stem, leaf, coarse root, and fine root biomass in interaction with encroachment ability and nitrogen-fixation ability (Zhou et al. 2021), with tree species as random effects. Where necessary, data were log-transformed to meet normality assumptions; final weights, initial weights, shoot elongation, root elongation, phosphatase activity, and competition intensity were log transformed. Extreme outliers were removed (1 root elongation data point and 1 competition intensity data point). All analyses were done in R (R Core Team 2021), with linear mixed-effects models performed in *lme4* (Bates *et al.* 2015) and figures created in *ggplot2* (Wickham 2016). Model selection was done via AIC selection by comparing best predictor variables for each of the response variables, with the best model selected as the simplest model within $\Delta AIC < 2$ of the overall minimum AIC (see supplementary materials for full model results). Post-hoc Tukey pairwise comparisons were calculated in two ways, depending on the type of model used for statistical analysis. All pairwise comparisons were calculated using the *emmeans* and *cld* functions from *emmeans* and *lsmeans* packages, respectively (Lenth et al. 2018, Lenth 2016), but grass pairwise comparisons were run as linear models while tree pairwise comparisons were run as linear mixed-effect models for consistency with statistical analyses.

Results

Grass Responses: Competition, Nutrients, and Phosphatase Activity

Grasses grew least in N_{Low} and P_{Low} treatments and most in N_{High} and P_{High} treatments, with larger responses to N_{High} than to P_{High} (Fig. 1a). In N_{Low} treatments, grasses allocated relatively more biomass belowground (Fig. 1d, $p < 0.001$, n = 130), irrespective of whether

grasses were grown with or without trees (Fig. 1a, c). Overall grass biomass was not significantly impacted by tree competition but increased with nutrient addition (Fig. 1a, c, $p < 0.001$, n = 130, for N addition and $p < 0.001$, n = 130 for P addition).

Grasses increased their root phosphatase activity in response to N addition in both N_{High}, P_{Low} and N_{High}, P_{High} nutrient treatments (Fig. 1e, $p < 0.001$, n = 130). Grass root phosphatase rates were high, especially in N_{High} treatments, irrespective of tree competition (Fig. 1e). Therefore, both grass growth and phosphatase activity did not seem to be impacted by tree competition but were responsive to nutrient additions, especially N.

Tree Responses: Competition, Nutrients, and Phosphatase Activity

In contrast with grass, trees were strongly affected by competition. Trees in competition with grasses grew less and had consistently lower biomass than when grown without grass (Fig. 1b, $p < 0.001$, n = 120, df = 213), while both N_{High} and P_{High} tended to increase tree growth and biomass (Fig. 1b, $p < 0.001$, n = 30, df = 213 for N addition, $p = 0.05$, n = 30, df = 213 for P addition). Our results confirmed our initial hypothesis that trees grown without grasses would accumulate more biomass than trees grown with grasses (Fig. 1b).

Trees grew larger in N_{High} treatments compared to N_{Low} treatments both in and out of competition (Fig. 1b, $p < 0.001$ n = 60, df = 213). In N_{Low} treatments, all tree species shifted biomass allocation belowground, but allocated more biomass belowground when competing with grasses (Fig. 1d, $p = 0.001$, n = 30, df = 215). Curiously, the addition of P under low N conditions (N_{Low}, P_{High}), induced an increase in belowground biomass allocation in trees (Fig. 1d), perhaps reflecting competition for N under conditions of high P availability.

Tree root phosphatase activity differed across nutrient and competition treatments, as trees increased root phosphatase activity in N_{High} treatments (Fig. 1f, $p < 0.001$, n = 120, df = 216)

and decreased root phosphatase activity when in competition with grasses (Fig. 1f, $p < 0.001$, n = 60, df = 216). Although tree root phosphatase activity was lower than grass root phosphatase activity, trees decreased root phosphatase activity when grown with grasses while grasses did not alter their root phosphatase activity when grown with trees (Fig. 1e-f), consistent with their growth responses.

Tree Responses: Biomass Allocation, Shoot Elongation, and Root Elongation

Trees, like grasses, were sensitive to N availability. Trees also allocated biomass differently in N_{Low} compared to N_{High} environments, with different stem, leaf, coarse root, and fine root (1-2 mm) fractions depending on N treatment (Fig. 2). Under N_{Low}, all trees increased belowground biomass, increasing coarse root and fine root (1-2 mm) mass fraction (Fig. 2c-d, $p < 0.001$, n = 120, df = 217), whereas under N_{High} treatments, trees instead increased stem and leaf mass fraction (Fig 2a-b, $p < 0.001$, n = 120, df = 217). However, in competition with grasses, the effects of N availability became more pronounced (Supp. Fig. 1): stem and leaf mass fraction decreased, and coarse root and fine root (1-2 mm) mass fraction increased under N_{Low} (Supp. Fig. 1, $p = 0.001$, n = 60, df = 215), while trees increased stem and leaf mass fraction (Fig. 2a-b, $p < 0.001$, n = 60, df = 217) and decreased coarse root and fine root (1-2 mm) mass fraction under N_{High} (Fig. 2c-d, $p < 0.001$, n = 60, df = 217). Overall, trees prioritized aboveground biomass when N was not limiting, whereas trees prioritized belowground growth when N was limiting, likely for nutrient foraging.

Additionally, trees changed their growth architecture under competition with grasses. Trees consistently increased their root and shoot elongation when grown with grasses (Fig. 3, $p < 0.001$, n = 60, df = 217), differentiating on their vertical axes by growing taller stems and longer

tap roots when in competition with grasses (Fig. 3), whereas trees grew shorter stems and tap roots when grown without grass (Fig. 3).

Tree-Grass Dynamics: Competition Intensity and Plant Functional Groups

Competition intensity varied by plant functional type (Fig. 4a, $p = 0.01$, n = 356, df = 50), with trees experiencing much higher competition intensity than grasses. Competition was most intense under P addition, in both N_{Low}, P_{High} and N_{High}, P_{High} treatments (Fig. 4, $p = 0.01$, n = 52, df = 50). Grasses experienced no difference in competition intensity in response to nutrients but grass total dry root biomass increased with additional N and P (Fig. 4b, $p < 0.001$, n = 356, df = 350). Tree total root biomass was significantly smaller than those of grasses regardless of N and P addition (Fig. 4b, $p < 0.01$, n = 356, df = 350). The discrepancy between grass and tree total root biomass likely facilitated increased competition intensity for trees, because grasses likely increased nutrient uptake through larger root biomass. Grass mass gain increased with N and P addition (Fig. 1a, $p < 0.01$, n = 132, df = 129), regardless of competition treatment, though N increased grass mass gain more than P. However, tree mass gain did not vary predictably but was significantly less than grass mass gain across all treatments (Fig. 4c, $p < 0.001$, n = 356, df = 354).

While our experimental design included nitrogen fixation ability and encroachment ability, both had marginal impacts on predicting growth. Woody encroachers gained more mass over the course of the experiment than non-encroaching species (Supp. Fig. 2, $p = 0.05$, n = 80, df = 2). Nitrogen fixers also gained more mass over the course of the experiment than non-fixers (Fig. 5a, $p = 0.01$, n = 120, df = 2), though non-fixers tended to have more fine-root (0-2 mm) biomass than fixers (Supp. Table 1, Supp. Fig. 5, $p < 0.001$, n = 120, df = 3.8). Nitrogen fixers require nitrogen-fixing microbes to form nodules, and though we did not purposefully inoculate

seedlings, we observed nodules in 7 pots during the final harvest, all on *Senegalia burkeii* trees. Nodules were not found on any other potentially nodulating tree species.

Discussion

We conducted a greenhouse experiment to test two general hypotheses for how trees and grasses might coexist in the face of competition for N and P. First, trees and grasses could specialize on different soil resources to minimize competition (Vitousek and Howarth 1991, Tjoelker et al. 2005). Second, trees and grasses could compete for the same resources, with grasses out-competing trees for all soil resources in the short-term but with trees nonetheless persisting via long-term investment in light competition and surviving fires and herbivory (Craine et al. 2008, Hoffman et al. 2011).

We found evidence to reject conclusively our first hypothesis and probable support for our second. Grasses were stronger competitors for N (Fig. 1a) and foraged more intensively for P (Fig. 1e), whereas tree saplings did not respond to increased P and experienced strong competition from grasses in every treatment (Fig. 1b). We even observed probable effects of water competition, even though water provision was abundant: trees elongated their roots in competition with grass, perhaps to explore deeper soil layers for water (Case et al. 2020, Wang et al. 2012). This pattern is consistent with a variety of studies that show grasses are not strongly influenced by tree competition but do respond to resource availability (Riginos 2009, February et al. 2013b, Moustakas et al. 2013). Our results also reflect the widespread observation that C_4 grasses are aggressive nutrient foragers belowground (Casper and Jackson 1997, Gibert et al. 2016) and are consistent with the suggestion that African savanna grasses are particularly efficient at extracting soil N and have higher N-use efficiency than trees (Bilbao and Medina 1990), in addition to having increased P-use efficiency and acquisition compared to trees (Rao et

al. 1997, Junior et al. 2020). Overall, this suggests that trees and grasses are probably not specialized on different soil resources. These belowground results are instead consistent with hypothesis 2a, suggesting that grasses are effective competitors for scarce belowground resources, likely via high root biomass allocation and investment in nutrient-acquiring enzymes like phosphatase to aggressively acquire soil N and P.

Past work has also suggested that trees might be more competitive in resource-rich environments (Hoffmann et al. 2012, Pellegrini 2016, Tomlinson et al. 2019; hypothesis 2b), which our results did not support. Rather, competition for belowground resources intensified for trees in resource rich settings. However, we did find that trees elongated their shoots in competition with grass (see also Holdo and Nippert 2022) and allocated more resources aboveground (see also Bloom et al. 1985, Poorter et al. 2012) in resource-rich environments, potentially reflecting the importance of light competition and/or fire in high-resource environments (Fig. 2a-d, Fig. 3). It is also consistent with the finding from field studies that trees are more competitive aboveground in resource-rich (wetter) settings (Moustakas et al 2013; Dohn et al 2013), although it remains unclear whether trees in more nutrient-rich settings are also more able to shade out grasses in their subcanopies. Overall, our results are likely consistent with the hypothesis (H2) that grasses are stronger competitors for scarce soil resources than trees (a component of hypothesis 2), whereas trees are stronger competitors above ground. However, we suggest further research to disentangle nutrient and soil effects on aboveground competition between trees and grasses.

Our study is among the first to consider root phosphatase activity by both trees and grasses in savanna. Past work in other ecosystems has shown that root phosphatase activity may be mediated by soil available P, with higher investment in phosphatase when P is more limiting (Batterman et al. 2018, Guilbeault-Mayers et al. 2020). Our results showed instead that N supply

was more important: both grasses and trees invested more strongly in phosphatase production when released from N limitation, irrespective of P supply (see Fig. 1e-f). We also note that trees produced phosphatase at a much lower rate than grasses and even downregulated phosphatase production when in competition with grasses, which is also surprising given that phosphatase might allow trees to compete more effectively with grasses under intense nutrient competition. One possibility is that trees in competition with grasses were so resource limited that they could not afford to produce phosphatase for nutrient foraging. An alternative is that grass root phosphatase activity was much higher than that of trees even in N_{Low} treatments that trees may have benefitted from phosphatase produced by grasses rather than producing the enzymes themselves.

While N-fixing ability and encroachment risk was incorporated into our experimental design, neither functional group responded differently to grass competition or nutrient availability, although N-fixers and woody encroachers tended to grow larger than non-fixers (Supp. Fig. 2), which may merit further investigation. This finding in large part reflects the fact that we did not inoculate with native rhizobia (though these can sometimes be transferred through seed coats (Faizah et al. 1980)). The only species found to nodulate during the experiment was *Senegalia burkei*, a non-encroaching species. Native soil microbial communities are important for tree growth, especially N-fixing trees (Vitousek et al. 2002), and microbial symbionts are known to be able to alter nodulation frequency or competitive outcomes if included (Cramer et al. 2007, 2010, Abbott et al. 2015, Hortal et al. 2017). Further experimentation, especially growing N-fixers with and without their inoculants, may yield additional insights. We also note that, although insignificant, N fixers trended towards having higher root phosphatase activity (Supp. Fig. 4), both with and without added nutrients, which is

consistent with the hypothesis that N-fixers can facilitate higher phosphatase activity (Houlton et al. 2008, Batterman et al. 2018).

Our findings may have important field implications: nutrients may facilitate aboveground growth, but this may in turn interact with ecological processes like grazing and fire (Redfern et al. 2006, Holdo et al. 2009, Staver et al. 2009, Wakeling et al. 2011, Hoffmann et al. 2020). Additionally, though soil texture was not studied in this experiment, soil texture has been found to play an important role in structuring savanna vegetation (Case & Staver 2018). Clay soils are often nutrient-rich with more grass biomass, less tree biomass, and more frequent and intense fires while the opposite trend is generally true on Kruger's granitic soils (Buitenwerf et al. 2014, Kulmatiski et al. 2017, Staver et al. 2017). These results have generally been interpreted as evidence for hydrological limitation of tree growth, but our results here raise the intriguing possibility that vegetation differences between nutrient-rich clays and nutrient-poor sands may be due to a combination of soil nutrients and hydrological differences, amplified by fire and herbivory (Staver et al. 2017, Case and Staver 2018). Studying the interplay between these variables could help elucidate these connections in field settings.

In sum, grasses are effective belowground competitors, consistently outcompeting trees for essential macronutrients N and P irrespective of nutrient supply rate, suppressing tree sapling growth both below- and aboveground. In low N and P environments, trees grow less because of fewer resources, investing more in root biomass than stem biomass, which could put saplings at risk in savanna fires because saplings are too nutrient limited to invest in aboveground biomass. Higher nutrient environments facilitate increased tree growth but intensify competition with grasses, potentially providing a chance for saplings to escape the fire trap but also increasing grass biomass and thus fire risk; the net outcome for trees will depend both on the relative magnitudes of these effects. As anthropogenic change continues, resulting in N and P deposition

alongside disturbances to nutrient cycles and dynamics, more available nutrients will likely fertilize C_4 grass production (Sankaran et al. 2008), resulting in increased fuel for fires. Conversely, if nutrient cycles are disturbed such that less nutrients are available to savanna vegetation, trees and grasses will likely increase belowground allocation, suppressing tree growth in low nutrient environments. While outcomes are uncertain, our results clearly demonstrate that soil nutrients are vital, though understudied, components of savannas that play a large potential role in savanna vegetation dynamics.

Data Availability Statement

Data (Biro et al., 2024) associated with this paper is available in the Dryad Digital Repository at https://doi.org/10.5061/dryad.8w9ghx3v3.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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. Arielle Biro, Michelle Y. Wong, and Yong Zhou all assisted in greenhouse harvesting and data collection. Arielle Biro and A. Carla Staver performed statistical analyses and wrote the manuscript with extensive feedback from all authors.

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Tables

Table 1. Tree and grass species and their encroachment group, phylogenetic family, and nitrogen-fixing nodulation ability.

Species Name	Encroachment Group	Family	Nitrogen-Fixation Ability
Senegalia burkei	Non-encroacher	Fabaceae	Nodulating
Schotia brachypetala	Non-encroacher	Fabaceae	Non-nodulating
Colophospermum mopane	Possible Encroacher	Fabaceae	Non-nodulating
Vachellia nilotica	Possible Encroacher	Fabaceae	Nodulating
Combretum apiculatum	Woody Encroacher	Combretaceae	Non-nodulating
Dichrostachys cinerea	Woody Encroacher	Fabaceae	Nodulating
Melinis repens	Grass	Poaceae	Non-nodulating

Figures

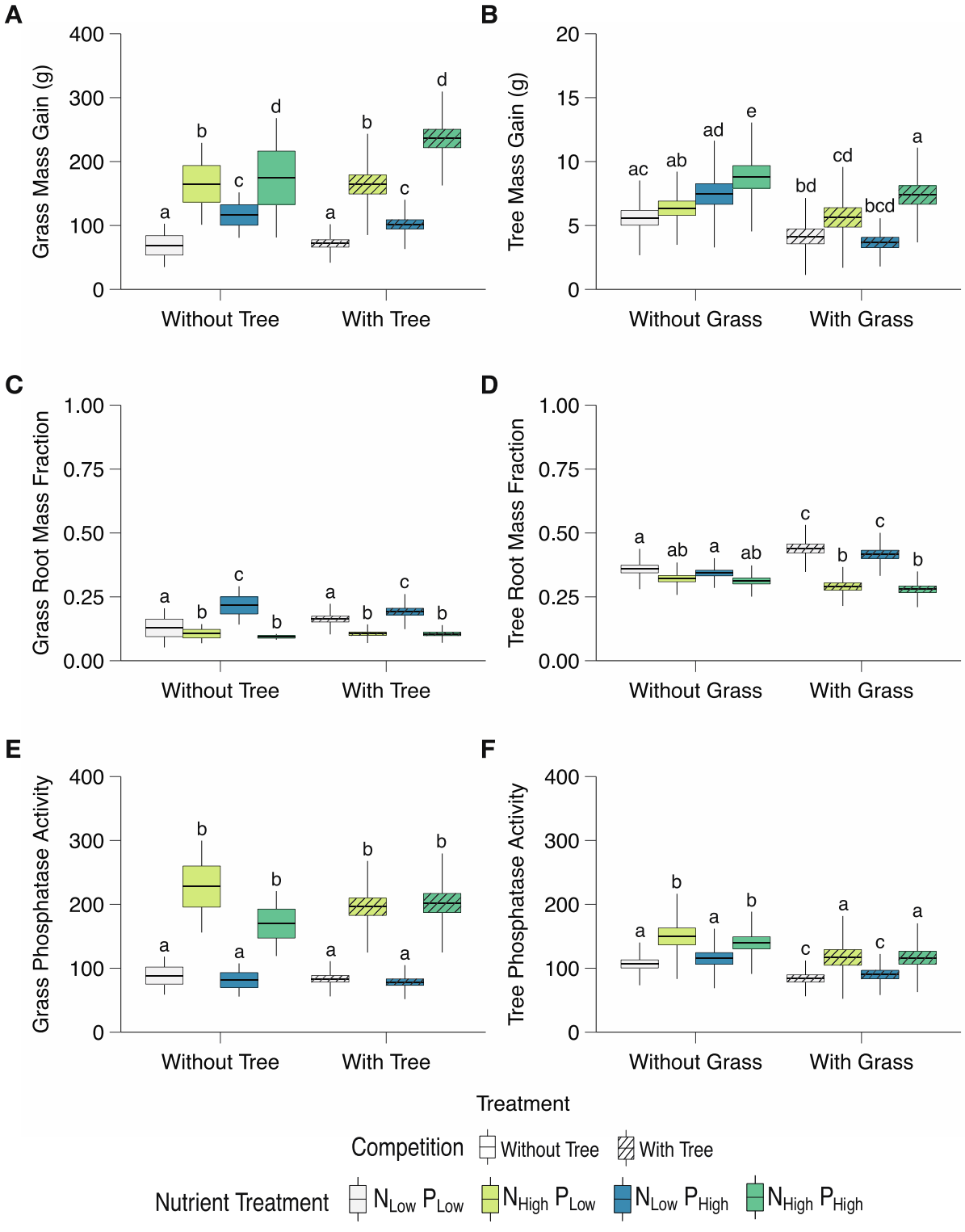
A
Grass Mass Gain (g)
400
300
200
100
0
a
b
c
d
a
b
c
d
Without Tree
With Tree
B
Tree Mass Gain (g)
20
15
10
5
0
ac
ab
ad
e
bd
cd
bcd
a
Without Grass
With Grass
C
Grass Root Mass Fraction
1.00
0.75
0.50
0.25
0.00
a
b
c
b
a
b
c
b
Without Tree
With Tree
D
Tree Root Mass Fraction
1.00
0.75
0.50
0.25
0.00
a
ab
a
ab
c
b
c
b
Without Grass
With Grass
E
Grass Phosphatase Activity
400
300
200
100
0
a
b
a
b
a
b
a
b
Without Tree
With Tree
F
Tree Phosphatase Activity
400
300
200
100
0
a
b
a
b
c
a
c
a
Without Grass
With Grass
Treatment
Competition
Without Tree
With Tree
Nutrient Treatment
N_Low P_Low
N_High P_Low
N_Low P_High
N_High P_High

Figure 1. Tree and grass responses at the end of the 16-week fertilization x competition experiment. (A) Total dry grass mass gain calculated by subtracting final grass dry biomass by initial grass dry biomass. (B) Total dry tree mass gain calculated by subtracting final tree dry biomass by initial tree dry biomass. (C) Grass root mass fraction, calculated by dividing total dry belowground grass biomass by total dry grass biomass. (D) Tree root mass fraction, calculated by dividing total dry belowground tree biomass by total dry tree biomass. (E) Grass root phosphatase activity measured in μmol of pNP per gram of dry root biomass per hour. (F) Tree root phosphatase activity measured in μmol of pNP per gram of dry root biomass per hour. Phosphatase activity for each plant was measured in triplicate and averaged. Values were rescaled to control for plotting by subtracting the species random effect values from variables of interest. Boxplots show standard errors and means, and whiskers represent standard deviations (n = 5 for grasses per nutrient and competition treatment, n = 5 for trees per nutrient and competition treatment). Differences were significant at a $p < 0.001$ level for grass total dry biomass between N_{Low} and N_{High} nutrient treatments, for grass root-to-shoot ratios between P_{Low} and P_{High}, and for tree total dry biomass, in response to nitrogen, phosphorus, and competition additions (see Supp. Table 1 for statistical results). Letters denote significant differences in pairwise comparisons among treatments, where bars that share letters are not significantly different at p = 0.05.

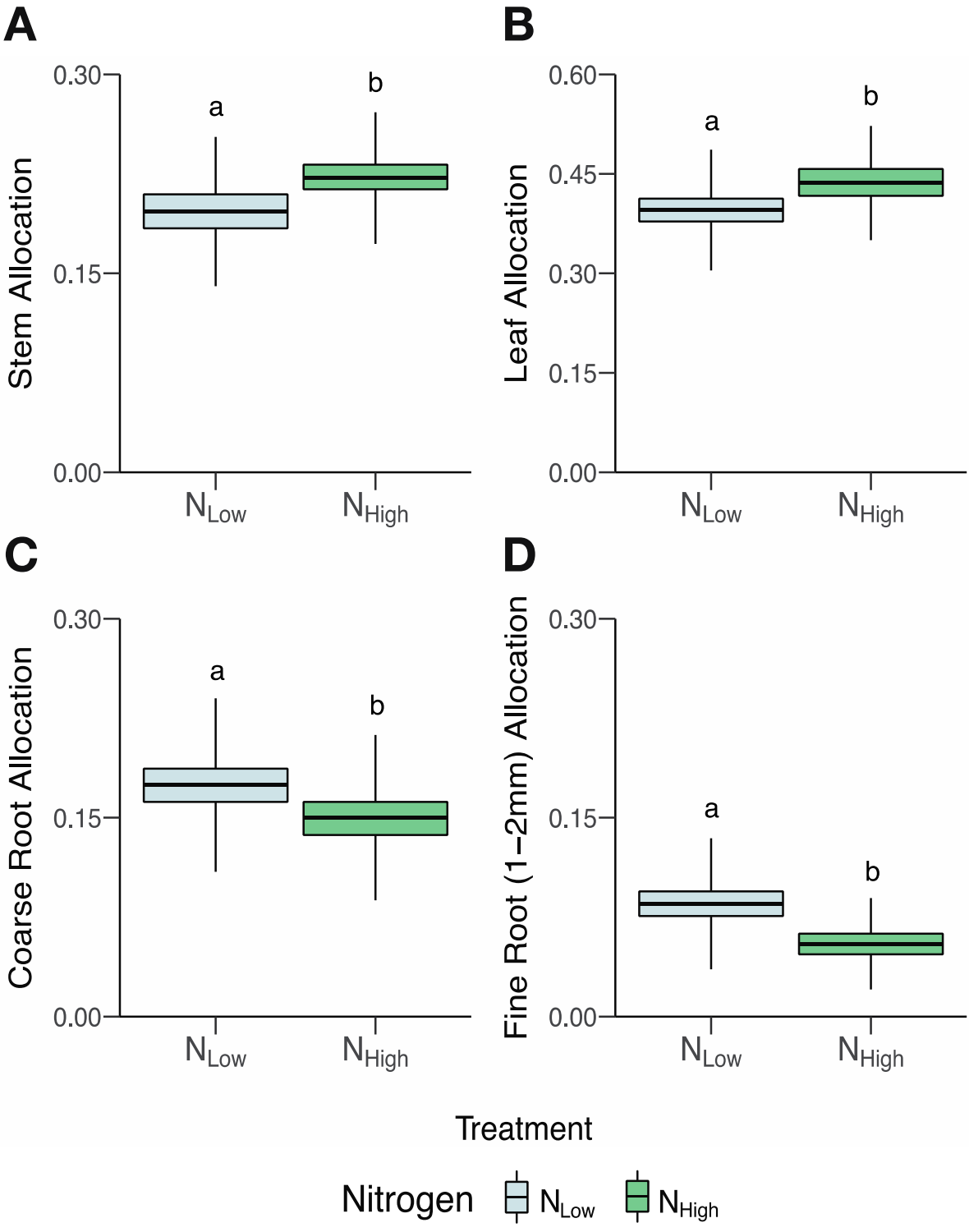
A
B
C
D
Stem Allocation
Leaf Allocation
Coarse Root Allocation
Fine Root (1−2mm) Allocation
0.30
0.15
0.00
0.60
0.45
0.30
0.15
0.00
a
b
N_Low
N_High
Treatment
Nitrogen
N_Low
N_High

Figure 2. Aboveground and belowground traits of trees across N_{Low} and N_{High} treatments. (A) Stem mass fraction was calculated by dividing stem dry biomass by total dry biomass. (B) Leaf mass fraction was calculated by dividing leaf dry biomass by total dry biomass. (C) Coarse root mass fraction was calculated by dividing coarse root dry biomass by total dry biomass. (D) Fine root (1-2 mm) mass fraction was calculated by dividing fine root (1-2 mm) dry biomass by total dry biomass. N_{Low} boxplots include N_{Low}, P_{Low} and N_{Low}, P_{High} treatments. N_{High} boxplots include N_{High}, P_{Low} and N_{High}, P_{High} treatments. Values were rescaled to control for plotting by subtracting the species random effect values from variables of interest. Boxplots show standard errors and means, and whiskers represent standard deviation values (n = 120 for trees per N treatment group). Letters denote significant differences in pairwise comparisons among treatments, where bars that share letters are not significantly different at p = 0.05.

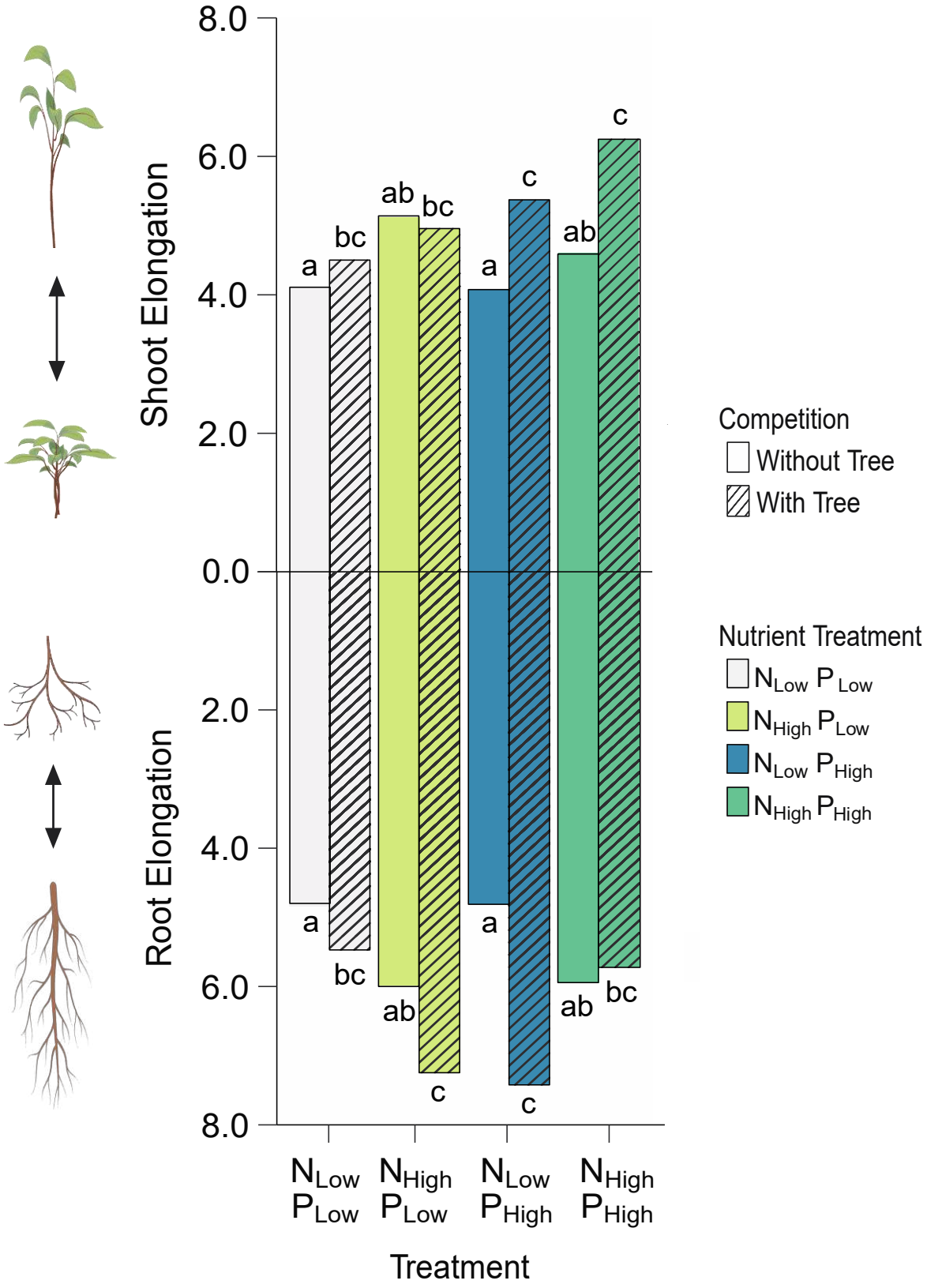
8.0
6.0
4.0
2.0
0.0
2.0
4.0
6.0
8.0
Shoot Elongation
Root Elongation
a
bc
ab
bc
a
c
ab
c
a
bc
ab
c
a
c
ab
bc
Competition
Without Tree
With Tree
Nutrient Treatment
N Low P Low
N High P Low
N Low P High
N High P High
N Low P Low
N High P Low
N Low P High
N High P High
Treatment

Figure 3. Shoot elongation and root elongation across nutrient treatments and competition treatments. Shoot elongation (β, where $Height = Diameter^{\beta}$), showing that trees in competition with grasses grew taller and skinnier than trees grown out of competition with grasses ($p < 0.001$), calculated by dividing the log transformed stem height by the log transformed stem basal diameter (in mm). Root elongation (β, where $Root\ Length = Root\ Diameter^{\beta}$), showing that trees in competition with grasses grew longer tap roots than trees grown out of competition with grasses ($p < 0.001$), calculated by dividing the log transformed root length by the log transformed root diameter. Columns represent shoot elongation and root elongation values. Letters denote significant differences in pairwise comparisons among treatments, where bars that share letters are not significantly different at $p = 0.05$.

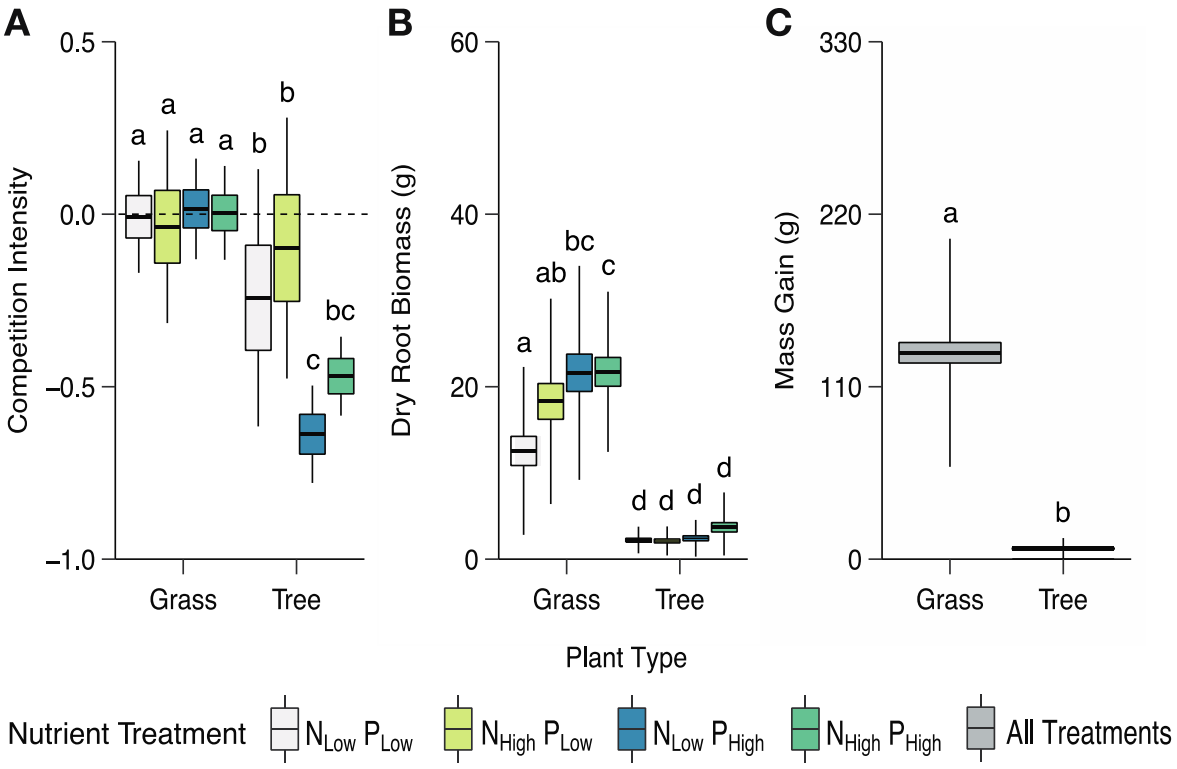

Figure 4. Competition intensity, total dry root biomass, and mass gain for grasses and trees across all nutrient treatments. (A) Competition intensity for grasses and trees across all four nutrient treatments, calculated by dividing the average mass gain of a tree or grass grown with competition by the average mass gain of a tree or grass grown out of competition and subtracted by one for each nutrient treatment within one species (*i.e.*, only individuals of the same species were compared). Negative values (< 0) indicate negative competition effects while positive values (> 0) indicate facultative effects of competition. (B) Total dry root biomass in grams for grasses and trees across all nutrient and competition treatments. (C) Mass gain for grasses and trees averaged across all nutrient and competition treatments, calculated by subtracting final weights from initial weights. Boxplots show standard errors and means, and whiskers represent standard deviation values (n = 20 for grasses per treatment group, n = 60 for trees per treatment

group). Letters denote significant differences in pairwise comparisons among treatments, where bars that share letters are not significantly different at $p = 0.05$.

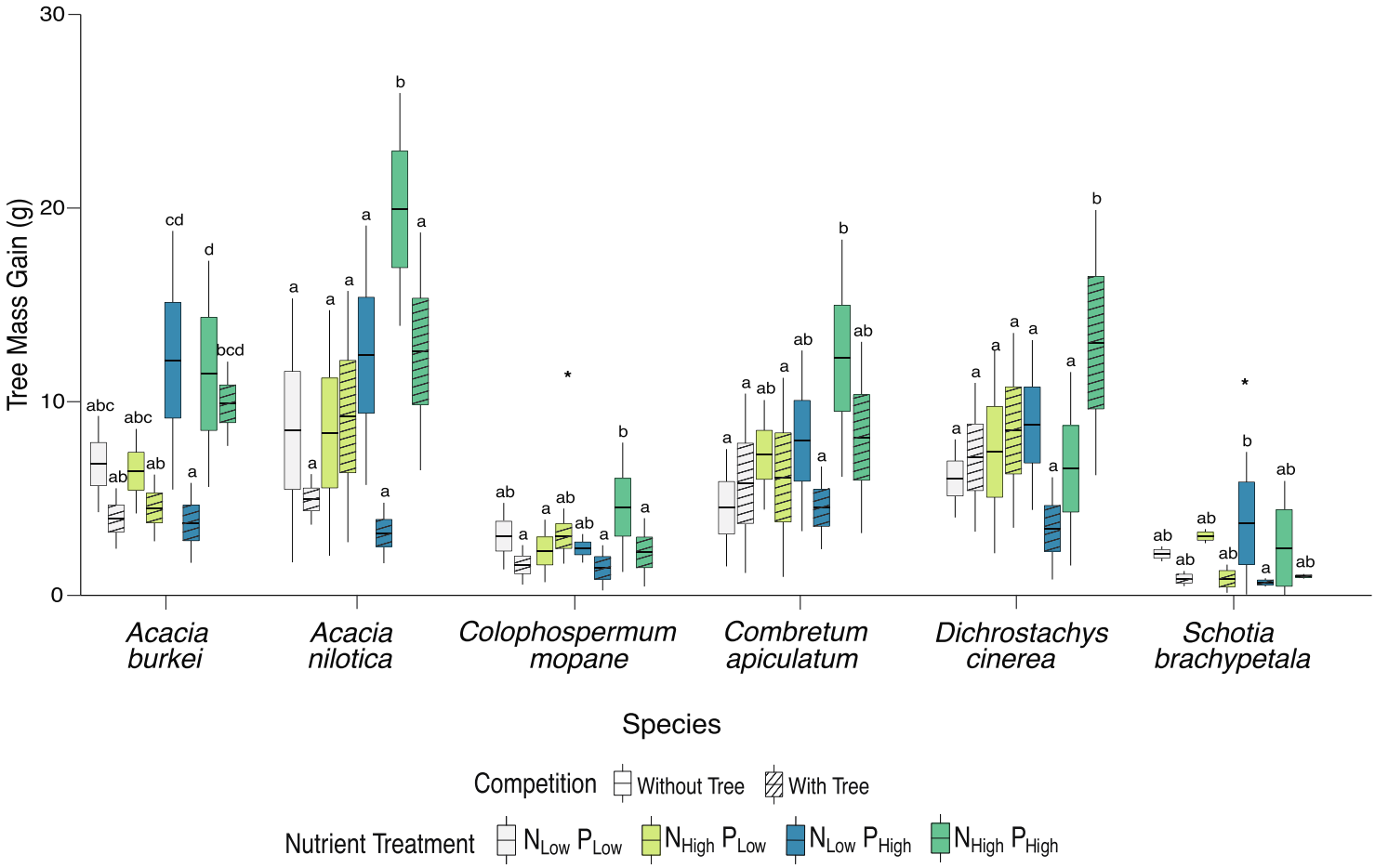

Figure 5. Tree mass gain (g) for each of the six tree species grown in the greenhouse experiment in fully factorial design with nutrient treatment and competition treatment shown (pairwise intraspecies comparisons are denoted by letters and interspecies comparison are denoted by asterisks. Only *C. mopane* and *S. brachypetala* were significantly different than the other four species grown). Mass gain was calculated by subtracting final weights from initial weights. Boxplots show ± standard errors and means, and whiskers represent ± standard deviation values (n = 60 for trees per treatment group). Letters denote significant differences in pairwise

comparisons among treatments, where bars that share letters are not significantly different at $p = 0.05$.