

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 nonlegumes¹

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PREMISE OF THE STUDY: The seedling stage is particularly vulnerable to resource limitation, with potential consequences for community composition. We investigated how light and soil variation affected early growth, biomass partitioning, morphology, and physiology of 22 tree species common in tropical dry forest, including eight legumes. Our hypothesis was that legume seedlings are better at taking advantage of increased resource availability, which contributes to their successful regeneration in tropical dry forests.

METHODS: We grew seedlings in a full-factorial design under two light levels in two soil types that differed in nutrient concentrations and soil moisture. We measured height biweekly and, at final harvest, biomass partitioning, internode segments, leaf carbon, nitrogen, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$.

KEY RESULTS: Legumes initially grew taller and maintained that height advantage over time under all experimental conditions. Legumes also had the highest final total biomass and water-use efficiency in the high-light and high-resource soil. For nitrogen-fixing legumes, the amount of nitrogen derived from fixation was highest in the richer soil. Although seed mass tended to be larger in legumes, seed size alone did not account for all the differences between legumes and nonlegumes. Both belowground and aboveground resources were limiting to early seedling growth and function.

CONCLUSIONS: Legumes may have a different regeneration niche, in that they germinate rapidly and grow taller than other species immediately after germination, maximizing their performance when light and belowground resources are readily available, and potentially permitting them to take advantage of high light, nutrient, and water availability at the beginning of the wet season.

KEY WORDS legumes; light availability; nitrogen fixation; soil resources; tree seedlings

The seedling stage is a particularly critical life-history stage in which conditions of resource limitation may have important consequences for community composition if species vary in response to resource availability. In wet tropical forests, the vast majority of studies investigating the effects of resource availability on seedling

performance have focused on light as a critical resource, because as little as 0.5–5.0% of sunlight reaches the forest understory in closed canopies of wet tropical forests (Augspurger, 1984; Brokaw, 1985; Sanford, 1989; Chazdon and Pearcy, 1991; Hubbell et al., 1999; Nicotra and Chazdon, 1999; Montgomery and Chazdon, 2001). Many experimental studies have demonstrated interspecific trade-offs between rapid growth in high light conditions and survivorship under low light (Kitajima, 1994), and indeed this is a cornerstone of the theory of gap phase succession (Denslow, 1987). By contrast, tropical dry forest (TDF) is characterized by lower annual rainfall, a dry season lasting 3–8 mo, and many deciduous species (Murphy and Lugo, 1986); collectively, this suggests that patterns of understory light availability in TDF contrast strongly with those in wet forests (Parker et al., 2005). Several seedling studies in TDF have shown that light may not be as limiting a resource there as in wet

¹ Manuscript received 24 July 2016; revision accepted 27 February 2017.

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doi:10.3732/ajb.1600276

tropical forests (Gerhardt, 1996; Markesteijn et al., 2007; Chaturvedi et al., 2013). For example, Gerhardt (1996) found that increasing light levels during the wet season improved seedling growth, but that the higher light levels during the dry season increased desiccation, reducing seedling survival in some species. Thus, water may be a more limiting resource than light in controlling seedling growth and survivorship in TDF (Gerhardt, 1996; Chaturvedi et al., 2013).

Plants take up both water and nutrients from the soil. Recent studies have shown that tropical tree species may partition soil nutrient gradients as well as light gradients (John et al., 2007; Baribault et al., 2012; Condit et al., 2013) and that seedlings of most species respond to fertilization with increased growth (Lawrence, 2003). Some studies have shown that higher soil moisture and nutrient availability are more important in early than in later stages of growth (Breugel et al., 2011; Chaturvedi et al., 2013). Moreover, resources can interact such that the availability of nutrients modifies the responses to variation in another resource. For example, low soil nutrient availability may constrain seedlings' ability to respond to high light availability (Huante et al., 1998), but different species may vary in their responses to diverse combinations of resources. Ceccon et al. (2003) found that seedlings of different species responded distinctly to full-factorial fertilization with nitrogen (N) and phosphorus (P), depending on light availability and bulk density of the topsoil; some of the species responded positively to the nutrient addition, whereas in others this treatment had no effect or had a negative effect.

Although tropical forests are known for their high plant biodiversity, they are also remarkable for the large abundance and diversity of trees in the family Fabaceae (i.e., the legumes; Gentry, 1988). Legumes differ from most other plant families in their ability to host symbiotic N-fixing bacteria (Corby, 1988). Beyond N fixation, tropical legume trees may also have distinct suites of traits including high water-use efficiency, high foliar N and carbon (C), rapid germination rates, and high photosynthetic rates (Adams et al., 2010; Powers and Tiffin, 2010; Sui et al., 2011; Reyes-García et al., 2012; Vargas G. et al., 2015; Adams et al., 2016), leading some researchers to incorporate them as a separate plant functional type in ecosystem models (Wang et al., 2007). However, field experiments underscore that, even for the legume tree species within the TDF biome, there is a great deal of interspecific variation in functional traits and the responses of N fixation to variation in resources (Gei and Powers, 2015; Bhaskar et al., 2016).

Very few studies have worked with multiple species (>20) in the tropics to simultaneously evaluate the effects of aboveground and belowground resources on seedling performance. In the present study, we investigated how light and soil affect early growth, biomass partitioning, morphology, and physiology (including N fixation) of 22 common TDF tree species, including eight legumes, under common conditions in a shadehouse. We grew seedlings in a full-factorial design under two light conditions in two soil types that differed in nutrient concentrations and water holding capacity. The tree species chosen reflected the composition of the plant communities in our study area in the TDF of northwestern Costa Rica (Powers et al., 2009), with eight species in Fabaceae and 14 species belonging to 11 other families. This sampling allowed us to test the hypothesis that legume seedlings are better able to take advantage of increased resource availability, which contributes to their apparently successful regeneration niche in TDF compared to other taxa, as some studies have suggested (Vargas G. et al., 2015). We were

particularly interested in whether legumes differ in their responses to resource availability (i.e., behave as a distinct plant functional group) or whether large interspecific variation within the legume family prevents broad generalizations. In particular, if N fixation is strongly limited by energy availability (Vitousek and Howarth, 1991) or if it is up-regulated in N-poor soils (Barron et al., 2011), we would expect all N-fixing legumes to have their highest investment in nodules and highest reliance on N fixation under conditions of high light and low nutrient availability.

MATERIALS AND METHODS

Site description—The study was conducted in Sector Santa Elena (hereafter “Santa Elena”) of Área de Conservación Guanacaste, in northwestern Costa Rica. Santa Elena has a mean annual temperature of 25°C, mean annual precipitation of ~1765 mm (range: 880–3030 mm), and a 6 mo dry season on average (Gillespie et al., 2000; Powers et al., 2009; Powers and Perez-Aviles, 2013; Becknell and Powers, 2014). Soil, seeds, and seedlings for the experiment were collected both at Santa Elena and at Parque Nacional Palo Verde (hereafter “Palo Verde”), which is also located in northwestern Costa Rica (10.35°N, 85.35°W). Palo Verde has a mean annual temperature of 25°C, mean annual precipitation of ~1444 mm (range: 714–2130 mm), and a 5 mo dry season on average (Becknell and Powers, 2014). Both Santa Elena and Palo Verde are protected areas, predominantly covered by regenerating TDF, and span much of the extent of what was originally Costa Rica's TDF biome (Gillespie et al., 2000; Powers et al., 2009; Powers and Perez-Aviles, 2013).

Soils—Soils throughout the Costa Rican TDF vary in parent materials and weathering stage, and hence in physical properties and chemical composition (Leiva et al., 2009; Becknell and Powers, 2014). We collected soils from both Palo Verde and Santa Elena to examine effects of high vs. low belowground resource availability on seedling performance. We analyzed samples of these two soils to confirm nutrient concentrations. Although we do not have taxonomic descriptions of these soils, the soils from Santa Elena are likely Inceptisols, developed from volcanic ash deposits (Ulate, 2001). Based on their known occurrence and dark color, the soils from Palo Verde would likely be classified as Vertisols or Mollicsols. To increase soil aeration and drainage of the pot environment, we combined 1/3 parts sand to 2/3 parts sieved soil in ~4 L black plastic bags (about 25 × 30 cm). Soil characteristics were measured on unreplicated bulk samples collected at the time of planting. We measured clay, sand, and silt concentrations using the hydrometer method on soil type before and after we added sand (see Supplemental Data with this article; Appendix S1). We measured Bray extractable P by extracting samples of 3 g air-dried soil in 25 mL of a 0.03N NH_4F and 0.025N HCl solution. Soil extracts were analyzed for PO_4^{3-} with spectrophotometry following Lajtha et al. (1999). Total C and N were measured via dry combustion on a PDZ Europa ANCA-GSL elemental analyzer, and cation exchange capacity was quantified via the summation method at the Research Analytical Laboratory, University of Minnesota. To minimize the differences between microbial communities in the different soils, we combined equal weights of Santa Elena and Palo Verde soils in 1 L water, shook it, and added 10 mL of this solution to each seedling bag 2 wk before planting. Volumetric soil moisture was measured in each bag at 0–5 cm depth four times over the

experiment with a SM150 Soil Moisture Sensor (Delta-T Devices, Cambridge, UK). We acknowledge that soil moisture may not be the best comparative metric of soil water availability on soils that vary in texture. The Palo Verde soil (high-resource soil) had ~10× higher concentrations of exchangeable cations and extractable P than the Santa Elena soil (low-resource soil), although total N did not differ appreciably.

Light treatments—Plants were grown under two different light treatments in a shade house (about 7 × 4 m) in a full-factorial manipulation of soil type and light environment. One side of the shade house was covered with a standard shade cloth that transmitted 50% (high light) of photosynthetically active radiation compared to open sky (quantified with an MQ-100 Quantum sensor; Apogee, Logan, Utah, USA), and the other side had a shade cloth that allowed for ~25% (low light) of full sunlight. The low-light treatment falls in between the average below-canopy dry-season percentage of visible sky (33.6%) and wet-season values (13.4%) for successional forests in this region (G. Derroire, unpublished data).

Tree species, planting, and growing conditions—We selected 22 common species of trees present in Costa Rican TDF (Powers et al., 2009), including eight species of legumes and 14 other species from 11 families (Appendix S2). The legume species we used include the three legume subfamilies (Caesalpinioideae, Mimosoideae, Papilionoideae). Average seed mass was measured on three representative seeds per species after drying at ~60°C for >48 h. For very small seeds, between 5 and 10 seeds were weighed together, and thus average mass was calculated. We also compiled a seed mass database for 87 common woody species from the TDF of northwestern Costa Rica (Appendix S3).

Of the 22 species, 19 were grown from seeds, but for three species (*Bursera simaruba* [L.] Sarg., *Licania arborea* Seem., and *Spondias mombin* L.) we used seedlings ~3 wk old, collected from the forest and transplanted into bags. Some of the species (*Crescentia alata* Kunth, *Dalbergia retusa* Hemsl., *Enterolobium cyclocarpum* [Jacq.] Griseb., *Guazuma ulmifolia* Lam., *Hymenaea courbaril* L., and *Albizia saman* [Jacq.] F. Muell.) received a scarification treatment before they were planted, to accelerate germination, by placing them in 100°C water for 30 s and then in ice water for 1 min, and repeating this process three times. Then seeds from all species were soaked in water overnight, including seeds that had received the scarification treatment. Two seeds of each species were planted per bag, with eight replicate bags per species per treatment, for a total of 704 plants.

After planting, seedling position within each light treatment was randomized. One week after germination, seedlings were thinned to one per bag. Germination was recorded once a week for the first 2 wk and then every 2 wk after that. Because we obtained low germination rates for some species, more seeds were added to those bags after scarification. When rainfall was scarce (i.e., lack of rain during 48 h), seedlings were watered via an overhead sprinkler system every other day to prevent desiccation. The experiment was conducted during the wet season, so environmental conditions were relatively similar throughout the growing period.

Stem height and internodes—Stem height of each seedling was measured to the nearest 0.5 cm every ~2 wk throughout the experiment. Following the final harvest, we counted and measured all internode segments on each stem.

Harvests—One month after the first planting, we did an early harvest of three individuals of each species by treatment combination. Four months after the first planting, the remaining seedlings were harvested over a 3 d period. For each harvest, the aboveground portion of the seedlings was removed and divided into leaves and stems, then the bags were cut open and their contents were carefully washed to extract roots. In the case of nodulating legume species, for the final harvest nodules were separated from the roots. All leaves, nodules, roots, and stems of each seedling were dried at ~60°C for >48 h and weighed.

Relative growth rate—We used the difference in biomass between the first harvest and the final harvest to calculate relative growth rate (RGR, in grams per day) as follows: $RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$, where W_1 and W_2 are the average biomass from the initial and final harvest, respectively, per species per treatment, and $t_2 - t_1$ is the number of days between the initial and final harvest. Unfortunately, because seedlings did not all germinate at the same time, they were different ages when we did the initial and final harvests, which could have had an influence on growth rate; but even with this drawback, these results can still shed light on initial seedling performance.

Foliar elements and isotopes—After drying and weighing the leaves, one leaf (including the petiole) from each individual of all legumes—and, because of limited funds, a subset of the other species that grew for comparable periods of time (*Guazuma ulmifolia*, *Luehea candida*, *Pachira quinata* [Jacq.] W. S. Alverson, and *Tabebuia rosea* [Bertol.] DC.)—were dried at 65°C for 48 h and ground to a fine powder. Leaf C and N concentrations and their stable isotopes $\delta^{13}C$ and $\delta^{15}N$ were measured on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon, Cheshire, UK) at the Stable Isotope Facility at the University of California, Davis.

Estimation of nitrogen fixation—Once seed reserves are exhausted, the N in legume seedlings comes from two main sources, uptake of mineral N from the soil or N fixation, or perhaps in some cases through ectomycorrhizal fungi as some legumes associate with them (Frioni et al., 1999). To estimate the percentage of plant N derived from fixation (%Ndfa), we used a simple mixing model based on stable N isotope signatures (i.e., $\delta^{15}N$ abundances) as follows (Shearer and Kohl, 1986): $\%Ndfa = (\delta^{15}N_{reference} - \delta^{15}N_{N_2-fixing}) / (\delta^{15}N_{reference} - B) \times 100$, where $\delta^{15}N_{reference}$ represents the mean value of the $\delta^{15}N$ of nonfixing reference species grown in the same soil type and light environment as the N_2 -fixing species, $\delta^{15}N_{N_2-fixing}$ is the ^{15}N abundance for each N_2 -fixing legume, and B is the average $\delta^{15}N$ for each species grown in sand (i.e., when plants are relying only on N_2 fixation to meet all N requirements). This model takes advantage of the difference in N isotopic composition of fixed N coming from air, and assumes that the $\delta^{15}N$ composition of reference plants reflects that of soil mineral sources. We used this equation to estimate %Ndfa of the six potential N_2 -fixing legume species. The two nonfixing legume species, *Caesalpinia eriostachys* Benth. and *Hymenaea courbaril*, were used as reference plants. The average difference in $\delta^{15}N$ abundance between N derived from the soil (as indexed by the reference plants) and that of the air (which, by convention, equals 0‰) for our data was 2.95‰, indicating the suitability of this method to our data. The B value of four of the N_2 -fixing legumes (*Dalbergia retusa*, *Enterolobium cyclocarpum*,

Gliricidia sepium (Jacq.) Kunth ex Walp., *Lysiloma divaricatum* Benth.) was calculated from seedlings of the same species grown in pure sand in a previous study (M. G. Gei et al., unpublished data). For *Lonchocarpus felipei* N. Zamora and *Albizia saman*, we used the B value of the most closely related legume for which we knew its ^{15}N abundance in sand (*Dalbergia retusa* and *Enterolobium cyclocarpum*, respectively).

Data analysis—We used t -tests to compare average seed mass for the 22 species used in the experiment and for the 87 common TDF species from our seed mass database. We log transformed seed mass in both instances to account for the fact that there were orders of magnitude of difference in the data. Because seed mass and legume vs. nonlegume status (hereafter “group”) were key variables driving the underlying performance of seedlings, independent of the applied treatments, we conducted analysis of covariance (ANCOVA). We ran the ANCOVAs with group and seed mass and the interaction between these two variables as the explanatory variables and initial height, height at a common age, the total biomass at the time of the final harvest, and the relative growth rate of biomass as the response variables. We log transformed seed mass in all the analyses in which it was included. Because seeds germinated at different times (or were planted as ~3 wk old seedlings), we compared seedling height among individuals after approximately the same number of weeks of growth (10–11 wk). These analyses were done in R version 3.3.1.

Our main goal was to determine whether legumes responded differently to the two light and soil treatments compared to the other species. The responses in which we were interested included initial height of the seedlings after 2 wk postgermination, height at a common age (after about 10–11 wk of growth, hereafter “final height”), stem internode length and number, leaf mass fraction (LMF), stem mass fraction (SMF), root mass fraction (RMF), nodule mass fraction (NMF), total biomass (biomass), $\delta^{13}\text{C}$ (as an integrated index of water-use efficiency), total foliar N (total N), concentration of foliar N (%N), and relative daily growth rate of biomass (RGR) between the initial to the final harvest. Response variables of interest were analyzed using a mixed model, with light environment, soil type, group, and seed mass as the main, fixed effects and species as a random effect nested within group. We included species as a random variable in our model to account for the differences among species. These analyses were conducted using the nlme R package. We log-transformed the following variables to meet assumptions of normally distributed residuals: final height, LMF, SMF, RMF, stem internode length, and %N. To understand how NMF and %Ndfa varied under different resource conditions for the N-fixing legumes, we used a full factorial analysis of variance with species, light level, and soil type as main effects and NMF and %Ndfa as response variables. In this case we included species as one of the main effects because we wanted to determine whether all legumes responded to the treatments in a similar fashion. We conducted Tukey’s HSD post hoc analyses for means separation among legume species to understand %Ndfa response to treatment combination. These analyses were done in R 3.2.1.

RESULTS

Soil variation—Even though seedlings were watered frequently, there were large and persistent differences in soil moisture between

soil types over the experiment that are likely due to the higher clay concentration in the high-resource soil (Appendices S1 and S4). Soil moisture in the high-resource soil averaged 24.8% water (range: 12.8–38.9%), whereas in the low-resource soil the average was 12.7% water (range: 6.1–28.5%). All measures of nutrient availability (e.g., Bray P, CEC, etc.) were higher in the Palo Verde soil (hereafter “high-nutrient soil”) than in the Santa Elena soil (hereafter “low-nutrient soil”; Appendix S1).

Seed mass and height growth—Seed mass varied among species for the 19 species grown from seed; however, on average, it was over one-third higher in legumes than in nonlegumes (0.72 g vs. 0.45 g), but this was only marginally statistically significant ($T = 1.861$, $df = 18$, $P = 0.08$; Appendices S5, S6, and S7). Furthermore, when we compared legume and nonlegume seed mass for 87 common dry-forest species we found the same trend. Legume seed mass was approximately one-third higher than that of nonlegumes (0.28 g vs. 0.20 g) and was marginally statistically significant ($T = 1.928$, $df = 86$, $P = 0.06$; Appendixes S3 and S7). Following germination for the species grown from seed, legumes grew more rapidly than nonlegumes ($T = 5.1032$, $df = 252$, $P < 0.001$): mean height of legumes at first measurement was 12.6 cm (range: 4.0–33.5 cm), whereas the mean for nonlegumes was 7.6 cm (range: 1.0–25.0 cm) (Appendix S6). Initial height differed between legumes and nonlegumes ($F_{1,213} = 46.70$, $P < 0.001$), but part of this was explained by seed mass ($F_{1,213} = 269.51$, $P < 0.001$); however, the significant interaction between group and seed mass ($F_{1,213} = 60.12$, $P < 0.001$) indicates that seed mass affected initial height in a different manner for legumes vs. nonlegumes. In general, species with larger seed mass were taller than species with smaller seed mass (Appendix S8), underscoring the importance of seed size for initial height growth.

When final height was analyzed, the main effects of light level and soil type and the two-way interaction between group by light level and seed mass by soil type affected seedling height, and the four-way interaction between all the fixed effects was marginally significant (Table 1), where the tallest seedlings were the legumes in the low light and high nutrient level, suggesting that legumes’ initial height advantage was maintained through time. This pattern was clear in the biweekly height measurements, in which legumes were consistently taller than nonlegumes (Fig. 1). Furthermore, when comparing only the effect of seed mass ($F_{1,361} = 81.78$, $P < 0.001$) and soil type ($F_{1,361} = 8.03$, $P < 0.01$) on final height, both of these variables affected seedling growth, but not the interaction between these two variables. However, even though the interaction between soil type and seed mass was not statistically significant, the seedlings with larger seeds seemed to be less affected by soil type, whereas seedlings with smaller seed mass grew taller in the high-nutrient soil than in the low-nutrient soil (Appendix S9). When we examined the effect of only seed mass and group on final height, we found a significant effect of group ($F_{1,361} = 35.21$, $P < 0.001$) and of seed mass ($F_{1,361} = 19.36$, $P < 0.001$), but not of the interaction between these two variables (Appendix S10). Seedlings with larger seed masses continued to be taller after 10–11 wk of growth, but legumes were taller than nonlegumes on average.

Total biomass and relative growth rate—Group, soil type, light level, seed mass, the two-way interactions group \times soil type and soil type \times seed mass, and the three-way interactions group \times soil type

TABLE 1. F-ratios from a mixed model with species as a random effect and legume or nonlegume status (group), light level (light), soil type treatment (soil), and seed mass (seed) as fixed effects.

Variable	Group	Soil	Light	Seed	Group × soil	Group × light	Group × seed	Soil × light	Soil × seed	Light × seed	Group × soil × light	Group × soil × seed	Group × light × seed	Soil × light × seed	Soil × seed	Light × seed	Group × soil × light × seed	Denominator df
Final height	2.435	4.115*	16.229***	0.005	1.348	6.322*	0.051	0.356\$	22.621***	0.015	2.333	3.224\$	2.822\$	0.084	0.84	0.084	2.880\$	334, 15
Biomass	10.206**	16.667***	5.489*	4.640*	5.186*	0.001	0.262	2.638	22.436***	3.229\$	0.002	14.500***	3.832\$	4.403*	4.403*	3.585\$	334, 15	
LMF	0.047	0.176	10.898**	0.879	3.579\$	2.252	0.000	1.250	5.860*	0.084	1.114	2.453	0.972	0.220	0.220	0.021	334, 15	
SMF	5.687*	0.049	4.031*	0.002	1.702	2.485	0.054	0.801	4.001*	0.235	0.153	0.003	0.637	0.027	0.027	0.135	334, 15	
RMF	3.068	0.294	14.887***	0.535	2.125	0.768	0.229	1.125	0.588	0.096	0.931	0.557	0.076	0.488	0.488	0.104	334, 15	
Internode number	0.150	0.371	1.252	4.252\$	0.476	0.374	8.772**	4.199\$	23.820***	2.791	0.676	11.482***	0.177	5.798*	5.798*	0.032	334, 15	
Internode length	1.960	4.650*	31.436***	0.242	0.0243	6.537*	2.357	0.000	3.839*	0.230	0.324	2.510	2.787	0.950	0.950	0.555	331, 15	
δ ¹³ C	0.113	6.272*	14.574***	0.171	3.331\$	1.558	0.067	0.573	0.337	1.214	0.007	0.737	0.469	0.480	0.480	0.001	201, 8	
%N	0.499	7.228**	23.508***	1.301	1.254	0.002	0.140	0.023	1.818	3.526\$	0.029	3.749\$	0.197	0.005	0.005	0.016	201, 8	
Total N	0.544	25.525***	6.818**	2.373	1.216	0.228	0.465	0.333	6.053*	1.043	0.154	0.300	0.220	0.400	0.400	0.249	201, 8	
RGR	0.782	0.003	0.270	4.850*	0.243	0.089	0.014	0.085	0.080	0.717	0.002	0.123	1.641	1.279	1.279	0.697	38, 13	
Numerator df	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	

Notes: Significance: \$ P < 0.10; * P < 0.05; ** P < 0.01; and *** P < 0.001. The first denominator df is for all the variables except group and seed mass, and the second one is for group and seed mass. LMF = leaf mass fraction, SMF = stem mass fraction, RMF = root mass fraction, N = nitrogen, RGR = relative growth rate (grams per day).

× seed mass and soil type × light level × seed mass caused a significant difference in average total biomass at final harvest. The seedlings grown in high-light and high-nutrient soil had larger final biomass, and the legumes had the highest biomass (mean = 9.81 g, range: 3.40–23.40 g), whereas nonlegumes in the low-light and low-nutrient soil had the lowest biomass (mean = 1.41 g, range: 0.04–3.36 g; Table 1). When we examined the effect of only seed mass and group on total biomass, group caused the greatest difference ($F_{1,361} = 48.48, P < 0.001$). However, seed mass ($F_{1,361} = 5.93, P < 0.05$) and the interaction between seed mass and group also caused a significant difference ($F_{1,361} = 4.02, P < 0.05$). Legumes had higher overall biomass than the nonlegumes; however, the legumes with smaller seeds had slightly higher biomass than the legumes with larger seeds, whereas nonlegume biomass was the same independent of seed mass (Appendix S11).

In the full factorial mixed model, only seed mass caused a significant effect on RGR (Table 1). Furthermore, when we compared RGR between seed mass and group we obtained the same results: only seed mass ($F_{1,63} = 18.13, P < 0.001$) had a significant effect on daily plant biomass increments, although group was marginally significant ($F_{1,63} = 3.40, P = 0.069$). Between the initial and final harvest, the seedlings with smaller seed masses had higher RGRs, and legumes, on average, had slightly higher values than nonlegumes (Appendix S12).

Biomass partitioning and morphology—Biomass partitioning (i.e., allocation of photosynthate to the structures of roots, stems, and leaves) for the final harvest was most strongly affected by light (Table 1), whereas group also had an effect in SMF, and LMF and SMF were affected by the interaction between seed mass and soil type. When we compared allocation between the initial and final harvests, in general, allocation to leaves increased under low light availability, whereas allocation to roots increased under high light conditions and to stems in legumes (Fig. 2). Also between the initial and final harvests, legumes initially allocated more to leaves and nonlegumes to roots, but by the final harvest nonlegumes had larger LMF and RMF on average (Appendix S13). Legumes had larger stem mass fractions both in the initial and the final harvest, consistent with their greater heights. The three-way interactions group × soil type × seed mass and soil type × light level × seed mass had a significant effect on the number of internodes in seedling stems, but in general the highest number of internodes was found in legumes grown in high-nutrient soil (Table 1; Appendix S14). Light level, soil type, the interaction between group and light level, and the interaction between soil type and seed mass also affected the length of the internodes. In general, seedlings with larger seed mass had longer internodes and were also taller. The seedlings with the longest internodes were legumes grown in low light in high-nutrient soil (Appendix S14 and Table 1).

Leaf water-use efficiency and nitrogen concentration—For a subset of species, water-use efficiency (WUE), as indexed by δ¹³C, differed significantly between light and soil conditions, with a significant interaction between soil type and group (Table 1 and Fig. 3). In general, seedlings growing in low light had more negative δ¹³C, and the lowest value of δ¹³C was in nonlegumes grown in high-nutrient soil in low light (mean = −31.16‰, range: −29.70‰ to −32.74‰). By contrast, legumes grown in high-resource soil in high light had the least negative δ¹³C values (mean = −29.33, range:

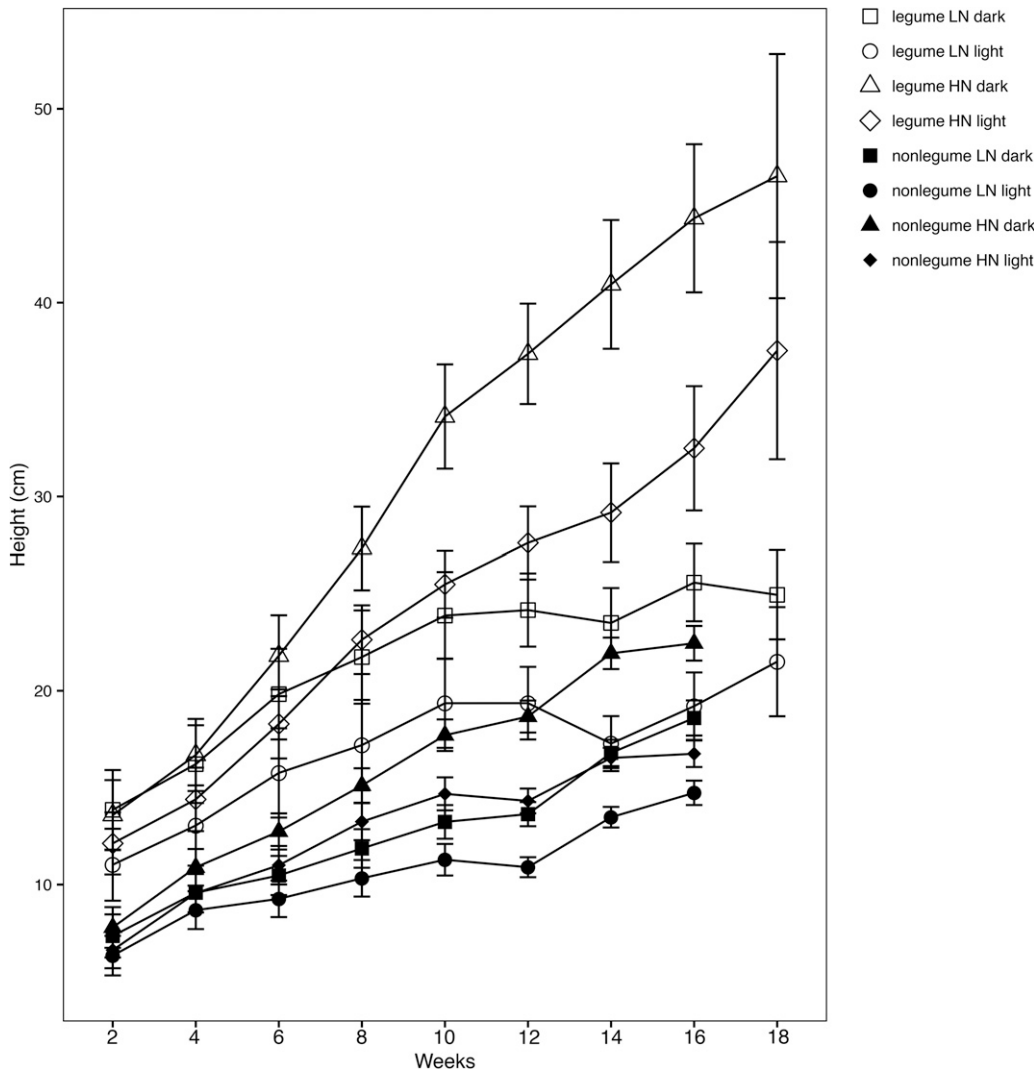


FIGURE 1 Mean height, measured every 2 wk, of seedlings of legume and nonlegume species growing in low-nutrient (LN) or high-nutrient (HN) soil and in 50% full sun (light) or 25% full sun (dark). Error bars indicate SE.

–27.13‰ to –31.39‰). In other words, while low light consistently decreased WUE across all taxa, the effects of soil resources differed for legumes compared to nonlegumes: WUE decreased with low soil resources in the legume taxa, but increased in the same soil for the nonlegumes.

Leaf N concentrations varied by a factor of 6 and depended on light and soil type (Table 1 and Fig. 3). In general, seedlings growing in lower light and in low-nutrient soil had higher percent foliar N (mean = 2.97%, range: 1.64–6.86%; Fig. 3). Seedlings with the lowest percent foliar N were the nonlegumes grown in high-nutrient soil in high light (mean = 1.52%, range: 1.09–2.10%). Total foliar N (i.e., foliar N concentration multiplied by leaf mass) varied by a factor of 8 and followed a similar trend as %N, with soil type and light level both affecting leaf total N; Table 1). The interaction between soil type and seed mass also had a lower, but significant, effect on total N (Table 1). Legumes grown in the high-nutrient soil had much higher values than the legumes grown in low-nutrient soils (Fig. 3). By contrast, there were no significant treatment effects of light or soil resources on total foliar N in the nonlegumes (Fig. 3). Light conditions were less important, but legumes in low light had

more total foliar N than those in high light (mean = 0.08, range: 0.02–0.19 g plant⁻¹; and mean = 0.07, range: 0.02–0.13 g plant⁻¹, respectively; Table 1 and Fig. 3).

Nodule mass fraction and nitrogen derived from fixation—On average, nodules comprised ~2% of the total biomass of N-fixing legumes (Appendix S5) and differed among species and soils, but not among light treatments (Appendix S12 and Table 2). Based on the ¹⁵N natural abundance method, seedlings acquired 7.6–100% of their N from fixation and the average value of Ndfa was 72.75% (Appendix S5). Furthermore, like NMF, Ndfa also differed among species and soils (Table 2; Appendix S15), and species responded differently to both soil type and light level. Percentage of N derived from fixation for *D. retusa*, *E. cyclocarpum*, *L. felipei*, and *L. divaricatum* was affected by soil type, but for *A. saman* light level instead of soil had a significant effect, and for *G. sepium* there was no significant effect of soil or light on %Ndfa (Fig. 4 and Table 2). Ndfa was consistently higher under high fertility and soil moisture, suggesting that belowground resources limited N fixation more than light in young seedlings (Fig. 4). The correspondence between NMF and Ndfa

was low. For example, on average, *L. felipei* had the highest NMF (0.024 g g⁻¹) and had the second-highest percentage of N derived from fixation (84.0%), but *G. sepium* had the second-lowest NMF (0.006 g g⁻¹) and the highest Ndfa (92.0%). By contrast, *L. divaricatum* had both the lowest average NMF (0.002 g g⁻¹) and the lowest Ndfa (53.0%).

DISCUSSION

Resource availability at the seedling stage is particularly important for determining seedling survival, and how different groups of plants respond to resources can constrain stand-level forest composition. Our study examined how variation in aboveground and belowground resources affected early seedling growth and performance for a large number of TDF tree species, with a special focus on comparing legumes, the most abundant and diverse family in the study region, to other taxa. Our hypothesis was that legume seedlings are better at taking advantage of increased resource availability, which contributes to their apparently successful regeneration

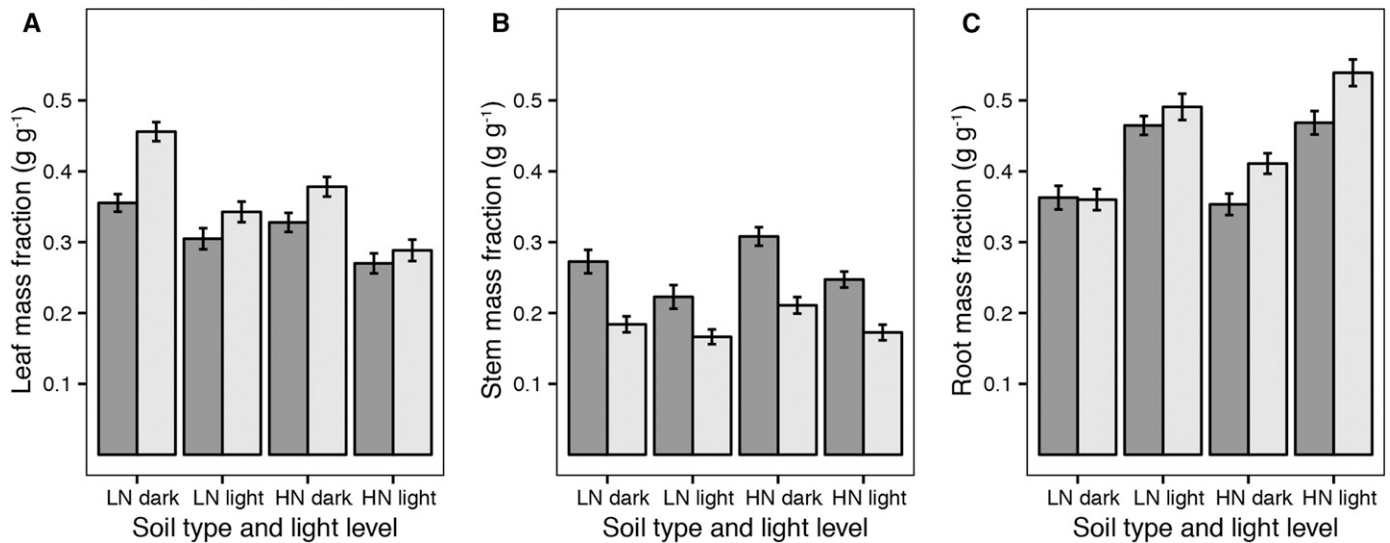


FIGURE 2 Mass fractions at the time of harvest of eight legumes species (dark gray) and 14 nonlegume species (light gray) grown in treatment combinations of low-nutrient (LN) or high-nutrient (HN) soil and 50% full sun (light) or 25% full sun (dark). (A) Leaf mass fraction was significantly affected by light level ($F_{1,334} = 10.90, P < 0.01$). (B) Stem mass fraction was significantly different between legumes and nonlegumes ($F_{1,15} = 5.69, P < 0.05$) and between light level levels ($F_{1,334} = 4.03, P < 0.05$). (C) Root mass fraction was significantly affected by light level ($F_{1,334} = 14.89, P < 0.05$). Error bars indicate SE.

niche in TDF compared to other taxa. We found that legumes had larger seed mass, grew faster immediately after germination, and maintained this initial height advantage over species from other families. Below, we discuss (1) how seed mass affects initial seedling performance, (2) whether variation in aboveground or belowground resources drives early seedling performance, (3) whether legumes have a distinct regeneration niche compared to diverse nonlegume taxa, and (4) environmental controls on symbiotic N fixation.

One of our objectives was to determine whether legume tree species behave as a distinct functional group (as used in ecosystem modeling; Bhaskar et al., 2016). Some authors have suggested that TDF legumes have a distinct regeneration niche compared to other tree species because they germinate more quickly than nonlegumes (Vargas G. et al., 2015). In our study, legumes had higher seed mass and faster postgermination growth rates, gaining a significant height advantage over nonlegume species during the first 2 wk of growth. Legumes had similar root and leaf mass fraction compared to other species, but differed in having greater stem mass fraction as a result of their greater height. Larger seed mass may have provided legumes with sufficient resources to grow more quickly immediately after germination (Slot et al., 2012). However, legume growth rates decelerated after 2 wk. Independent of whether a seedling was a legume or not, seed mass played an important role in initial seedling performance. Seedlings with larger seeds had greater initial and final heights; however, in general, seed mass was not as relevant for total final biomass, and this could have been influenced by the fact that seedlings with smaller seeds had higher RGR. Larger seed mass may play an important role in the initial establishment of seedlings (Foster, 1986; Jurado and Westoby, 1992), in particular if the growth period is limited to often unpredictable rainfall patterns, which is the case in our study site. Similar trends have been found in other studies, in which species with larger seeds were more successful in early stages of establishment in adverse environments

(Moles and Westoby, 2004) and in environments that experience a dry season (Dalling and Hubbell, 2002). Additionally, our findings that smaller-seeded species had higher RGR also coincides with previous studies' findings that species with smaller seeds have higher RGR than species with larger seeds (Fenner, 1983; Shipley and Peters, 1990).

By contrast, independent of seed mass, nonlegume species did not grow as fast initially but maintained a steadier increase in height over time. Similar trends have been found in other species. For example, Jurado and Westoby (1992) found that arid species with larger seeds had larger seedlings 10 d after germination in relation to species with smaller seeds; however, seed mass alone does not explain our results, because legumes responded differently depending on resource availability. Collectively, our findings that legumes grow quickly after germination, coupled with faster germination rates in relation to other TDF species (Vargas G. et al., 2015), suggest a possible mechanism to explain their high abundance in our study site, where legumes represent 18% of the stems and basal area (Powers et al., 2009). This rapid germination and initial growth may allow legumes to take advantage of resources quickly and to grow for longer periods during the wet season. This may give legumes a competitive advantage over other species in highly seasonal environments such as TDF, where water and nutrients are often limiting and the majority of annual germination and growth occurs during the wet months (Ceccon et al., 2006), potentially increasing legume survival at the seedling stage, similar to the findings of Menge and Chazdon (2016) in a tropical wet forest. Although we only grew the seedlings for a short period and are unable to extrapolate our results to the sapling and adult stages, distinct regeneration niches are just one possible explanation for legume success in TDF, given that other studies have suggested that adult trees of legumes in these forests have distinct traits beyond high foliar N, such as high WUE (Powers and Tiffin, 2010). Similarly, in a TDF in Mexico,

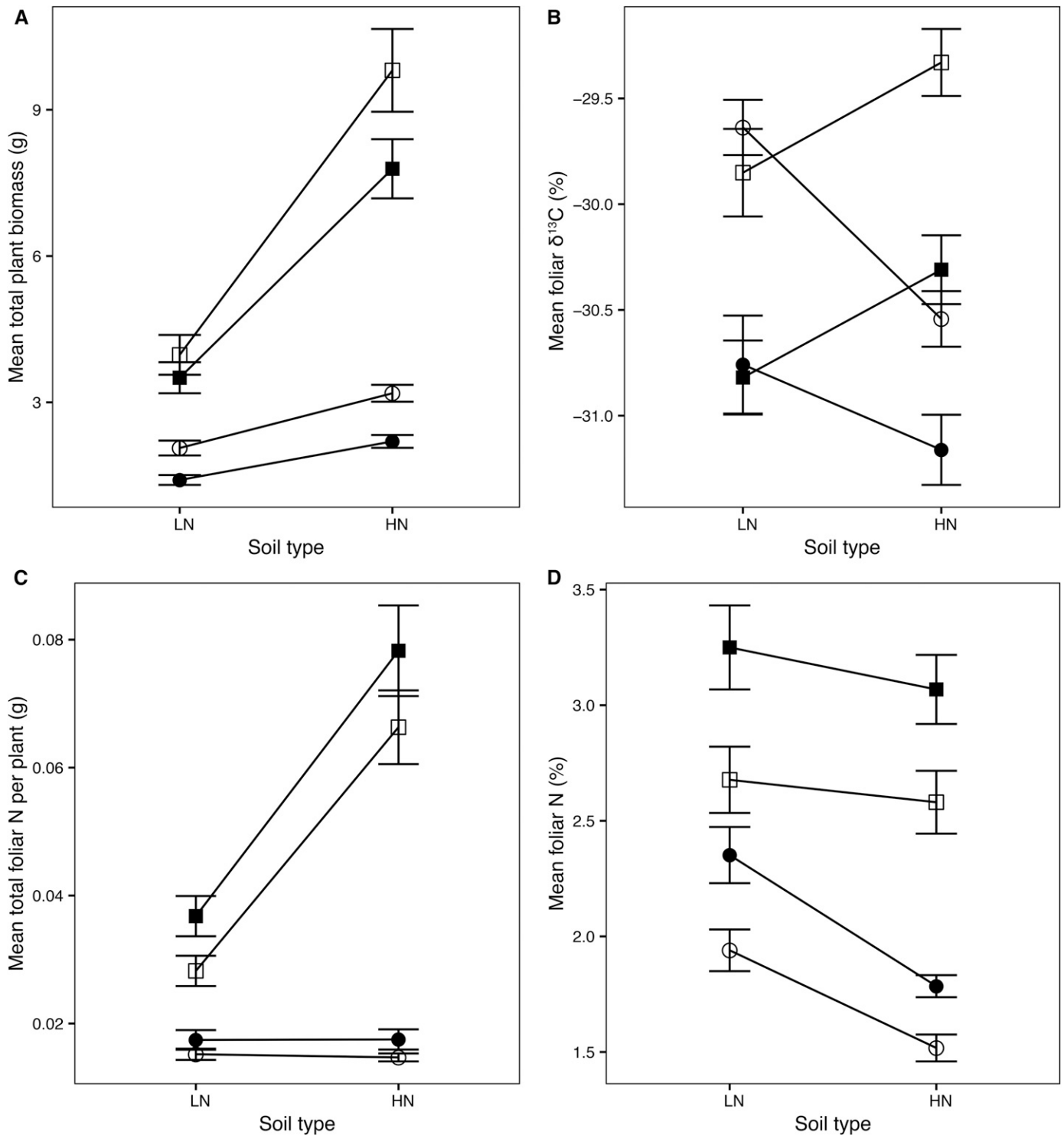


FIGURE 3 (A) Mean total biomass at the time of harvest of eight legume species (squares) and 14 nonlegume species (circles) grown in treatment combinations of low-nutrient (LN) or high-nutrient (HN) soil and 50% full sun (open symbols) or 25% full sun (filled symbols). There were significant effects of legume vs. nonlegume status ($F_{1,15} = 10.21, P < 0.001$), light level ($F_{1,15} = 5.49, P < 0.001$), nutrient level ($F_{1,15} = 16.69, P < 0.001$), and legume vs. nonlegume \times nutrient level interaction ($F_{1,15} = 5.19, P < 0.001$). (B) Mean foliar $\delta^{13}\text{C}$ at the time of harvest of a subset of species ($n = 12$). There were significant effects of light level ($F_{1,201} = 14.57, P < 0.001$) and nutrient level ($F_{1,201} = 6.27, P < 0.001$). (C) Mean total foliar N at the time of harvest of a subset of species. There were significant effects of light level ($F_{1,201} = 6.81, P < 0.01$) and nutrient level ($F_{1,201} = 25.53, P < 0.001$). (D) Mean foliar nitrogen at the time of harvest of a subset of species. There were significant effects of light level ($F_{1,201} = 23.51, P < 0.001$) and nutrient level ($F_{1,201} = 7.23, P < 0.01$). Error bars indicate SE.

TABLE 2. *F*-ratios from a linear model for nitrogen-fixing legume species with species ($n = 22$), light level (light), and soil type treatment (soil) as fixed effects.

Variable	Species	Light	Soil	Species × light	Species × soil	Light × soil	Species × light × soil	Denominator df
NMF	15.3920***	0.4259	7.881**	1.799	2.450*	4.583*	0.450	84
Ndfa	18.604***	1.206	116.132***	5.714***	4.893***	2.979	2.579	87
Numerator df	5	1	1	5	5	1	5	

Notes: Significance: \$ $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. NMF = nodule mass fraction, Ndfa = nitrogen derived from fixation.

Pineda-García et al. (2015) found that N-fixing legume tree seedlings were not only able to maintain leaf function under drought, but were also able to sustain high growth rates when water was readily available.

For many of the response variables we measured, interactions between group (legume vs. nonlegume status) and light or between group and soil type were significant, suggesting that legume responses to variation in resource availability differ from those of nonlegume tree species. In general, all species in our study had higher WUE as indexed by $\delta^{13}\text{C}$ in the high light level, presumably because higher light permitted increased photosynthesis rates in relation to water loss. By contrast, legumes growing in the high-resource soil had higher WUE and much higher biomass than legumes growing in the low-resource soil, whereas nonlegumes used water more efficiently in the poor soil compared to legumes. One possible explanation for higher WUE efficiency and biomass in legumes in nutrient-rich (and presumably wetter) soils is that legumes are better able to take advantage of additional belowground resources compared to other taxa. Consistent with this explanation, all N-fixing legumes up-regulated fixation in the high-resource soil, which suggests that this process was limited by soil nutrients and/or water, and that legumes use additional nutrients like P to maximize N acquisition and growth. This translated into higher biomass production (by almost threefold) in high-resource soil compared to low-resource soil, and apparently higher WUE under conditions of high aboveground and belowground resources. Also, even though the nonlegumes had higher WUE in the low-nutrient, low-moisture soil, final seedling biomass was not dramatically different between the two soil types.

All of our results are consistent with a growing body of work that suggests that legumes have different functional trait values and trait correlations compared to nonlegume taxa (Powers and Tiffin, 2010; Reyes-García et al., 2012; Adams et al., 2016; Menge and Chazdon, 2016). It is firmly established that legumes have higher foliar N concentrations (Powers and Tiffin, 2010; Adams et al., 2016), but at the same time they have higher WUE (Reyes-García et al., 2012; Adams et al., 2016) and growth under drought (Pineda-García et al., 2015). In our experiment, foliar N concentrations were affected by both light and soil (Table 1 and Fig. 3), but the only interactions that were significant with legume vs. nonlegume status involved soil type. Higher WUE when belowground resources are more abundant and higher photosynthesis rates may give legume seedlings an advantage over nonlegumes during the wet season in environments where water availability varies strongly seasonally and where water is often a limited resource even during the wet season. Consistent with this, in a recent meta-analysis, Adams et al. (2016) found that legumes had higher foliar N and WUE across a diversity of arid environments, indicating a general trend of increased performance of legumes in environments with limited water. Pellegrini et al. (2016) also found that N-fixing legumes were more abundant in arid tropical environments than in wetter ones, further supporting

the hypothesis that legumes have a competitive advantage in drier environments.

In addition to allowing us to compare a large number of legume species to nonlegumes, our study investigated how symbiotic N fixation varied in response to resources and whether all N-fixing legume taxa responded in similar or individualistic ways. Both nodule mass fraction and the percentage of N derived from fixation (Ndfa) varied considerably among species. While several of the resource × species interactions were significant, all species growing in high-resource soils consistently fixed more N than individuals in low-resource soils, and there was no evidence that N fixation differed by light treatment (Table 2). Together, these results suggest that (1) N fixation in TDF seedlings seems to not be constrained by light limitation; (2) despite higher soil water and nutrients in the high-resource soil treatment, availability of mineral N in either soil was not sufficient to down-regulate fixation; and (3), water and/or nutrients like P or micronutrients like molybdenum might limit N fixation in this landscape, as found elsewhere (Barron et al., 2008; Batterman et al., 2013). Thus, even though there is broad variation in Ndfa and allocation to nodules among the N-fixing legume species, as well as variation in functional traits such as wood density (Powers and Tiffin, 2010), legume species respond to changes in resources in remarkably similar ways.

CONCLUSIONS

Our results show that seed mass is an important factor in initial seedling growth and suggest that species with larger seed mass will initially grow taller following germination, but that species with smaller seeds will later have higher relative growth rates. Our results also show that both belowground and aboveground resources were limiting to early growth and function of tree seedlings in this TDF and that species differed in their responses to resource availability. In this forest, legumes have a different regeneration niche than taxa in other families, germinating rapidly and growing taller than other species immediately after germination. Higher seed mass in legumes only partially explains these results. Legumes are capable of maximizing performance when light and belowground resources are readily available, which could permit them to take advantage of higher levels of light, nutrients, and water available at the beginning of the wet season in a tropical dry forest.

ACKNOWLEDGEMENTS

The authors thank R. Montgomery, D. Moeller, and P. Kennedy for comments that improved the manuscript. We also gratefully acknowledge excellent assistance from D. Perez-Aviles in the field, logistical assistance from R. Blanco and M. M. Chavarria of the Area de Conservación Guanacaste, and financial support from U.S.

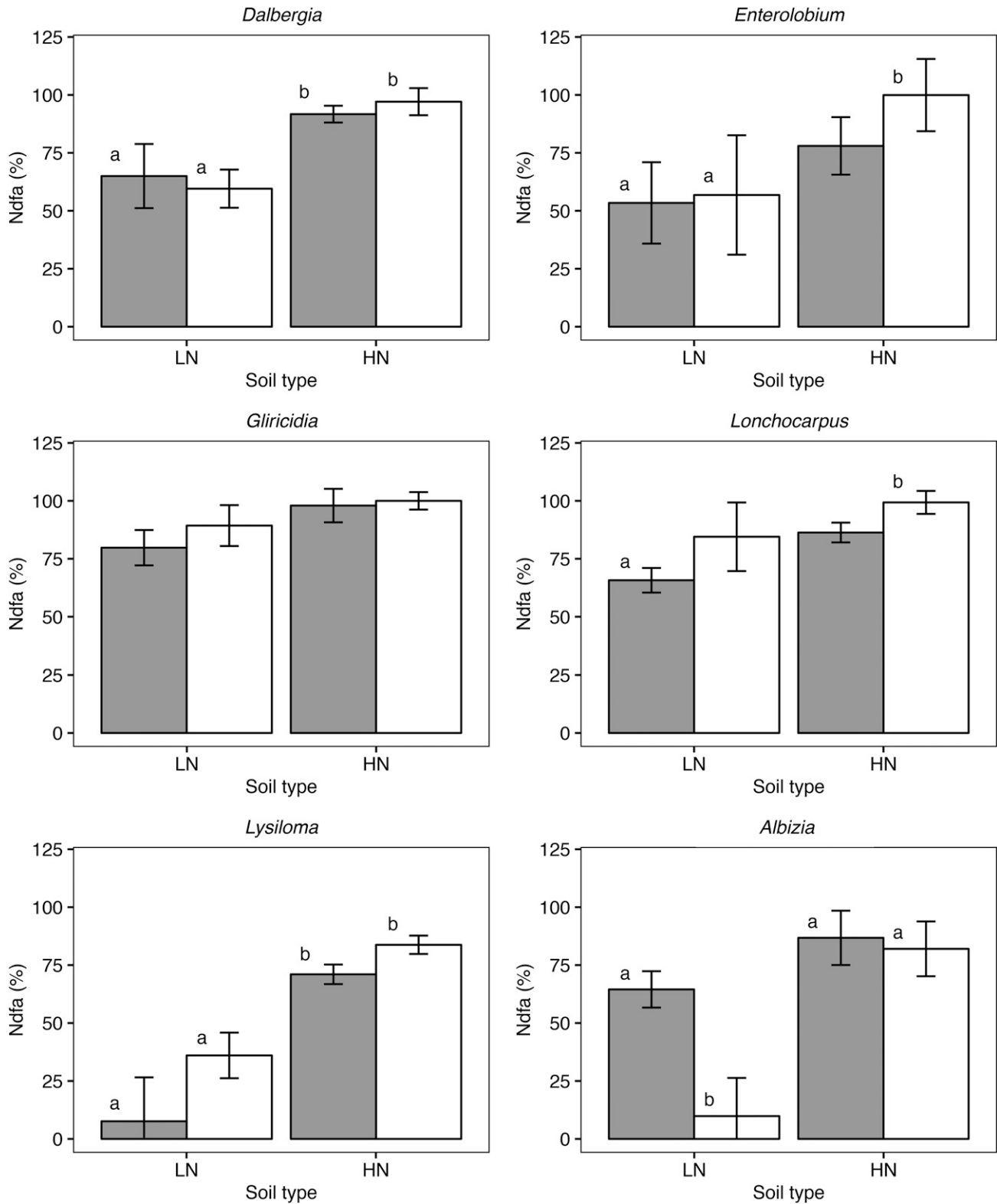


FIGURE 4 Estimates of the percentage of plant nitrogen derived from the air (Ndfa) at the time of harvest for six nitrogen-fixing legume species grown in treatment combinations of low-nutrient (LN) or high-nutrient (HN) soil and 25% full sun (gray) or 50% full sun (white). There were significant effects of species ($F_{5,84} = 18.60, P < 0.001$), nutrient level ($F_{1,84} = 116.13, P < 0.001$), species \times light level interaction ($F_{5,84} = 5.71, P < 0.001$), and species \times nutrient level interaction ($F_{5,84} = 4.89, P < 0.001$). Significant differences within each species (Tukey's HSD test, $P < 0.05$) are indicated by different letters. Error bars indicate SE.

National Science Foundation CAREER Award DEB-1053237 to J.S.P. We thank two anonymous reviewers for comments that greatly improved this work.

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