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Title: Water, nutrients, or both? Effects of multiple belowground resources on early seedling growth, physiology, and symbionts in eight species of tropical dry forest trees

*Erick Calderón-Morales, Leland K.Werden, Bonnie G. Waring, Roberto Cordero, and Jennifer S. Powers*

Introduction

Plants require multiple belowground resources including water and a variety of nutrients. Differences in both how plants acquire these resources and how they respond to variability in the availability of soil-borne resources contribute to niche differentiation and the maintenance of diversity (Levine and HilleRisLambers 2009, Holste et al. 2011). Because the seedling stage is a vulnerable time in trees’ life histories during which populations suffer elevated mortality (Ribbens et al. 1994), many studies have evaluated how water and nutrients affect seedling growth rates (Baraloto et al. 2006), allocation to roots versus shoots (Burslem et al. 1996, Canham et al. 1996), physiological process rates (Graciano et al. 2005), and relationships with symbionts such as mycorrhizae and nitrogen fixing bacteria in Fabaceae (Birhane et al. 2015). These studies typically find that responses to multiple belowground resources are often species-specific and also may depend upon whether above- or belowground organs are considered (Freschet et al. 2013). Thus, we still lack a general framework for understanding the joint effects of belowground resources on seedling performance, particularly in environments with large seasonal or temporal variability in soil moisture and nutrient availability (Lodge et al. 1994), such as seasonally dry tropical forests.

DESCRIBE THE TYPES OF RESPONSES… (Table 1).

Paragraph 2 introduce TDF..

Seedling growth in TDF is strongly constrained by water availability and is typically restricted to the wet season (Gerhardt 1993, Maza-Villalobos et al. 2013). However, studies have also shown that TDF seedlings respond positively to increased nutrient availability (Huante et al. 1995a, Huante et al. 1995b, Ceccon et al. 2003). Surprisingly, few studies have investigated how nutrients and soil water jointly affect tropical dry forest tree seedlings. There are a number of possible responses to increased water and nutrients, from additive, synergistic, antagonistic, or no response.

\* SYNERGISTIC if plant didn't respond to each resource individually, but does respond to them added together, OR if LRR for both resources together is outside the 95% CI for an individual resource

\*\*\* what does it mean if the resources are not balanced? Can a plant take advantage of added water or nutrients, if it does not have the other resource?

Paragraph 3 Goals and Specific Objectives

We used a diverse sample of tropical dry forest tree species from different functional groups in a seedling pot experiment to answer the following questions: 1) how do biomass allocation and functional traits change during seedling development, 2)

how do added water and/or nutrients affect growth, physiology, water use efficiency, functional traits, and relationships with symbionts, and are these responses additive, antagonistic or synergistic, and 3) which traits are correlated, and does this vary among species? We predicted that

Methods

***Study site and species***

Our experiment took place during between June to November, 2015, in a shade house located at the Estacion Experimental Forestal Horizontes, Area de Conservación Guanacaste, Costa Rica (10°420 46″ N, 85°350 44″). Rainfall is highly seasonal, with a dry period of 5-6 months. Mean annual precipitation at nearby Sector Santa Rosa is 1729 mm and mean temperature is 25 C (Becknell and Powers 2014), although Horizontes likely has lower rainfall than Santa Rosa (Gutiérrez-Leitón 2013). Our study occurred during one of the driest years on record (Cooley et al. 2019), wherein rainy season rainfall was less than X of average due to the very strong El Niño Southern Oscillation (ENSO) event of 2015; thus we considered ambient rainfall as a drought. We used 8 species that belong to 5 different families: *Enterolobium cyclocarpum, Gliricidia sepium, Hymenea courbaril, Dalbergia retusa, Swietenia macrophylla, Simarouba glauca, Tabebuia ochraceae* and *Pachira quinata* (Table 2). Collectively, these species represent the full range of functional trait variation that defines different tropical dry forest plant functional types in ecosystem simulation models (Xu et al 2016), including nitrogen (N) fixing and non-N fixing legume species.

***Seed germination, growing conditions***

The shade house was covered with a 50% shade cloth. All the seeds sown directly into pots containing a soil mixture of 2 parts of locally collected soil and 1 part sand. To improve germination rates, we applied two different treatments to the seeds. *Enterolobium* seeds were subject to water baths alternating from boiling to cold water for 30 seconds for breaking the seed coat. All other species were soaked in water overnight.

Seeds were sown directly into pots in early June, 2015. Following germination and establishment, pots were thinned to one seedling per pot. All plants received supplemental water when needed. After ~12 weeks, an initial harvest of four individual per species was made (see Methods below). Watering and/or fertilizer treatments on the remaining seedlings were started two days later, and were imposed for 12 weeks (N = 4 plants per species per treatment) prior to harvest.

***Watering and fertilization treatments***

At 12 weeks, plants were randomly assigned to one of four treatments: control (no additions), nutrient addition, water addition, water + nutrient addition. Water additions consisted of 500 mL added every two weeks. The nutrient addition treatment consisted of a commercial fertilizer containing NPK and complete micronutrients. Nutrient addition rates were corresponded to 150 kg N ha-1 yr-1, prorated to the duration of the experiment (3 months). Fertilizer was dissolved in water and added to the surface soil in the pots every two weeks in 20 mL doses with a syringe.

***Soil moisture***

Soil moisture readings were taken in all pots immediately prior to watering treatments and then ~1 day following watering, and these measurements were repeated six equally spaced times during the growing season after the treatments began. This allowed us to quantify the magnitude of the watering effect on soil moisture, and also allowed us to whether elevated soil moisture in the watered pots persisted beyond two weeks. These measurements were made with a SM150 Soil Moisture Sensor (Delta-T Devices, Ltd, Cambridge, England).

***Height measurements and harvest***

Height was measured

***Physiological measurements and gas exchange***

Erick.. can you fill in this detail?

***Statistical analysis***

We first compared seedlings in the initial harvest at ~12 weeks to seedlings from the control treatments (i.e. no added water or nutrients) harvested at ~24 weeks. We used two factor analyses of variance (ANOVAs) with harvest date (early or late in the growing season) and species as the main effects, and total dry biomass, relative allocation to roots, stems and leaves, specific leaf area, foliar N concentrations, and foliar 13C (as an integrated metric of water use efficiency) as response variables. This allowed us to interpret any changes in seedling allocation or traits after we imposed the watering and fertilization treatments within the context of developmental changes over the growing season. Next, to understand how

ANOVA and log ratio analysis….

Finally, we calculated pairwise Spearman’s rank correlation coefficients to investigate correlations among growth, functional traits, and physiological variables. We calculated these for legumes and non-legume taxa separately, as numerous studies have suggested that the direction and magnitude of trait correlations differ between legumes and non-legumes (Powers and Tiffin, 2010; Pineda-Garcia et al 2016, Adams et al 2016).

RESULTS

**Adding nutrients, water or both does not increase the number and weight of nodules**

*Soil moisture treatments*

*Changes in biomass, allocation, and functional traits during development*

DISCUSSION

Is water or nutrients more important for early seedling growth and physiology?

Variation in responses among functional groups, and trait correlations

Evergreen species of savanna trees don’t allocate as much belowground, because they don’t have to reflush leaves every year compared to deciduous taxa and as a consequence, relative growth rates are driven by different traits (Tomlinson et al. 2014).

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Table 1 Measurements, units, and predictions about how added water or nutrients affects values

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | Units | Predicted Response to Added Nutrients | Predicted Response to Added Water |
| Final biomass | grams | ↑ | ↑ |
| Leaf mass fraction | % | ↑ | ↓ |
| Stem mass fraction | % | ↑ | ↑ |
| Root mass fraction | % | ↓ | ↑ |
| Height growth rate | cm | ↑ | ↑ |
| Final height | cm | ↑ | ↑ |
|  |  |  |  |
| Photosynthesis rate | µmol m-2 s-1 | ↑ | ↑ |
| Conductance | mol m-2 s-1 | ↓ | ↑ |
| Instantaneous WUE | µmol / mol | ↑ | ↓ |
| Foliar stable carbon isotope concentration | ‰ |  |  |
| Foliar nitrogen concentration | % | ↑ | ↓ |
|  |  |  |  |
| Mycorrhizal colonization | % | ↓ | ↔ |
| Nodule number (N-fixing legumes only) |  | ↓ | ↑ |
| Nodule mass (N-fixing legumes only) |  | ↓ | ↑ |

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Table 2. Taxonomic identity, wood density, specific leaf area, leaf habit, and plant functional group classification of eight common species of tropical dry forest tree used in the pot experiment. Trait data are from Powers and Tiffin (2010).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Family | Species | Wood density (g cm-3) | Specific leaf area (cm2 g-1) | Leaf habit |
| Bignoniaceae | *Tabebuia ochraceae* | 0.80 | 75.8 | deciduous |
| Fabaceae—  N fixing | *Dalbergia retusa* | 0.80 | 67.7 | deciduous |
|  | *Enterolobium cyclocarpum* | 0.38 | 145.5 | deciduous |
|  | *Gliricidia sepium* | 0.78 | 137.8 | deciduous |
| Fabaceae—non-fixing | *Hymenaea courbaril* | 0.84 | 69.4 | semi-deciduous |
| Malvaceae | *Pachira quinata* | 0.38 | 105.6 | deciduous |
| Meliaceae | *Swietenia macrophylla* | 0.67 | 68.7 | deciduous |
| Simaroubaceae | *Simarouba glauca* | 0.41 | 54.8 | evergreen |

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Table 3. Results from ANOVAs

Table X. Final summary table (with examples)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variable | *Tabebuia ochraceae* | *Dalbergia retusa* | *Enterolobium cyclocarpum* | *Gliricidia sepium* | *Hymenaea courbaril* | *Pachira quinata* | *Swietenia macrophylla* | *Simarouba glauca* |
| Final biomass | Nutrient limited | Additive co-limitation |  |  |  |  |  | No response |
| Leaf mass fraction |  |  |  |  |  |  |  |  |
| Stem mass fraction |  |  |  |  |  |  |  |  |
| Root mass fraction |  |  |  |  |  |  |  |  |
| Height growth rate |  |  |  |  |  |  |  |  |
| Final height |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
| Photosynthesis rate |  |  |  |  |  |  |  |  |
| Conductance |  |  |  |  |  |  |  |  |
| Instantaneous WUE |  |  |  |  |  |  |  |  |
| Foliar stable carbon isotope concentration |  |  |  |  |  |  |  |  |
| Foliar nitrogen concentration |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
| Mycorrhizal colonization |  |  |  |  |  |  |  |  |
| Nodule number (N-fixing legumes only) |  |  |  |  |  |  |  |  |
| Nodule mass (N-fixing legumes only) |  |  |  |  |  |  |  |  |

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