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Physiological and Growth Responses of Tropical Dry Forest Tree Seedlings to Water and Nutrient Additions: Comparisons Between Nitrogen Fixers and Non-Fixers

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ABSTRACT

Belowground resources are key determinants of seedling growth and survival in tropical forests. Nutrients and light may limit plant growth the most in tropical wet forests, whereas water may limit plant growth more in tropical dry forests. Nitrogen (N)-fixing species play an important role in the nitrogen and carbon cycles across tropical dry forests. However, studies investigating the joint effects of water and nutrients on the physiology and performance of N-fixing species are scarce. We implemented a full factorial shade house experiment that manipulated water and nutrients (NPK 20:20:20 and complete micronutrients) using eight tree species representing N-fixing and non-fixing tree species in the tropical dry forest of Costa Rica to determine: (1) How plant responses to water and nutrient availability vary between N-fixing and non-fixing tree species?; and (2) How nutrient and/or water availability influences seedling water- and nutrient-use traits? We found that growth and physiological responses to water and nutrient addition depended directly on the capacity of species to fix atmospheric N₂. N-fixing species responded more strongly to nutrient addition, accumulating 67% more total biomass on average (approximately double that of non-fixing taxa) and increasing average height growth rate by 41%. N-fixing species accumulated more biomass without compromising water-use efficiency, taking full advantage of the increased nutrient availability. Interestingly, results from our experiment show that increased water availability rarely influenced tropical dry forest seedling performance, whereas nutrient availability had a strong effect on biomass and growth. Overall, our results highlight the ability of N-fixing seedlings to take advantage of local soil resource heterogeneity, which may help to explain the dominance of N-fixing trees in tropical dry forests.

1 | Introduction

Plants require multiple belowground resources, including water and a variety of nutrients, to grow (Wright 2019).

Differences in how plants acquire these resources and how they respond to variation in their availability contribute to niche differentiation and the maintenance of diversity (Holste et al. 2011; Levine and HilleRis Lambers 2009). Research has

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focused on the seedling stage, as it is a vulnerable ontogenetic phase during which tree populations suffer elevated mortality, acting as a bottleneck for future plant community composition (Ribbens et al. 1994; Uriarte et al. 2018). Studies have generally shown that responses to the availability of belowground resources are often species-specific and may differ between above- and below-ground organs (Freschet et al. 2013). Thus, we still lack a general framework for understanding the joint effects of belowground resources (i.e., water and nutrients) on seedling performance, particularly in environments with high seasonal or temporal variability in soil moisture and nutrient availability, such as tropical dry forests (Waring et al. 2019). Understanding the effects of water and nutrient availability on tropical dry forest species is particularly important because these forests are expected to experience more intense and unpredictable climatic events, such as droughts (Allen et al. 2017), which may alter belowground resource availability and influence plant performance and function in tropical dry forests in the near future (Waring et al. 2019).

Seedling growth is thought to be strongly constrained by water availability and restricted to the wet season mostly in dry tropical forests (Gerhardt 1993; Maza-Villalobos et al. 2013). Indeed, experiments using tropical wet and dry forest seedlings have shown that water additions can increase growth and survival of tropical tree species (Marod et al. 2004; McLaren and McDonald 2003; Paine et al. 2009; Thaxton et al. 2012). However, several studies have shown that nutrient availability may also play an important role alongside water in determining seedling establishment and performance at early ontogenetic stages in tropical dry forest, as shown by Ceccon et al. (2004) and Chaturvedi et al. (2013), challenging the idea that tropical dry forests are only limited by water availability (Li et al. 2024; Medvigy et al. 2019).

At the plant level, high nutrient availability can increase leaf nitrogen (N) and phosphorus (P) concentrations (Santiago 2015), which generally leads to responses in water- and nutrient-use traits such as higher maximum CO₂ assimilation rates (A_{\max} ; Thompson et al. 1992), higher photosynthetic nitrogen use efficiency (*PNUE*; Hidaka and Kitayama 2009) and higher water-use efficiency (*WUE*; Raven et al. 2004; Smith-Martin et al. 2017), promoting the effective use of belowground resources and enhancing seedling carbon accumulation (Pasquini and Santiago 2012). These adjustments in carbon acquisition traits coupled with the efficient use of belowground resources following nutrient addition may explain higher growth and survival rates observed in nutrient addition experiments in a Mexican tropical dry forest (Huante et al. 1995; Salinas-Peba et al. 2014). Yet, nutrient availability is heavily influenced by organic matter turnover (Kreuzwieser and Gessler 2010). At the start of the wet season in tropical dry forests, there is a pulse of nutrient availability as increased water availability stimulates microbial activity, and with this, the decomposition of leaf litter (Murphy and Lugo 1986). Indeed, it has been shown that soluble N and P compounds peak at the end of the dry season, which become available to plants and microorganisms with the first rains (Anaya et al. 2007; Campo et al. 1998).

Across the Neotropics, legume tree species (Leguminosae) are one of the most dominant taxonomic groups (Gei et al. 2018).

A possible explanation for the dominance of N-fixing species in these regions could be related to the capacity of legume species to form a symbiotic association with rhizobia, a group of bacteria that has the ability to fix atmospheric nitrogen (N-fixing) (Gei et al. 2018). Nitrogen fixation enhances the *WUE* of N-fixing species by increasing leaf N concentrations (Powers and Tiffin 2010), leading to high CO₂ assimilation rates and low water losses due to reduced stomatal conductance (g_s). This increased *WUE* has been hypothesized to confer N-fixing species a competitive advantage over non-fixing species in environments where water is a limiting factor (Adams et al. 2016). Yet, N fixation is an energetically costly process (Gutschick 1981) which may be sensitive to nutrient and water availability. In fact, N-fixing species from the tropical dry forest can display a facultative strategy (Sheffer et al. 2015), downregulating N fixation when soil N concentration is high, and upregulating fixation when soil P concentrations increase (Waring et al. 2019), spurring faster growth rates in N-fixing species (Toro et al. 2023). In contrast, the role of water in determining N fixation rates is still not well understood (Dovrat and Sheffer 2019). While N-fixing species are important components in wet environments such as the tropical rainforest (Taylor et al. 2019), the abundance of N-fixing species peaks in water-limited environments such as tropical dry forests (Steidinger et al. 2019) indicating that N fixation might be highly sensitive to soil moisture conditions (Pellegrini et al. 2016; Valliyodan et al. 2016; Wurzbarger and Ford Miniat 2014).

During the seasonal transition between the dry to wet season in tropical dry forest, seedlings are exposed to high nutrient input from the microbial activity that decomposes leaf litter, as well as high levels of water availability for a few weeks (Campo et al. 1998; Singh et al. 1989), yet few studies have directly investigated how increased availability of nutrients and water can affect the performance and physiological traits of tropical dry forest tree seedlings (Santiago 2015). How these belowground resources individually and interactively affect tropical dry forest seedling performance across a broad range of functional types, including N-fixing and non-fixing species, is not well resolved. To address this, we examined how water and nutrient addition affected (1) seedling growth and biomass allocation, and (2) seedling ecophysiological traits of eight tropical dry forest tree species in north-west Costa Rica. We used three N-fixing legume species and five non-fixing species that encompass a wide range of trait variation for this region in a shade house experiment to answer the following questions: (Q1) How do responses to water and nutrient availability vary between N-fixing and non-fixing tree species? (Q2) How does increased nutrient and/or water availability influence seedling water- and nutrient-use traits and the capacity for N fixation?

Based on previous studies in this region (Smith-Martin et al. 2017; Toro et al. 2023), we expected that N-fixing legumes would take advantage of the increased resource availability by modulating their functional characteristics toward more acquisitive (i.e., higher leaf N concentrations and maximum photosynthetic rates) and efficient trait values (i.e., lower g_s , higher *PNUE*, and *WUE*). These dynamics might lead these species to have higher performance (i.e., growth and biomass accumulation) in comparison with non-fixing species (Adams et al. 2016; Gei et al. 2018; Pineda-García et al. 2015; Steidinger et al. 2019),

potentially helping to explain the dominance of N-fixing legumes across tropical dry forests.

2 | Methods

2.1 | Study Site and Species Selection

Our experiment took place between June–November 2015, in a shade house at Estación Experimental Forestal Horizontes, Área de Conservación Guanacaste, Costa Rica (10°42'04" N, 85°35'04" W). Rainfall is highly seasonal at this site, with a 5–6-month dry season. Mean annual precipitation at nearby Sector Santa Rosa is 1729 mm, and mean annual temperature is 25°C (Becknell and Powers 2014), with Horizontes likely having slightly lower rainfall than Santa Rosa (Werden et al. 2020). Our study occurred during one of the driest years on record (Cooley et al. 2019); wet season rainfall was less than ~50% of average due to a very strong El Niño Southern Oscillation (ENSO) event in 2015 (Powers et al. 2020), thus ambient rainfall conditions during the experiment represent a strong drought.

We selected eight tree species from five different families that are locally abundant for the shade house experiment. The Fabaceae included *Enterolobium cyclocarpum*, *Gliricidia sepium*, and *Dalbergia retusa*, all of which fix nitrogen (N-fixing), as well as the non-nitrogen fixer *Hymenaea courbaril*. The other non-fixing families and species we studied included Meliaceae: *Swietenia macrophylla*, Simaroubaceae: *Simarouba glauca*, Bignoniaceae: *Tabebuia ochraceae*, and Bombacaceae: *Pachira quinata*. Collectively, these species represent the full range of functional trait variation that defines tropical dry forest plant functional types in ecosystem simulation models built for the region (Xu et al. 2016), including N-fixing and non-fixing legume species, and evergreen and deciduous leaf phenology (Table 1).

2.2 | Seed Germination and Growing Conditions

Seeds were collected from trees growing in and around Estación Experimental Forestal Horizontes. All seeds were sown directly into pots (10 cm × 10 cm × 20 cm) containing a 2 kg

homogenized mixture of two parts locally collected soil and one part sand and placed in a shade house covered with shade cloth that allowed ~50% of sunlight and permitted precipitation to pass through. Soil was collected from a nearby naturally regenerating forest patch of approximately 30 years old with a land-use history of primarily cattle grazing since deforestation typical of the broader region. Average total soil P concentrations for samples collected ~300 m from where we collected soil for this experiment were ~458 mg/kg, suggesting that the soils we used were intermediate in P concentrations when compared globally (Yang et al. 2013). To improve germination rates, we applied two different treatments to the seeds: *E. cyclocarpum* seeds were subject to water baths alternating from boiling to cold water for 30 s to break the seed coat. All other species were soaked in water overnight before being sown. Seeds were sown directly into pots in early June 2015. Following germination and establishment, pots were thinned to one seedling per pot. All plants were watered to soil capacity when needed during this period. After ~8 weeks, watering and/or fertilizer treatments began and continued for 12 weeks more prior to harvest.

2.3 | Watering and Fertilization Treatments

At 8 weeks, seedlings were randomly assigned to one of four treatments (128 seedlings in total): control (no additions), nutrient addition (plus nutrients), water addition (plus water), and water and nutrient addition (plus water + nutrients). With these treatments, we tried to simulate the environmental conditions found during the early seasonal transition between the dry and wet season in tropical dry forest (Anaya et al. 2007; Campo et al. 1998) with the aim of quantifying seedling biomass and physiological responses to an increased availability in water and nutrients (and their interaction).

Water additions consisted of adding 500 mL to each pot every 2 weeks, which aimed to represent a 30% increase over the mean rainfall of August, September, and October in Costa Rica. Our goals were not to distinguish between the effects of different nutrient elements; rather, we aimed to boost the fertility of the soil overall. Thus, in the nutrient addition treatment, we applied a commercial fertilizer (NPK 20:20:20 and complete

TABLE 1 | Tropical dry forest species used in the water and nutrient addition experiment, grouped by capacity to fix nitrogen leaf habit and the total number of plants measured.

Genus species	Family	Leaf habit	Leaf type	Nitrogen fixer	Number of plants measured
<i>Dalbergia retusa</i>	Fabaceae	Deciduous	Compound	Yes	14
<i>Gliricidia sepium</i>	Fabaceae	Deciduous	Compound	Yes	13
<i>Enterolobium cyclocarpum</i>	Fabaceae	Deciduous	Compound	Yes	15
<i>Hymenaea courbaril</i>	Fabaceae	Semi-deciduous	Compound	No	16
<i>Pachira quinata</i>	Malvaceae	Deciduous	Compound	No	15
<i>Simarouba glauca</i>	Simaroubaceae	Evergreen	Compound	No	15
<i>Tabebuia ochraceae</i>	Bignoniaceae	Deciduous	Compound	No	14
<i>Swietenia macrophylla</i>	Meliaceae	Deciduous	Compound	No	15

micronutrients) to each pot at a rate equivalent to 150 kg N, P, and K ha⁻¹ year⁻¹, but prorated over the duration of the experiment (~3 months). This nutrient addition rate matches the rates applied by Waring et al. (2019) in a stand-level experiment in the same region. A total of 0.0375 g of fertilizer was dissolved in 20 mL of water and added to the potted soil surface every 2 weeks with a syringe. The water and nutrient addition treatment was a combination of the nutrient or water addition treatments above; our control seedlings received no water or nutrient additions. Soil moisture (SM150 Soil Moisture Sensor; Delta-T Devices Ltd., Cambridge, England) was measured in all pots (0–5 cm soil depth) immediately prior to watering treatments and ~1 day after watering; these measurements were repeated over six equally spaced time periods (every 2 weeks) during the experiment. This allowed us to quantify the magnitude of the watering effect on soil moisture and determine whether elevated soil moisture in the watered pots persisted beyond 2 weeks (Figure S1).

2.4 | Functional Trait Measurements

Due to a small amount of seedling mortality not related to the treatments applied, we measured traits on a total of 117 seedlings out of 128 originally planted (Table 1). We measured six physiological traits related to carbon and nutrient acquisition and resource-use efficiency for each seedling. Prior to harvesting the plants, we measured maximum photosynthetic capacity at 1200 PAR (A_{\max} ; $\mu\text{mol m}^{-2}\text{s}^{-1}$) and stomatal conductance (g_s ; $\text{mol m}^{-2}\text{s}^{-1}$) with a LCi portable photosynthesis system (ADC Bioscientific Ltd. Hoddesdon, UK). After measuring gas exchange, one leaf per plant was scanned to obtain its area, then dried at ~60°C for 48 h to later obtain the specific leaf area (SLA; cm^2g^{-1}). This same leaf was ground to a fine powder and wrapped in tin capsules to quantify leaf N concentration ($N\text{-area}$; g/m^2) on a PDZ Europa ANCA-GSL elemental analyzer (Stable Isotope Facility at the University of California, Davis). Finally, we calculated photosynthetic nitrogen use efficiency (PNUE; $\mu\text{mol mol}^{-1}\text{s}^{-1}$) as A_{\max} divided by $N\text{-area}$ (dos Santos et al. 2006; Hidaka and Kitayama 2009) and intrinsic water-use efficiency ($iWUE$; $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$) as A_{\max} divided by leaf transpiration rate.

2.5 | Plant Performance: Biomass and Growth

Every 2 weeks, we measured the height (cm) of each seedling to calculate relative height growth (RGR_h ; $\log(\text{cm}) \text{ days}^{-1}$). We calculated RGR_h as follows:

$$\text{RGR}_h = \log(H_f) - \log(H_i) / t_2 - t_1$$

where H_i is initial plant height, H_f is the final plant height, and t_1 and t_2 are the dates when the experiment started and finished, respectively. After taking gas exchange measurements, we harvested each seedling to determine its above- and belowground biomass. First, we separated plants into leaves and stems, then the roots were carefully separated and washed. For the N-fixing species (*E. cyclocarpum*, *G. sepium*, and *D. retusa*) all nodules were counted, and a random sample between 3 and 18 nodules was taken from each plant depending on nodule availability.

Roots, stems, leaves, and the nodule samples were dried at ~60°C for 72 h until they reached a constant mass; then their biomass (g) was quantified. Nodule samples were weighed on a precision balance at the University of Minnesota to estimate nodule dry mass. From this, we calculated the root to shoot ratio for each seedling and estimated the total nodule mass per plant by multiplying the total number of nodules found on the roots of each plant by the average nodule dry weight. Also, nodule mass fraction was calculated as the total nodule mass divided by the total plants' dry weight (Smith-Martin et al. 2017).

2.6 | Statistical Analyses

To determine how plant responses to water and nutrients varied between N-fixing and non-fixing species (Q1), we built linear mixed models using the nlme R package with a Gaussian error distribution (Pinheiro et al. 2021), with treatment (no addition, plus water, plus nutrients, or plus water + nutrients), tree species N-fixing status (N-fixing vs. non-fixing), and their interaction as fixed effects. We constructed three separate models as specified below using the following plant performance variables as responses: total seedling biomass, RGR_h , and root to shoot ratio:

$$\text{Plant performance} \sim \text{Treatment} \times \text{N-fixing status} + \text{Initial seedling height} + (1 | \text{species}) \quad (1)$$

We used N-fixing status as a fixed effect in our analyses as we hypothesized that N-fixing and non-fixing species would respond differently to treatments (Adams et al. 2016; Pineda-García et al. 2015; Smith-Martin et al. 2017). We used initial seedling height as a covariate to control for height differences between plants at the beginning of the experiment, and we included species ($N=8$) as a random effect to account for species-level variation.

To investigate how water and/or nutrient addition influenced functional trait values and the relationships with N-fixing status as a functional group (Q2), we first performed a principal components analysis to understand how traits were coordinated within N-fixing and non-fixing species. We scaled and centered each trait for each individual seedling ($N\text{-area}$, SLA, A_{\max} , $iWUE$, g_s , and PNUE) and extracted the main principal component axes that explained most of the variation. Then, we used the same mixed modeling approach used in Equation (1), but instead using $N\text{-area}$, SLA, and A_{\max} (as metrics of resource acquisition), $iWUE$, g_s , and PNUE (as integrated metrics of plant resource use efficiency) as response variables. This allowed us to detect any differences in these traits between treatments after we imposed the watering and fertilization treatments within the context of developmental changes over a short growing season (~3 months). To determine the effects of water and/or nutrient addition on N-fixing capacity (using the number of nodules, the total nodule mass per plant, and nodule mass fraction as proxies). Given that five out of eight species in our experiment do not fix nitrogen (Table 1), we first fitted zero-inflated models with a negative binomial distribution to address the possible influence of the zero values on our models; however, these models performed worse in terms of their AIC when compared to the models with a Gaussian distribution. Therefore, we decided to use a subset of the data that included only the N-fixing species and built our models using a Gaussian error distribution. For

these models, we used nodule count, the estimated total nodule mass, and the nodule mass fraction as response variables and treatment and initial height as fixed effects. Species (*E. cyclocarpum*, *D. retusa*, and *G. sepium*) was included as a random effect, ensuring the estimation of the variance of the random effects terms was nonzero (Gomes 2022).

To address non-normality and the presence of outliers, we log transformed the response variables *PNUE*, *iWUE*, *N-area*, nodule mass fraction, and number of nodules. To detect significant model terms and to account for the unbalanced number of seedlings within treatments, we performed likelihood ratio *F*-tests on type-III ANOVAs for all models. We used Tukey's HSD post hoc tests to detect differences between groups (*emmeans* package; Lenth 2022). All analyses were performed using R version 4.2 (R Core Team 2022).

3 | Results

3.1 | (Q1) How Do Plant Responses to Water and Nutrients Availability Vary Between N-Fixing and Non-Fixing Tree Species?

Soil moisture data showed that soil moisture rarely differed between treatment groups before starting the experiment (Figure S1A). After experimental irrigation, our watering treatment increased soil water availability by ~15% on average compared to unwatered treatments (no additions and plus nutrients treatments; Figure S1B) even though we aimed to increase it by 30%.

The effects of treatments on total plant biomass, RGR_h , and root to shoot ratio depended on N-fixing status (Table 2). Overall, N-fixing species accumulated more biomass with the addition of nutrients than with the addition of water (Figure 1A). Total biomass

for N-fixing species increased by 68% in both nutrient addition treatments when compared to the no additions treatment (no additions vs. plus nutrients: $t = -6.38$, $p < 0.001$; no additions vs. plus water + nutrients: $t = -6.67$, $p < 0.001$), while the watering treatment increased total N-fixer biomass only by 32% (no additions vs. plus water: $t = -3.17$, $p = 0.04$). Growth rates of N-fixing species followed a similar pattern when nutrients were added (Figure 1B). The RGR_h of N-fixing species increased by 41% on average in both nutrient addition treatments (no additions vs. plus nutrients: $t = -4.89$, $p < 0.001$; no additions vs. plus water + nutrients: $t = -5.13$, $p < 0.001$). The nutrient addition treatment shifted biomass toward roots as the root to shoot ratio of N-fixing species increased by 40% compared to the no additions treatment ($t = -3.41$, $p = 0.02$; Figure 1C), but root to shoot ratios did not differ between the other treatments and the no additions treatment ($p > 0.05$).

On the other hand, non-fixing species only responded when nutrients were added, gaining biomass in these cases, but their responses were lower on average compared to N-fixing species (Figure 1A). Total biomass for non-fixing species increased by 33% in the nutrient addition treatment (no additions vs. plus nutrients: $t = -3.09$, $p < 0.05$) and 39% in the water and nutrient treatment (no additions vs. plus water: $t = -3.78$, $p < 0.01$), while the plus water treatment did not have a significant effect. Also, the *RGR* and the root to shoot ratio were not influenced by any of the treatments for non-fixing species ($p > 0.05$; Figure 1B,C).

3.2 | (Q2) How Does Increased Nutrient and/or Water Availability Influence Seedling Water- and Nutrient-Use Traits and the Capacity for N Fixation?

The first three principal component axes explained 89% of the variability in the trait data. The first principal component axis (PC1) accounted for 43% of total variation and showed strong

TABLE 2 | *F*-ratios from mixed models for each of the response variables listed, with fixed effects for group (N-fixer or non-fixer status) and treatment (no additions, plus nutrients, plus water, and plus water + nutrients), and a species-level random effect.

Response variable	Treatment	Group	Treatment × Group	R_c^2	R_m^2
Total biomass	5.76**	1.42 ^{ns}	4.90**	0.80	0.49
RGR_h	4.81**	3.91 ^{ns}	3.23*	0.90	0.52
log(Root to shoot ratio)	0.70 ^{ns}	0.03 ^{ns}	4.50**	0.86	0.04
log(<i>iWUE</i>)	26.88***	4.09 ^{ns}	4.67**	0.72	0.56
log(<i>PNUE</i>)	3.62*	0.52 ^{ns}	1.79 ^{ns}	0.49	0.20
log(<i>N-area</i>)	7.37***	15.96**	2.38 ^{ns}	0.87	0.58
SLA	0.25 ^{ns}	0.43 ^{ns}	1.80 ^{ns}	0.58	0.17
g_s	18.66***	6.40*	1.70 ^{ns}	0.47	0.39
A_{max}	0.26 ^{ns}	7.97**	7.38***	0.94	0.50
Nodule mass fraction	0.85 ^{ns}	—	—	0.78	0.09
Total nodule mass	4.62**	—	—	0.41	0.14
Nodule count	11.33***	—	—	0.95	0.05

Note: R_c^2 and R_m^2 represent the conditional and marginal *R* squared for each model, respectively. Significance: ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

loadings on A_{\max} , N -area, and reflects the capacity of species to gain carbon (Figure 3). The second principal component axis (PC2) accounted for 24% of total variation, having $PNUE$ and N -area as the strongest loadings (Figure S2B). Finally, the third principal component axis (PC3) accounted for 22% and showed strong loadings on g_s and SLA (Figure S2B).

Of the six traits measured, only $iWUE$ and A_{\max} responded to the treatments, and the magnitude and direction of these responses depended on N-fixing status (Table 2). N-fixing species increased A_{\max} by 16% in the plus water treatment (no additions vs. plus water: $t = -3.46$, $p = 0.0175$) while their $iWUE$ did not change in any of the treatments applied (Figure 2). For non-fixer species, we found that water addition decreased $iWUE$ by approximately 50% (no additions vs. plus water: $t = 6.21$, $p < 0.0001$; no additions vs. plus water + nutrients, $t = 6.84$, $p < 0.0001$) while A_{\max} did not change significantly between any treatments (Figure 2).

We also found significant treatment effects on stomatal conductance (g_s), leaf N concentration per unit of area (N -area), and

photosynthetic N use efficiency ($PNUE$) regardless of species' N-fixing status (Figure 3). When water was added, g_s increased in all individuals between 58% (no additions vs. plus water + nutrients: $t = -4.40$, $p = 0.0002$) and 69% on average (no additions vs. plus water: $t = -5.19$, $p < 0.0001$). While nutrient addition decreased the $PNUE$ by 18% (no additions vs. plus nutrients: $t = 2.82$, $p = 0.03$) and increased N -area by 17% (no additions vs. plus nutrients: $t = -3.28$, $p = 0.008$), SLA did not change across the treatments. Lastly, we found that N-fixing species had double the leaf N concentrations (non-fixing/N-fixing: $t = -3.86$, $p = 0.008$) and double the $iWUE$ (non-fixing/N-fixing: $t = -3.98$, $p = 0.0073$) compared to non-fixing species regardless of the treatment applied.

Resource addition also affected N-fixation potential, as indexed by total plant nodule mass and nodule counts (Table 2). Nodule mass increased by 65% on average when nutrients were added (no additions vs. plus nutrients: $t = -2.73$, $p = 0.044$; no additions vs. plus water + nutrients: $t = -3.05$, $p = 0.02$). Additionally, nodule counts increased by 20% when water and nutrients were

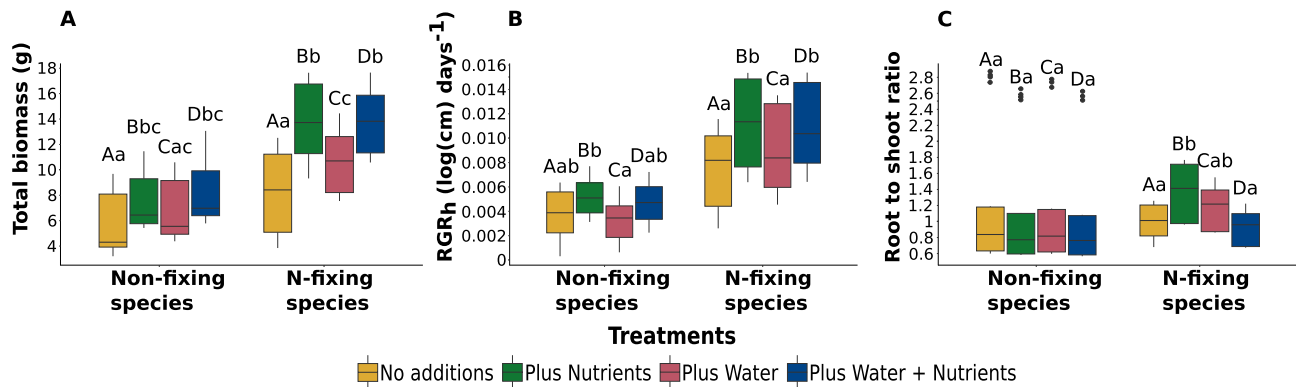


FIGURE 1 | Boxplots showing the lower quartile, median and the upper quartile for (A) total biomass, (B) relative growth rate, and (C) root to shoot ratio of non-fixing and N-fixing species grown in treatment combinations of no additions (yellow), plus nutrients (green), plus water (pink) and plus water + nutrients (dark blue) treatments. Dots below letters represent outliers. There were significant interactions between treatment and N-fixer status for all variables (Table 2). Significant differences (Tukey's HSD; $p < 0.05$) between the non-fixing and N-Fixing species groups for each treatment are indicated with upper case letters. Significant differences within the non-fixing or N-fixing species groups for each treatment are indicated by lower case letters.

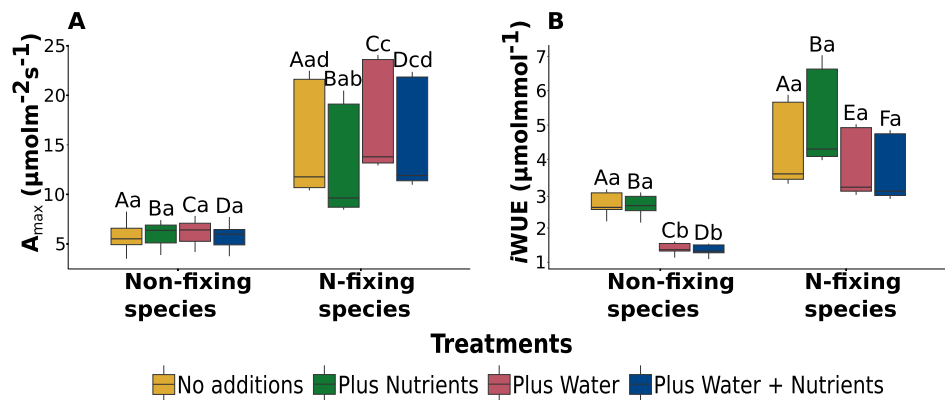


FIGURE 2 | Boxplots showing the lower quartile, median and the upper quartile for traits with a significant interaction between treatment and N-fixing status. (A) Maximum photosynthetic capacity (A_{\max} ; $\mu\text{mol m}^{-2}\text{s}^{-1}$) and (B) instantaneous water-use efficiency ($iWUE$; $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$). $iWUE$ was back-transformed from logarithmic transformation for plotting. Significant differences (Tukey's HSD; $p < 0.05$) between the non-fixing and N-fixing species groups for each treatment are indicated with upper case letters. Significant differences within the non-fixing or N-fixing species groups for each treatment are indicated by lower case letters.

added together (no additions vs. plus water + nutrients: $t = -2.95$, $p = 0.025$) and decreased by 15% when only nutrients were added (Figure 4A). However, this result was marginally significant (no additions vs. plus nutrients: $t = 2.62$, $p = 0.056$). We did not find an effect of the plus water treatment on nodule counts nor on total plant nodule mass (Figure 4), and nodule mass fractions did not respond to any of the treatments applied (Table 2).

4 | Discussion

As extreme climatic events such as droughts that might alter resource availability and dynamics become more common, it is important to understand how they affect plants at early life history stages and whether additional factors like nutrients modulate the responses to water availability. Results from our shade house experiment show that responses to nutrient addition were stronger on tropical dry forest seedling biomass and growth than on water addition. Moreover, we demonstrate that N-fixing species had a high capacity to take advantage of increased resource availability, particularly for nutrients, and to a lesser degree for water. Additionally, traits related to resource use ($iWUE$, g_s , and $PNUE$) of individual seedlings shifted in a coordinated way with the addition of belowground resources toward more acquisitive and less resource-use efficient strategies, with the exception that

N-fixing species maintained water-use efficiency across treatments. These results may help to explain N-fixer dominance in tropical dry forests because they highlight the ability of N-fixing seedlings to adapt to local soil resource heterogeneity.

4.1 | Water and Nutrients Influence Seedling Growth and Biomass Allocation

We found that nutrients appear to play a larger role than water in the early stages of tropical dry forest seedling growth and biomass accumulation, even in a very dry year, and that responses to the additional resources depended on the capacity of species to fix nitrogen. When nutrients were added either alone or in combination with watering, all species responded positively; however, N-fixing species grew faster, accumulated high amounts of total biomass, and invested more in roots than in leaves. These results corroborate the findings reported by Smith-Martin et al. (2017) who found that responses to soil type and light availability differ between legume and non-legume tropical dry forest tree species. Our results using seedlings under controlled conditions were similar to the findings of a large-scale ecosystem experiment using many of the same species and similar treatments, which found stronger responses of trees to nutrient additions than to reductions in soil water availability

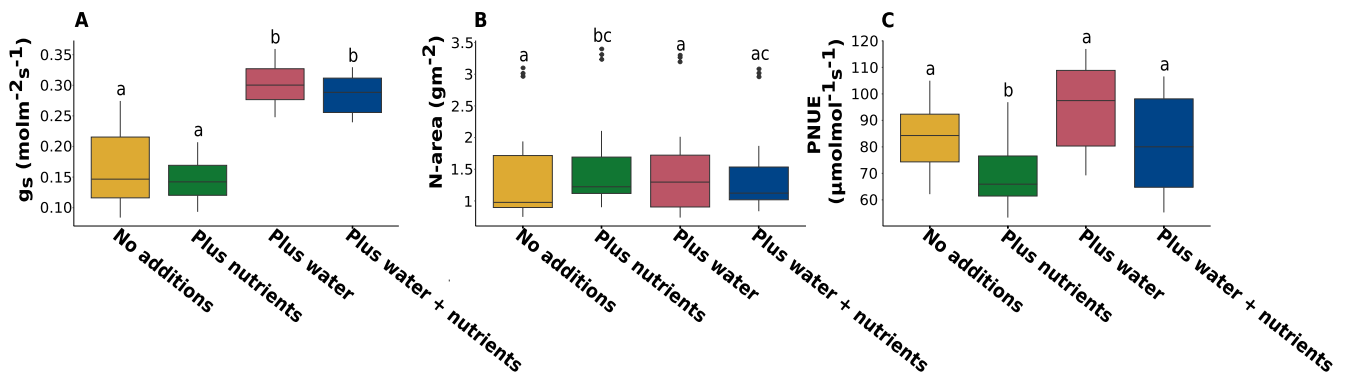


FIGURE 3 | Boxplots showing the lower quartile, median and the upper quartile for traits with a significant treatment effect. (A) stomatal conductance (g_s ; mol m⁻² s⁻¹), (B) leaf nitrogen concentration per unit of area (N -area; g/m²), and (C) photosynthetic nitrogen use efficiency ($PNUE$; μmol mol⁻¹ s⁻¹). Dots below letters represent outliers. N -area and $PNUE$ were back-transformed from logarithmic transformation for plotting. Significant differences between treatments (Tukey's HSD; $p < 0.05$) are indicated by lower case letters.

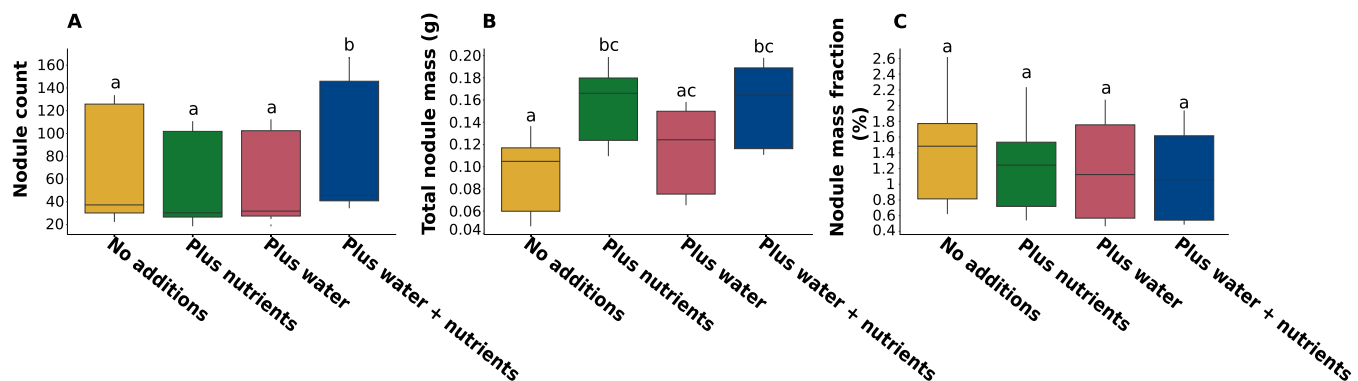


FIGURE 4 | Boxplots showing the lower quartile, median, and the upper quartile for (A) number of root nodules found in each seedling (B) estimated total nodule mass, and (C) nodule mass fraction. Significant differences between treatments (Tukey's HSD; $p < 0.05$) are indicated by lower case letters.

(Vargas G et al. 2023). Additionally, in the water addition treatment (plus water) only N-fixing species responded by increasing their total biomass, whereas non-fixing species did not. This result supports the findings of Nielsen et al. (2019) who found that chaparral shrub species with lower growth rates had lower total biomass accumulation with the addition of water than when nutrients were added.

Nutrient supply in tropical dry forest is highly sensitive to changes in water availability due to the positive effects of water availability on microbial communities that decompose leaf litter (Waring et al. 2021). Therefore, our results could play an important role in explaining seedling establishment and growth in these ecosystems. The capacity of N-fixing species to germinate quickly (Vargas G et al. 2015), to increase N fixation when light increases (Taylor and Menge 2018), to inhibit the growth of neighbor trees (Taylor et al. 2017), and to take advantage of high nutrient availability, as we have demonstrated, could all give N-fixing species a competitive advantage over non-fixing species when establishing in early successional forest.

4.2 | Nutrient and Water Availability Influence Seedling Water-Use and Carbon Assimilation Traits and N Fixation Potential

It has been proposed that seedlings coordinate their leaf traits toward a more acquisitive strategy as a short-term response to greater resource availability (Santiago 2015; Werden et al. 2020). Indeed, we found similar responses; but this strategy was highly associated with the capacity to fix nitrogen (Figure S2A). When nutrients were added alone, all species responded by increasing their leaf N concentrations while also decreasing their *PNUE* (Figure 3B,C). This could reflect a coordinated trait shift of tropical dry forest species toward a more acquisitive strategy characterized by leaves with shorter life spans, which corroborates the results by Cordell et al. (2001) who found a consistent trade-off between *PNUE* and leaf life span in fertilized plots in Hawaii. We also found consistent increases in stomatal conductance in all the tree species when water was added. This result might indicate that seedlings, regardless of their functional group, avoid heat by using transpiration as a strategy to control leaf temperature in hot environments, as shown by Aparecido et al. (2020) and Marchin et al. (2023).

We hypothesized that water-use efficiency and photosynthetic rates vary depending on N-fixing status. As expected, these two important traits for resource use and acquisition differed depending on the capacity to fix N but not in the direction that we predicted. Non-fixing species water-use efficiency was heavily reduced by the addition of water, which corroborates the results found by Nielsen et al. (2019) that showed that in chaparral shrub species, water-use efficiency is reduced with water addition. This result might be explained by the increases in stomatal conductance experienced by all seedlings to potentially avoid excessive leaf temperatures (Figure 3A). Yet despite the fact that N-fixing species had comparable stomatal conductance values to non-fixing species when water was added, N-fixing species maintained the same levels of water-use efficiency across all treatments and were twice as efficient on average as non-fixing, regardless of the treatment applied. This was potentially

achieved by an adjustment of photosynthetic rates in concert with leaf transpiration rates as seen in the plus water treatment (Figure 2).

We acknowledge that nodule count, nodule mass fraction, and estimated total plant nodule mass are imperfect proxies for N fixation. Given that caveat, in the case of symbiotic N-fixation, nutrient addition alone decreased the number of nodules (Figure 4A), corroborating the results found by McCulloch et al. (2021), Waring et al. (2019), and Vargas G et al. (2023) suggesting that high levels of soil N may downregulate nodulation (McCulloch and Porder 2021). However, nodule mass increased in both of the nutrient treatments (Figure 4B), which aligns with patterns observed in increasing biomass accumulation and growth rates of N-fixing species when nutrients were added (Figure 1A,B). Moreover, water addition did not significantly affect nodule counts or estimated total nodule mass. Our results suggest that nitrogen fixation might be a nutrient-limited process in these soils, as we observed increments in nodule mass in the nutrient treatments (Figure 4B). We hypothesize that this result could be due to the addition of phosphorus, as shown by Toro et al. (2023).

In general, N-fixing species are characterized by having high foliar N concentrations and high water-use efficiency (Powers and Tiffin 2010). These characteristics may explain why N-fixing species may maintain a competitive advantage over non-fixing species when resources necessary for growth are made available (Adams et al. 2016; Gei et al. 2018). Our results underscore this dynamic, as we found that N-fixing species were associated with high carbon uptake traits that enabled them to take advantage of elevated levels of belowground resources, especially nutrients, while non-fixing species showed a lower relative response to the same belowground resource additions (Figure 1). These results may explain the observed difference in biomass and growth rates between N-fixer and non-fixer species within this tropical dry forest system.

There were clear, different effects of nutrient versus water addition on N fixation (Figure 4). When nutrients were added, N-fixing species responded by upregulating nitrogen fixation, which was coordinated with a fast acquisition strategy with high mean water-use efficiency for rapid biomass accumulation. Overall, our results suggest that N-fixing species take advantage of increased resource availability as quickly as possible. These strategies could optimize seedling establishment, growth, and biomass gain in tropical dry forest, where belowground resource availability is seasonally pulsed. In the transition between dry and wet season in the tropical dry forest, these types of strategies are important because forest foliage expansion occurs quickly, coinciding with high nutrient availability from decomposing litterfall and increased microbial activity (Campo et al. 1998), high levels of light in the understory, and quickly increasing soil moisture. Under these rapidly changing conditions, N-fixing species may have a competitive advantage over non-fixing species as the N-fixing species we examined appear to have the capacity to coordinate their traits toward more acquisitive strategies while retaining their efficient water-use strategy across a variety of contexts, with the aim of maximizing growth and biomass accumulation before the canopy closes.

In pot experiments, it can be difficult to impose nutrient and watering treatments that are comparable in magnitude, especially given the role of soil moisture in regulating nutrient availability. Our treatments were designed to represent large pulses of nutrients and/or water. Our soil moisture data indicated that we succeeded in immediately boosting soil moisture ~15% over ambient values; although this was not sustained over 2 weeks. We acknowledge that this may have influenced our results, and we suggest that future experiments use multiple levels of nutrients and/or water, as these may elicit different responses (e.g., Toro et al. 2023).

5 | Conclusion

We found that a diverse group of tropical dry forest seedlings responded more to nutrient availability compared to water. Moreover, our experiment revealed fundamentally different ways that N-fixing tropical dry forest species respond to heterogeneity in belowground resources compared to non-N-fixing species. N-fixing legume seedlings generally grow rapidly compared to other taxa in the ontogenetic stages post-germination, and our work shows that they also have larger responses to nutrients compared to non-fixing. Moreover, we found that N-fixing species in this system appear not to fully conform to the acquisitive/conservative trait syndrome dichotomy, as they exhibited acquisitive growth characteristics with the capacity to maintain the same levels of resource use efficiency across water and nutrient addition treatments. Together, these results suggest that N-fixing species might have a competitive advantage in tropical dry forests at the seedling stage as they are able to adapt to local soil resource heterogeneity.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data, code, and all the R packages versions required for reproducing the findings in this study are available in the Zenodo repository: <https://doi.org/10.5281/zenodo.14219731>.

References

Adams, M. A., T. L. Turnbull, J. I. Sprent, and N. Buchmann. 2016. "Legumes Are Different: Leaf Nitrogen, Photosynthesis, and Water Use Efficiency." *Proceedings of the National Academy of Sciences* 113, no. 15: 4098–4103. <https://doi.org/10.1073/pnas.1523936113>.

Allen, K., J. M. Dupuy, M. G. Gei, et al. 2017. "Will Seasonally Dry Tropical Forests Be Sensitive or Resistant to Future Changes in Rainfall Regimes?" *Environmental Research Letters* 12, no. 2: e023001. <https://doi.org/10.1088/1748-9326/aa5968>.

Anaya, C. A., F. García-Oliva, and V. J. Jaramillo. 2007. "Rainfall and Labile Carbon Availability Control Litter Nitrogen Dynamics in a Tropical Dry Forest." *Oecologia* 150, no. 4: 602–610. <https://doi.org/10.1007/s00442-006-0564-3>.

Aparecido, L. M. T., S. Woo, C. Suazo, K. R. Hultine, and B. Blonder. 2020. "High Water Use in Desert Plants Exposed to Extreme Heat." *Ecology Letters* 23, no. 8: 1189–1200. <https://doi.org/10.1111/ele.13516>.

Becknell, J. M., and J. S. Powers. 2014. "Stand Age and Soils as Drivers of Plant Functional Traits and Aboveground Biomass in Secondary Tropical Dry Forest." *Canadian Journal of Forest Research* 44, no. 6: 604–613. <https://doi.org/10.1139/cjfr-2013-0331>.

Campo, J., V. J. Jaramillo, and J. M. Maass. 1998. "Pulses of Soil Phosphorus Availability in a Mexican Tropical Dry Forest: Effects of Seasonality and Level of Wetting." *Oecologia* 115, no. 1–2: 167–172. <https://doi.org/10.1007/s004420050504>.

Ceccon, E., S. Sánchez, and J. Campo. 2004. "Tree Seedling Dynamics in Two Abandoned Tropical Dry Forests of Differing Successional Status in Yucatán, Mexico: A Field Experiment With N and P Fertilization." *Plant Ecology* 170, no. 2: 277–285. <https://doi.org/10.1023/B:VEGE.0000021699.63151.47>.

Chaturvedi, R. K., A. S. Raghubanshi, and J. S. Singh. 2013. "Growth of Tree Seedlings in a Tropical Dry Forest in Relation to Soil Moisture and Leaf Traits." *Journal of Plant Ecology* 6, no. 2: 158–170. <https://doi.org/10.1093/jpe/rt025>.

Cooley, S. S., C. A. Williams, J. B. Fisher, G. H. Halverson, J. Perret, and C. M. Lee. 2019. "Assessing Regional Drought Impacts on Vegetation and Evapotranspiration: A Case Study in Guanacaste, Costa Rica." *Ecological Applications* 29, no. 2: e01834. <https://doi.org/10.1002/eap.1834>.

Cordell, S., G. Goldstein, F. C. Meinzer, and P. M. Vitousek. 2001. "Regulation of Leaf Life-Span and Nutrient-Use Efficiency of *Metrosideros polymorpha* Trees at Two Extremes of a Long Chronosequence in Hawaii." *Oecologia* 127, no. 2: 198–206. <https://doi.org/10.1007/s004420000588>.

dos Santos, U. M., J. F. de Carvalho Gonçalves, and T. R. Feldpausch. 2006. "Growth, Leaf Nutrient Concentration and Photosynthetic Nutrient Use Efficiency in Tropical Tree Species Planted in Degraded Areas in Central Amazonia." *Forest Ecology and Management* 226, no. 1–3: 299–309. <https://doi.org/10.1016/j.foreco.2006.01.042>.

Dovrat, G., and E. Sheffer. 2019. "Symbiotic Dinitrogen Fixation Is Seasonal and Strongly Regulated in Water-Limited Environments." *New Phytologist* 221, no. 4: 1866–1877. <https://doi.org/10.1111/nph.15526>.

Freschet, G. T., P. J. Bellingham, P. O. Lyver, K. I. Bonner, and D. A. Wardle. 2013. "Plasticity in Above- and Belowground Resource Acquisition Traits in Response to Single and Multiple Environmental Factors in Three Tree Species." *Ecology and Evolution* 3, no. 4: 1065–1078. <https://doi.org/10.1002/ece3.520>.

Gei, M., D. M. A. Rozendaal, L. Poorter, et al. 2018. "Legume Abundance Along Successional and Rainfall Gradients in Neotropical Forests." *Nature Ecology & Evolution* 2, no. 7: 1104–1111. <https://doi.org/10.1038/s41559-018-0559-6>.

Gerhardt, K. 1993. "Tree Seedling Development in Tropical Dry Abandoned Pasture and Secondary Forest in Costa Rica." *Journal of Vegetation Science* 4, no. 1: 95–102. <https://doi.org/10.2307/3235736>.

Gomes, D. G. E. 2022. "Should I Use Fixed Effects or Random Effects When I Have Fewer Than Five Levels of a Grouping Factor in a Mixed-Effects Model?" *PeerJ* 10: e12794. <https://doi.org/10.7717/peerj.12794>.

Gutschick, V. P. 1981. "Evolved Strategies in Nitrogen Acquisition by Plants." *American Naturalist* 118, no. 5: 607–637. <https://doi.org/10.1086/283858>.

Hidaka, A., and K. Kitayama. 2009. "Divergent Patterns of Photosynthetic Phosphorus-Use Efficiency Versus Nitrogen-Use Efficiency of Tree

- Leaves Along Nutrient-Availability Gradients." *Journal of Ecology* 97, no. 5: 984–991. <https://doi.org/10.1111/j.1365-2745.2009.01540.x>.
- Holste, E. K., R. K. Kobe, and C. F. Vriesendorp. 2011. "Seedling Growth Responses to Soil Resources in the Understory of a Wet Tropical Forest." *Ecology* 92, no. 9: 1828–1838. <https://doi.org/10.1890/10-1697.1>.
- Huante, P., E. Rincon, and I. Acosta. 1995. "Nutrient Availability and Growth Rate of 34 Woody Species From a Tropical Deciduous Forest in Mexico." *Functional Ecology* 9, no. 6: 849. <https://doi.org/10.2307/2389982>.
- Kreuzwieser, J., and A. Gessler. 2010. "Global Climate Change and Tree Nutrition: Influence of Water Availability." *Tree Physiology* 30, no. 9: 1221–1234. <https://doi.org/10.1093/treephys/tpq055>.
- Lenth, R. V. 2022. "emmeans: Estimated Marginal Means, Aka Least-Squares Means [Manual]." <https://CRAN.R-project.org/package=emmeans>.
- Levine, J. M., and J. HilleRis Lambers. 2009. "The Importance of Niches for the Maintenance of Species Diversity." *Nature* 461, no. 7261: 254–257. <https://doi.org/10.1038/nature08251>.
- Li, S., B. Waring, J. Powers, and D. Medvigy. 2024. "Tropical Dry Forest Response to Nutrient Fertilization: A Model Validation and Sensitivity Analysis." *Biogeosciences* 21, no. 2: 455–471. <https://doi.org/10.5194/bg-21-455-2024>.
- Marchin, R. M., B. E. Medlyn, M. G. Tjoelker, and D. S. Ellsworth. 2023. "Decoupling Between Stomatal Conductance and Photosynthesis Occurs Under Extreme Heat in Broadleaf Tree Species Regardless of Water Access." *Global Change Biology* 29, no. 22: 6319–6335. <https://doi.org/10.1111/gcb.16929>.
- Marod, D., U. Kutintara, H. Tanaka, and T. Nakashizuka. 2004. "Effects of Drought and Fire on Seedling Survival and Growth Under Contrasting Light Conditions in a Seasonal Tropical Forest." *Journal of Vegetation Science* 15, no. 5: 691–700. <https://doi.org/10.1111/j.1654-1103.2004.tb02311.x>.
- Maza-Villalobos, S., L. Poorter, and M. Martínez-Ramos. 2013. "Effects of ENSO and Temporal Rainfall Variation on the Dynamics of Successional Communities in Old-Field Succession of a Tropical Dry Forest." *PLoS One* 8, no. 12: e82040. <https://doi.org/10.1371/journal.pone.0082040>.
- McCulloch, L. A., D. Piotta, and S. Porder. 2021. "Drought and Soil Nutrients Effects on Symbiotic Nitrogen Fixation in Seedlings From Eight Neotropical Legume Species." *Biotropica* 53, no. 2: 703–713. <https://doi.org/10.1111/btp.12911>.
- McCulloch, L. A., and S. Porder. 2021. "Light Fuels While Nitrogen Suppresses Symbiotic Nitrogen Fixation Hotspots in Neotropical Canopy Gap Seedlings." *New Phytologist* 231, no. 5: 1734–1745. <https://doi.org/10.1111/nph.17519>.
- McLaren, K. P., and M. A. McDonald. 2003. "The Effects of Moisture and Shade on Seed Germination and Seedling Survival in a Tropical Dry Forest in Jamaica." *Forest Ecology and Management* 183, no. 1–3: 61–75. [https://doi.org/10.1016/S0378-1127\(03\)00100-2](https://doi.org/10.1016/S0378-1127(03)00100-2).
- Medvigy, D., G. Wang, Q. Zhu, et al. 2019. "Observed Variation in Soil Properties Can Drive Large Variation in Modelled Forest Functioning and Composition During Tropical Forest Secondary Succession." *New Phytologist* 223, no. 4: 1820–1833. <https://doi.org/10.1111/nph.15848>.
- Murphy, P. G., and A. E. Lugo. 1986. "Ecology of Tropical Dry Forest." *Annual Review of Ecology and Systematics* 17: 67–88.
- Nielsen, R. L., J. J. James, and R. E. Drenovsky. 2019. "Functional Traits Explain Variation in Chaparral Shrub Sensitivity to Altered Water and Nutrient Availability." *Frontiers in Plant Science* 10: 505. <https://doi.org/10.3389/fpls.2019.00505>.
- Paine, C. E. T., K. E. Harms, and J. Ramos. 2009. "Supplemental Irrigation Increases Seedling Performance and Diversity in a Tropical Forest." *Journal of Tropical Ecology* 25, no. 2: 171–180. <https://doi.org/10.1017/S0266467408005798>.
- Pasquini, S. C., and L. S. Santiago. 2012. "Nutrients Limit Photosynthesis in Seedlings of a Lowland Tropical Forest Tree Species." *Oecologia* 168, no. 2: 311–319. <https://doi.org/10.1007/s00442-011-2099-5>.
- Pellegrini, A. F. A., A. C. Staver, L. O. Hedin, T. Charles-Dominique, and A. Tourgee. 2016. "Aridity, Not Fire, Favors Nitrogen-Fixing Plants Across Tropical Savanna and Forest Biomes." *Ecology* 97, no. 9: 2177–2183. <https://doi.org/10.1002/ecs.1504>.
- Pineda-García, F., H. Paz, F. C. Meinzer, and G. Angeles. 2015. "Exploiting Water Versus Tolerating Drought: Water-Use Strategies of Trees in a Secondary Successional Tropical Dry Forest." *Tree Physiology* 35: tpv124. <https://doi.org/10.1093/treephys/tpv124>.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2021. "nlme: Linear and Nonlinear Mixed Effects Models." <https://CRAN.R-project.org/package=nlme>.
- Powers, J. S., and P. Tiffin. 2010. "Plant Functional Type Classifications in Tropical Dry Forests in Costa Rica: Leaf Habit Versus Taxonomic Approaches: Leaf Habit and Functional Traits of Dry Forest Trees." *Functional Ecology* 24, no. 4: 927–936. <https://doi.org/10.1111/j.1365-2435.2010.01701.x>.
- Powers, J. S., G. G. Vargas, T. J. Brodribb, et al. 2020. "A Catastrophic Tropical Drought Kills Hydraulically Vulnerable Tree Species." *Global Change Biology* 26, no. 5: 3122–3133. <https://doi.org/10.1111/gcb.15037>.
- R Core Team. 2022. "R: A Language and Environment for Statistical Computing." <https://www.R-project.org/>.
- Raven, J. A., L. L. Handley, and B. Wollenweber. 2004. "Plant Nutrition and Water Use Efficiency." In *Water Use Efficiency in Plant Biology*, 171–197. Blackwell and CRC Press.
- Ribbens, E., J. A. Silander, and S. W. Pacala. 1994. "Seedling Recruitment in Forests: Calibrating Models to Predict Patterns of Tree Seedling Dispersion." *Ecology* 75, no. 6: 1794–1806. <https://doi.org/10.2307/1939638>.
- Salinas-Peba, L., V. Parra-Tabla, J. Campo, and M. A. Munguia-Rosas. 2014. "Survival and Growth of Dominant Tree Seedlings in Seasonally Tropical Dry Forests of Yucatan: Site and Fertilization Effects." *Journal of Plant Ecology* 7, no. 5: 470–479. <https://doi.org/10.1093/jpe/rtt055>.
- Santiago, L. S. 2015. "Nutrient Limitation of Eco-Physiological Processes in Tropical Trees." *Trees* 29, no. 5: 1291–1300. <https://doi.org/10.1007/s00468-015-1260-x>.
- Sheffer, E., S. A. Batterman, S. A. Levin, and L. O. Hedin. 2015. "Biome-Scale Nitrogen Fixation Strategies Selected by Climatic Constraints on Nitrogen Cycle." *Nature Plants* 1, no. 12: 1–6. <https://doi.org/10.1038/nplants.2015.182>.
- Singh, J. S., A. S. Raghubanshi, R. S. Singh, and S. C. Srivastava. 1989. "Microbial Biomass Acts as a Source of Plant Nutrients in Dry Tropical Forest and Savanna." *Nature* 338, no. 6215: 499–500.
- Smith-Martin, C. M., M. G. Gei, E. Bergstrom, et al. 2017. "Effects of Soil Type and Light on Height Growth, Biomass Partitioning, and Nitrogen Dynamics on 22 Species of Tropical Dry Forest Tree Seedlings: Comparisons Between Legumes and Nonlegumes." *American Journal of Botany* 104, no. 3: 399–410. <https://doi.org/10.3732/ajb.1600276>.
- Steidinger, B. S., T. W. Crowther, J. Liang, et al. 2019. "Climatic Controls of Decomposition Drive the Global Biogeography of Forest-Tree Symbioses." *Nature* 569, no. 7756: 404–408. <https://doi.org/10.1038/s41586-019-1128-0>.
- Taylor, B. N., R. L. Chazdon, B. Bachelot, and D. N. L. Menge. 2017. "Nitrogen-Fixing Trees Inhibit Growth of Regenerating Costa Rican Rainforests." *Proceedings of the National Academy of Sciences* 114, no. 33: 8817–8822. <https://doi.org/10.1073/pnas.1707094114>.

- Taylor, B. N., R. L. Chazdon, and D. N. L. Menge. 2019. "Successional Dynamics of Nitrogen Fixation and Forest Growth in Regenerating Costa Rican Rainforests." *Ecology* 100, no. 4: e02637. <https://doi.org/10.1002/ecy.2637>.
- Taylor, B. N., and D. N. L. Menge. 2018. "Light Regulates Tropical Symbiotic Nitrogen Fixation More Strongly Than Soil Nitrogen." *Nature Plants* 4, no. 9: 655–661. <https://doi.org/10.1038/s41477-018-0231-9>.
- Thaxton, J. M., S. Cordell, R. J. Cabin, and D. R. Sandquist. 2012. "Non-Native Grass Removal and Shade Increase Soil Moisture and Seedling Performance During Hawaiian Dry Forest Restoration." *Restoration Ecology* 20, no. 4: 475–482. <https://doi.org/10.1111/j.1526-100X.2011.00793.x>.
- Thompson, W., L. Huang, and P. Kriedemann. 1992. "Photosynthetic Response to Light and Nutrients in Sun-Tolerant and Shade-Tolerant Rainforest Trees. II. Leaf gas Exchange and Component Processes of Photosynthesis." *Functional Plant Biology* 19, no. 1: 19. <https://doi.org/10.1071/PP9920019>.
- Toro, L., D. Pereira-Arias, D. Perez-Aviles, et al. 2023. "Phosphorus Limitation of Early Growth Differs Between Nitrogen-Fixing and Nonfixing Dry Tropical Forest Tree Species." *New Phytologist* 237, no. 3: 766–779. <https://doi.org/10.1111/nph.18612>.
- Uriarte, M., R. Muscarella, and J. K. Zimmerman. 2018. "Environmental Heterogeneity and Biotic Interactions Mediate Climate Impacts on Tropical Forest Regeneration." *Global Change Biology* 24, no. 2: e692–e704. <https://doi.org/10.1111/gcb.14000>.
- Valliyodan, B., H. Ye, L. Song, M. Murphy, J. G. Shannon, and H. T. Nguyen. 2016. "Genetic Diversity and Genomic Strategies for Improving Drought and Waterlogging Tolerance in Soybeans." *Journal of Experimental Botany* 67: erw433. <https://doi.org/10.1093/jxb/erw433>.
- Vargas G, G., D. Pérez-Aviles, N. Raczka, et al. 2023. "Throughfall Exclusion and Fertilization Effects on Tropical Dry Forest Tree Plantations, a Large-Scale Experiment." *Biogeosciences* 20, no. 11: 2143–2160.
- Vargas G, G., L. K. Werden, and J. S. Powers. 2015. "Explaining Legume Success in Tropical Dry Forests Based on Seed Germination Niches: A New Hypothesis." *Biotropica* 47, no. 3: 277–280. <https://doi.org/10.1111/btp.12210>.
- Waring, B. G., M. E. de Guzman, D. V. Du, et al. 2021. "Soil Biogeochemistry Across Central and South American Tropical Dry Forests." *Ecological Monographs* 91, no. 3: e01453. <https://doi.org/10.1002/ecm.1453>.
- Waring, B. G., D. Pérez-Aviles, J. G. Murray, and J. S. Powers. 2019. "Plant Community Responses to Stand-Level Nutrient Fertilization in a Secondary Tropical Dry Forest." *Ecology* 100, no. 6: e02691. <https://doi.org/10.1002/ecy.2691>.
- Werden, L. K., E. Calderón-Morales, J. P. Alvarado, L. M. Gutiérrez, D. A. Nedveck, and J. S. Powers. 2020. "Using Large-Scale Tropical Dry Forest Restoration to Test Successional Theory." *Ecological Applications* 30, no. 6: e02116. <https://doi.org/10.1002/eap.2116>.
- Wright, S. J. 2019. "Plant Responses to Nutrient Addition Experiments Conducted in Tropical Forests." *Ecological Monographs* 89, no. 4: 1382. <https://doi.org/10.1002/ecm.1382>.
- Wurzburger, N., and C. Ford Miniati. 2014. "Drought Enhances Symbiotic Dinitrogen Fixation and Competitive Ability of a Temperate Forest Tree." *Oecologia* 174, no. 4: 1117–1126. <https://doi.org/10.1007/s00442-013-2851-0>.
- Xu, X., D. Medvigy, J. S. Powers, J. M. Becknell, and K. Guan. 2016. "Diversity in Plant Hydraulic Traits Explains Seasonal and Inter-Annual Variations of Vegetation Dynamics in Seasonally Dry Tropical Forests." *New Phytologist* 212, no. 1: 80–95. <https://doi.org/10.1111/nph.14009>.
- Yang, X., W. M. Post, P. E. Thornton, and A. Jain. 2013. "The Distribution of Soil Phosphorus for Global Biogeochemical Modeling." *Biogeosciences* 10: 2525–2537. <https://doi.org/10.5194/bg-10-2525-2>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figures S1–S3:** btp70072-sup-0001-FigureS1-S3.docx.