Title

Above and belowground demographic and functional trait responses to biochar addition in seedlings of six tropical dry forest tree species

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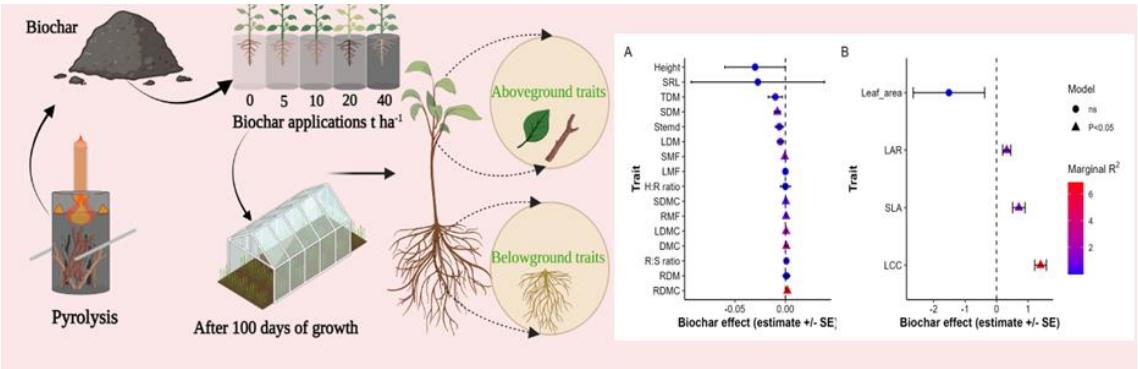
Abstract

The addition of biochar as a soil amendment has a great potential for ecological restoration and long-term carbon (C) storage. However, few studies have evaluated the functional trait responses of tree seedlings to increasing levels of biochar and almost no information is available for threatened ecosystems like tropical dry forests (TDF). Here, we conducted a greenhouse experiment to quantify effects of rates of biochar (0, 5, 10, 20, and 40 t ha^{-1}) on demographic and functional traits of six tree species commonly used in TDF restoration programs. After 100 days of growth, we found no negative effects of biochar on seedling survival and only in two of the species the highest dose applied slightly reduced the final biomass achieved. The addition of biochar increased leaf chlorophyll content (LCC) and specific leaf area (SLA) of all species. Greater variations in above-and below-ground trait responses to biochar were due more to inter-specific (53%) and intra-specific (37%) differences than main effects of biochar across species (10%), although we found that 53% of the variation in the LCC was due to the addition of biochar. We found a positive effect of biochar on morphological traits related to C gain and physiological tolerance to drought (dry mass content of root, leaf, and stem, LCC, SLA, and leaf area ratio). Therefore, we suggest that applications of biochar of up to 40 t ha^{-1} may improve growth capacity and drought resistance in the studied TDF tree species during field establishment, while contributing to long-term soil sequestration of atmospheric C.

Abstract Highlights:

- No negative effects of biochar on seedling survival and growth even at the highest dose applied.
- The application of biochar at the nursery stage has positive effects on traits related to drought tolerance.
- The addition of biochar as a soil amendment has can be safely incorporated into tropical dry forest restoration programs in large-scale.

Graphical abstract



Keywords: biochar, biomass allocation, intraspecific trait variability, plasticity, tropical dry forests

Introduction

Reducing the risks of a 1.5-°C rise in global temperatures requires a drastic reduction in greenhouse gas emissions along with greater sequestration of excess atmospheric carbon dioxide (CO_2) (IPCC, 2018) through the restoration and regeneration of natural forests (Bastin et al., 2019; Cook-Patton et al., 2020; Chazdon et al., 2016; Griscom et al., 2017; Lewis et al., 2019). Given soils represent the largest terrestrial reservoir of organic carbon (C), with a high storage capacity (Georgiou et al., 2022), the development of priority actions for the management of C in forest ecosystems is likely to be key to the long-term mitigation of impacts of climate change.

Biochar has been used as a soil amendment in agricultural ecosystems to increase productivity, with positive impacts on soil C stocks (Biederman & Harpole, 2013; Liu et al., 2013); as a result, it has been suggested that soil applications of biochar may improve the success rates of forest restoration projects (Lehmann & Joseph, 2015; Muhammad Irfan et al., 2017; Thomas & Gale, 2015; Wang et al., 2016). For example, biochar may contribute to soil C sequestration, due to its negative emissions of approximately 0.7 Gt C-eq/yr (Smith, 2016) and CO_2 capture equivalents of 1.8–11.9 Gt CO2-eq/year (Lee et al., 2010; Woolf et al., 2010). There is evidence for long-term effects of applications of biochar sequestration of C in soil (Wang et al., 2016), due to length of residence time and content of available C (108 days and 3%, respectively) and

recalcitrant C (556 years and 97%, respectively) (Wang et al., 2016); there are reports of extreme mean residence times for soil sequestration of recalcitrant C from biochar of >1000 years (Cheng et al., 2008; Rakshit et al., 2012). However, residence time of C depends on a range of factors, including raw materials and pyrolysis temperatures used in biochar production, soil type, and climate conditions (Amoah-Antwi et al., 2020).

The addition of biochar to agricultural systems has been shown to lead to 10–30% increases in crop biomass (Biederman & Harpole, 2013; Liu et al., 2013), with greater increases reported for pioneer herbaceous plant species (30–37%; Gale et al. (2017) and woody plants (c. 41%; (Thomas & Gale, 2015). These impacts on productivity are likely due to effects of biochar on soil and rhizosphere conditions, such as increases in available phosphorous (P) and microbial biomass of agricultural soils (Gao et al., 2019), greater cation exchange capacity, pH, content of total and organic C, and total nitrogen (N), and C/N ratios in agricultural soils a global scale (Dai et al., 2020), and increases in annual plant root P concentrations, and numbers of root-associated microbes and root nodules (Xiang et al., 2017). However, there is evidence for inconsistency in these positive effects of biochar across soil types, climate, and plant strategies; for example, addition of biochar to acidic, low fertility tropical soils increased crop yields by 25%, whereas there were no impacts of applications to neutral, high fertility temperate soils (Jeffery et al. (2017), while inter-specific variations in direction of responses have been reported for pioneer herbaceous plants (Gale et al. (2017). Nevertheless, it is possible that the addition of biochar to soils of reforestation, afforestation and forest restoration projects may elicit positive impacts on soil fertility, particularly in tropical ecosystems where degradation of soils has led to high levels of nutrient deficiency (Thomas & Gale, 2015).

Although growth and litter production of two 4-year old forest tree species have been shown to be unaffected by biochar application (Gonzalez Sarango et al., 2021), studies have shown that simultaneous additions of biochar and inorganic fertilizer increase height, diameter, and above- and below-ground biomass, including leaf production, in forest plant species (Lefebvre et al., 2019), while biochar applications have led to greater increases in tree seedling quality than applications of inorganic fertilizer (Fagbenro et al., 2015) and, in soils with high levels of salinity, addition of biochar improves productivity of tree seedlings (Drake et al., 2016).

Measurement of impacts on plants of biochar addition have largely been based on demographic trait metrics, such as growth and survival in seedlings, rather than a functional trait-based approach that would facilitate an understanding of response mechanisms and allow improved selection of species for forest restoration programs to optimize seedling establishment according to prevailing environmental conditions (Werden et al., 2018). For example, functional traits of leaf mass fraction and specific leaf area are predictors of photosynthetic capacity, as they are

related to light interception and water loss through transpiration (Markesteijn & Poorter, 2009), stem characteristics, such as diameter and the ratio of height to diameter, are predictors of survival and growth, as they are related to light capture, water transport, and resistance to pest and weather damage (Haase, 2008; Poorter, 1999), and root traits, such as root mass fraction and specific root length are related to water and nutrient uptake that are important for the capture and storage of water, nutrients, and seedling support (Poorter & Markesteijn, 2008), while the ratio of roots to shoots biomass reflects the balance between water loss by transpiration (shoot) and water grain through absorption (root) and dry matter content of leaf, stem and root tissues tend to be related to physiological drought tolerance (Hacke et al., 2001; Jacobsen et al., 2005). However, quantification of plant demographic and functional trait responses to biochar addition to threatened ecosystems in which restoration programs are urgently needed, such as tropical dry forests (TDFs), is currently lacking.

Thus, the aim of our study was to test for direction of effects of biochar-mediated increases in C capture on TDF tree species seedling growth and survival, and functional trait responses, to better inform selection of species for restoration programs. We conducted a greenhouse experiment to test for demographic and trait responses to contrasting rates of biochar addition in seedlings of six tree species commonly used in TDF restoration programs. We measured intraspecific trait variability (ITV), as individual, rather than species-level responses to biochar addition that better predicts seedling survival and growth (Poorter et al., 2018), to assess whether tree species phenotypic plasticity may be a useful indicator of suitability of target species for forest restoration programs (Lanuza et al., 2020).

Materials and Methods

Study site

The experiment was conducted in a greenhouse at the National Autonomous University of Nicaragua-Managua El Limón Experimental Station (13°03'044″ N, 86°21'057″ W), located at 888 m a.s.l. in northwestern Nicaragua. The dry tropical climate of the region is characterized by an average annual temperature and rainfall of 23.1 °C and 892 mm/year, respectively, and an annual water deficit of -385.4 mm/year (Ruiz-Gómez et al., 2021).

Experimental design

Dry wood feedstock (<5 cm diameter) (90% *Vachellia pennatula* Schltdl. & Cham. Seigler & Ebinger; 10% mix of other tree species locally used as firewood) was pyrolyzed in a top-lit updraft gasifier reactor at 700–1000 °C. Once the biochar had cooled to room temperature, it was crushed using a manual mill and screened using a 2-mm sieve prior to use.

We collected seeds of six, locally abundant TDF tree species (*Crescentia alata* Kunth, *Cordia alliodora* Ruiz & Pav. Oken, *Cedrela odorata* L., *Swietenia humilis* Zucc., *Tabebuia rosea* Bertol. DC., and *Guazuma ulmifolia* Lam.) from local single mother trees to reduce genotypic differences and minimize intraspecific trait variability and germinated the seeds over 20-25 days in a homogenous substrate. Then, we transplanted single seedlings of each species to nursery bags filled with sieved local vertisol topsoil (~15 cm depth, 0.5-cm sieve) to which treatments of biochar equivalent to 5, 10, 20, or 40 t ha^{-1}; we included an untreated control of topsoil only. The nursery bags were arranged in a factorial design (6 species × 5 treatments) with 20 replicates, to account for variation in light and temperature conditions. The seedlings were irrigated at field capacity (350 ml) twice a week and 3g of NPK (12-30-10) fertilizer was added 30 days after transplantation. After 100 days, we removed the seedlings from the nursery bags and gently washed the roots in tap water to remove substrate, prior to analysis of trait data.

Trait measurement and calculation

Mass (g) of seedling leaf, stem, and root material was measured fresh and following drying in an oven at 60°C for 48 h, or until constant weight, using an analytical balance with 0.001g precision. We calculated the mass fraction of leaf, stem, and root material (LMF, SMF, RMF, respectively) as the dry mass of each component/total seedling dry mass (g g^{-1}) (Amissah et al., 2022; Poorter et al., 2012) and leaf, stem, and root dry mass content (LDMC, SDMC, RDMC, respectively) was calculated as respective tissue dry/fresh mass (mg g^{-1}).

Specific root length (SRL) was calculated as the length of fine roots/root dry mass (cm g^{-1}) and root-to-shoot ratio was calculated as root mass/stem + leaf mass. Stem diameter (mm) immediately below the cotyledon scar was measured using calipers, seedling height (cm) was measured from the cotyledon scar to the base or tip of the terminal bud, or the end of the growing tip, if no bud was formed, and seedling robustness was calculated as the ratio between height and diameter at the root neck (Haase, 2008). Leaf chlorophyll content (LCC) (µmol cm^{-2}) was measured from 10 seedlings per species using a chlorophyll content meter (CCM-200 Plus, Opti-Sciences, USA) between 08:00 and 14:00 hrs and mean leaf area of fresh leaves from 10 seedlings per species that were digitized using a desktop scanner (HP Scanjet 5590, USA) was calculated using Image J software (Schneider et al., 2012). We calculated leaf area ratio (LAR) as total leaf area/plant mass (cm^2 g^{-1}) and specific leaf area (SLA) as leaf area/leaf mass (cm^2 g^{-1}) (Pérez-Harguindeguy et al., 2013; Poorter et al., 2012).

Data analysis

We calculated median, range (5^{th} to 95^{th} percentiles), and coefficients of variation for all measured demographic and functional traits across species and treatments (Table 1). Species trait responses to biochar were tested using general linear models, with species and biochar

treatment as fixed-effects terms, and trait responses across species were tested using general mixed-effects models, with treatment as a fixed-effect term and species as a random factor; analyses were conducted using the *lme4* R package (Bates et al., 2015) and quality of model fit was evaluated using the *performance* R package (Lüdecke et al., 2021).

For each species, we calculated a simple plasticity index (PI), defined as the highest phenotypic value divided by the lowest value (Poorter et al., 2012), to summarize the relationship between trait variability and response to biochar treatment, where PI = 1 indicates no change in response to rate of biochar application. We conducted a variance partitioning analysis using a series of nested linear mixed-effects models to estimate intra- and inter-specific variation in trait responses to biochar application rates, where separate linear mixed-effects models were fitted for each trait, with species as a random factor (for intra- and inter-specific trait variation), followed by treatment nested within species as a random factor (within species variation in trait response to biochar rate) (Vilà-Cabrera et al., 2015). All statistical analyses were conducted using R version 4.1.1 (R Core Team, 2021).

Results

Effects on demographic traits

We found that effects of biochar addition on all demographic and functional traits varied among species (Table 2). Across species, there were no effects of biochar seedling mortality ($F = 1.29$; $P = 0.31$), while application of biochar at 40 t ha^{-1} led to decreases in total dry mass of *C. alliodora* and *C. odorata* seedlings, increases in LMF in *C. alata*, decreases in SMF in C. *alliodora* and *T. rosea*, and increases in RMF in *T. rosea* (Figure 1).

The addition of biochar at 40 t ha^{-1} increased SLA in *C. odorata* and *G. ulmifolia* (Figure 2A) and addition per se increased LCC in all six species (Figure 2B). Overall, and controlling for species variability, the addition of biochar led to small decreases in SDM and SMF, and small to moderate increases in SDMC, RMF, LDMC, DMC, RDMC, LAR, SLA, and LCC (Figure 3; Table S1).

Effects on functional traits

There was wide variation in ranges of functional traits (Table 1). For example, SDM and LCC varied c. 13-fold (0.36–4.8 g and 22–285.5 μmol m^2, respectively), leaf area varied 8.5-fold (184.7–1569.3 cm^2), LAR varied 4.1-fold (45.0–182.3 cm^2 g^{-2}), and SLA varied 2.75-fold (166.5–457.3 cm^2 g^{-2}). There was greater variation in belowground than aboveground traits, including 14-fold variations in RDM and SRL (0.36–5.16 g and 4.65–65.3 cm g^{-2}, respectively).

Plasticity of trait responses

There was species variation in plasticity of trait responses to the addition of biochar, where those of *G. ulmifolia* tended to be least plastic, and LDM, SDM and LCC were the most plastic aboveground traits, while RDM and the SRL were the most plastic belowground traits across the other five species (Figure 4).

Inter- and intra-specific variation in trait responses to the addition of biochar were greater than across-species variation in trait responses to biochar (Figure 5). We found that an average of 53% of the variation in trait responses to the addition of biochar were due to inter-specific differences, ranging from 13% for SRL to 78% for height and H:D ratios, while intra-specific differences accounted for an average of 37% of variation in trait responses and main effects of biochar accounted for an average of 10% of the variation in species trait responses, ranging to up to 58% for LCC, thus confirming that this trait was particularly sensitive to the addition of biochar.

Discussion

We quantified demographic and functional trait responses in seedlings of six TDF tree species to increasing application rates of biochar and found contrasting impacts between the two trait types. While there were no effects on seedling survival and limited impacts on growth in two species under applications of 40 t biochar ha^{-1}, seedling functional traits were more sensitive to the addition of biochar. Soil addition of biochar increased LCC, LAR, and SLA, indicating an improvement in photosynthetic capacity of the seedlings (Markesteijn & Poorter, 2009), while there were moderate increases in DMC of root, stem, and leaf material, possibly indicating an improvement in physiological tolerance to drought conditions (Hacke et al., 2001; Jacobsen et al., 2005). A large proportion of the variation in trait values was explained by inter-specific differences in trait responses (53%), while differences in intra-species trait responses explained on average 37% of the variation trait values. Despite this potential for adaptive phenotypic plasticity the experimental addition of biochar only accounted for a 10% of trait variability on average, with the notable exception of the LCC where up to 53% of its variation was due to biochar. Hence, biochar addition did not negatively affect the growth or the trait expression patterns of the seedlings of the six TDF species studied. Altogether, our findings suggest that biochar addition can be safely incorporated in large-scale TDF restoration programs without compromising seedling establishment and thus contributing to long-term C sequestration in the soil.

Overall effects of biochar on tree seedling demographic and functional traits

We found positive main effects of biochar on key growth-related morphological traits (RDMC, LCC, SLA, LAR, RMF, LDMC, SDMC, DMC) across species (Figure 3), where biochar addition explained 6.9% of the variation in RDMC (Table S1), indicating physiological

tolerance to drought (Hacke et al., 2001; Jacobsen et al., 2005). The positive effects of biochar on the foliar traits (SLA and LAR) and root traits (RMF) is likely to improve field establishment of seedlings, as they are related to light capture, control of water losses through transpiration, and the capture and storage of water, nutrients, and seedling support, respectively (Markesteijn & Poorter, 2009).

Across species, we found no main effects of biochar on TDM, LDM, RDM, LMF, Stemd, Height, or SRL and negative effects on SDM and SMF, in contrast to findings for impacts on crops (Biederman & Harpole, 2013; Liu et al., 2013), woody plants (Thomas & Gale, 2015), and pioneer herbaceous species (Gale et al., 2017). These differences between our findings and those of previous studies may be due to the lack of simultaneous additions of biochar and fertilizer in our experiment that have been shown to increase plant height, stem diameter, total number of leaves, and above- and below-ground biomass (Lefebvre et al., 2019). However, reports of lack of effects of combined applications of biochar and fertilizer on plant growth (Gonzalez Sarango et al., 2021) indicate highly variable plant responses to biochar, that may depend on species.

Species responses to biochar

Our data show inter-specific variation in trait responses to addition of biochar, where there was greater allocation to aboveground biomass (high TDM, SDM, SLA) in *G. ulmifolia*, while in *C. alata* there were reductions in RMF, SMF, and increases in LMF and SLA. The allocation of biomass to plant organs varies with species, ontogeny, and environmental conditions (Poorter & Nagel, 2000). Dry forest species, such as *C. alata*, limit water losses through reductions in amount of transpiration tissues (lower Leaf_area, SLA, LAR) and improved access to water in deeper soil layers (higher RMF) (Poorter & Markesteijn, 2008), whereas fast-growing species, such as *G. ulmifolia*, are characterized by acquisitive foliar traits and greater allocation of biomass to aboveground structures under high levels of nutrient availability and greater allocation of biomass to belowground structures under nutrient limitation (Lanuza et al., 2020). Thus, our data show that seedlings of TDF species modulate biomass allocation, in response to shifts in resource availability, allowing adaptation to variations in forest conditions.

Our results indicate that greater variation in above- and below-ground trait responses to biochar was due to inter-specific, rather than intra-specific (ITV) differences and we found that addition of biochar increased the plasticity index of above- and below-ground functional traits (SRL, SDM, RDM, LDM, LCC). We found that ITV of our experiment was slightly higher than that reported by Poorter et al. (2018); Siefert et al. (2015), and similar to levels for three dry forest species subjected to contrasting levels of nutrients, irrigation, and herbivory (Lanuza et al.,

2020), indicating these species may show high level of adaptability to shifts in environmental conditions (Poorter et al., 2018).

We found that addition of biochar accounted for an average of 10% variation in trait responses across species, yet explained 53% of species variation in LCC, while the overall proportion of variation in LCC explained by biochar was 5.9%, indicating the sensitivity of this functional trait to water stress, given drought affects photosynthesis (Khaleghi et al., 2012). Biochar improves water retention capacity, by increasing interpore volume of soils (Liao & Thomas, 2019), and applications of biochar have been shown to improve water use efficiency of pioneer herbaceous seedlings by 44% (Gale et al., 2017), but also reduce leaf N content and LCC in tomato seedlings (Akhtar et al., 2014).

The proportions of inter-specific and intra-specific variation in trait responses to biochar addition ranged between 13 and 18% to 78%, respectively. Traits related to tissue quality and toughness (DMC of root, stem, leaf) are expected to express low levels of ITV, as they tend to be phylogenetically conservative (Chave et al., 2006); this was evident in our study for SDMC and RDMC, but was higher (61%) for LDMC. We found a low ITV for SLA, supporting findings reported by Poorter et al. (2018), but in contrast to controlled studies that show marked responses in leaf traits to shifts in ambient light levels, to enhance light capture (Poorter et al., 2009; Sterck et al., 2013).

Our data show high levels LMF, SRL, and LAR in *T. rosea*, *C. odorata*, and *S. humilis* and low levels of DMC of leaf, stem, and root material that are correlated with physiological tolerance to drought (Hacke et al., 2001; Jacobsen et al., 2005). Thus, it is likely that these species may be susceptible to water deficit, in contrast to *G. ulmifolia* that was characterized by high levels of DMC, height and robustness (H:D ratio), indicating adaptations for light capture, water transport, support, and tolerance to wind damage and drought conditions (Haase, 2008; Poorter, 1999).

We found that above-ground (LMF) and below-ground (RMF) trait responses to biochar mirrored relative allocations of biomass, as reported by Lanuza et al. (2020) for dry forest seedlings subjected to contrasting levels of fertilization and that are similar to responses to drought conditions, where species tend to reduce biomass allocation of LAR and LMF and increase allocation to RMF (Markesteijn & Poorter, 2009; Poorter & Markesteijn, 2008). Competition for above-ground and below-ground resources tends to be dynamic during the seedling stage, when acquisition of sufficient water, nutrients, and light is essential for sustained growth (Ågren et al., 2012; Fatichi et al., 2014; McMurtrie et al., 2008; Poorter et al., 2012).

Previous research has shown limited effects of RMF and LMF on below- and above-ground resource foraging, respectively (Poorter & Nagel, 2000). Our study showed that limited

investment in root biomass (low RMF), as found for *C. odorata*, *S. humilis* and *T. rosea*, may be offset by cost-effective root growth, as indicated by large root length per unit of biomass invested (high SRL), whereas low biomass investment in leaf material (low LMF), as found for *G. ulmifolia*, may be offset by large leaf area per unit of leaf biomass invested (high SLA). This compensation strategy in above- and below-ground biomass allocation has been demonstrated in response to drought conditions (Markesteijn & Poorter, 2009).

Conclusions

Our analysis shows that variations in above-and below-ground trait responses to biochar were due more to inter- and intra-specific differences than to main effects of biochar across species, indicating strong inherited effects of species. While we found heterogenous species responses to biochar addition, there were few overall effects; the few positive effects were for traits related to drought tolerance. There was greater variation in responses of below-ground traits than above-ground traits to biochar, where there were tradeoffs in biomass allocation to LMF and RMF structures. Our findings will contribute to the selection of species suitable for dry forest reforestation projects, based on plasticity of drought tolerance traits, and show that the application of biochar at the nursery stage improves allocation of biomass towards traits related to seedling growth and survival, while directly contributing to long-term soil sequestration of atmospheric C.

Authors contributions

Oscar R. Lanuza designed and conducted the experiments with supervision of Guille Peguero. Oscar R. Lanuza performed the data analysis with supervision of Guille Peguero. Oscar R. Lanuza and Guille Peguero led the writing with contributions and ideas of Josep M. Espelta and Josep Peñuelas. All authors edited, critically reviewed and finally approved the manuscript.

Declarations

Data availability

The data and code supporting the results of this study will made available at an open-access data repository once the paper is accepted.

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Competing interests

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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558

559 **Tables and Figures**

560 **Table 1.** Median values, 5–95th percentile ranges, and coefficients of variation (CV) of traits in
561 seedlings of six tropical dry forest tree species grown with the addition of biochar.

Traits	Abbreviation	Units	Median	Range (5–95th percentile)	CV
	Height	cm	36	15–77	46.91
Stem diameter	Stemd	mm	6.6	4.16–10.16	25.67
Leaf chlorophyll content	LCC	μmol m^2	129.2	22–285.5	56.45
Stem dry mass	SDM	g	2.19	0.36–4.8	58.53
Root dry mass	RDM	g	2.31	0.36–5.16	58.39
Leaf dry mass	LDM	g	3.08	0.75–4.91	41.49
Total dry mass	TDM	g	8.15	1.5–13.13	44.25
Dry matter content	DMC	%	0.29	0.19–0.41	23.09
Roof mass fraction	RMF	g g^{-1}	0.28	0.17–0.53	34.47
Stem mass fraction	SMF	g g^{-1}	0.28	0.18–0.39	23.55
Leaf mass fraction	LMF	g g^{-1}	0.41	0.26–0.57	24.42
Leaf dry matter content	LDMC	mg g^{-1}	0.28	0.18–0.39	22.6
Stem dry matter content	SDMC	mg g^{-1}	0.3	0.18–0.42	26.11
Root dry matter content	RDMC	mg g^{-1}	0.33	0.21–0.51	30.15
Leaf area	Leaf_area	cm^2	752.14	184.73–1569.3	54.19
Leaf area ratio	LAR	cm^2 g^{-1}	109.57	45.01–182.37	36.97
Specific leaf area	SLA	cm^2 g^{-2}	247.1	166.52–457.3	33.15
Specific root length	SRL	cm g^{-1}	10.8	4.65–65.27	128.07
Root to shoot ratio	R:S ratio	n/a	0.38	0.21–1.11	57.12
Height to diameter ratio	H:D ratio	n/a	4.79	2.73–12.72	52.99

562

563 **Table 2.** General linear model analysis of treatment × species interaction effects on tropical dry
564 forest tree species traits. See Table 1 for trait descriptions.

Trait	**Df**	**F**	***P*-value**	**R^2**
Height	20	6.99	<0.0001	0.79
Stemd	20	3.75	<0.0001	0.52
LCC	20	17.70	<0.0001	0.79
SDM	20	4.91	<0.0001	0.64
RDM	20	2.82	<0.0001	0.53
LDM	20	4.49	<0.0001	0.43
TDM	20	3.99	<0.0001	0.52
DMC	20	4.31	<0.0001	0.65
RMF	20	3.36	<0.0001	0.71
SMF	20	2.95	<0.0001	0.54
LMF	20	2.93	<0.0001	0.69
LDMC	20	5.56	<0.0001	0.39
SDMC	20	3.40	<0.0001	0.76
RDMC	20	6.42	<0.0001	0.67
Leaf_area	20	2.73	0.0001	0.70
LAR	20	1.79	0.0218	0.52
SLA	20	1.72	0.0305	0.76
SRL	20	1.90	0.0105	0.24
R:S ratio	20	3.03	<0.0001	0.70
H:D ratio	20	4.01	<0.0001	0.81

565

Figures

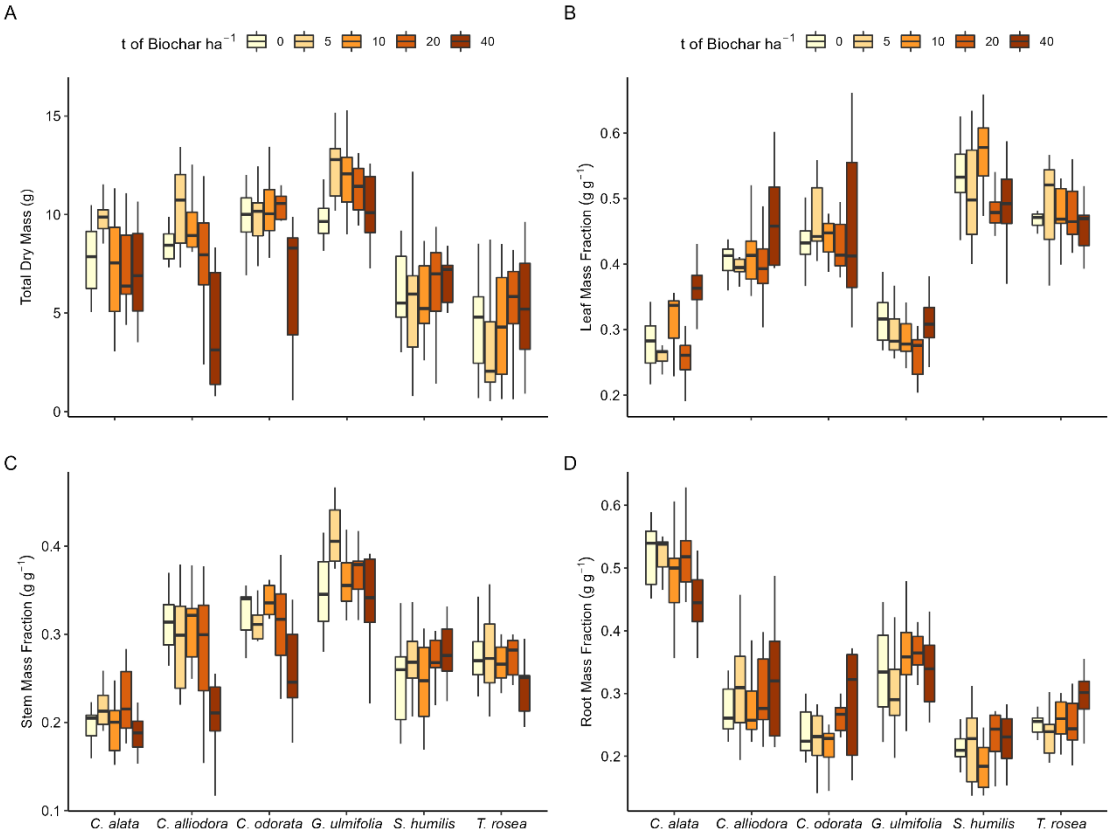
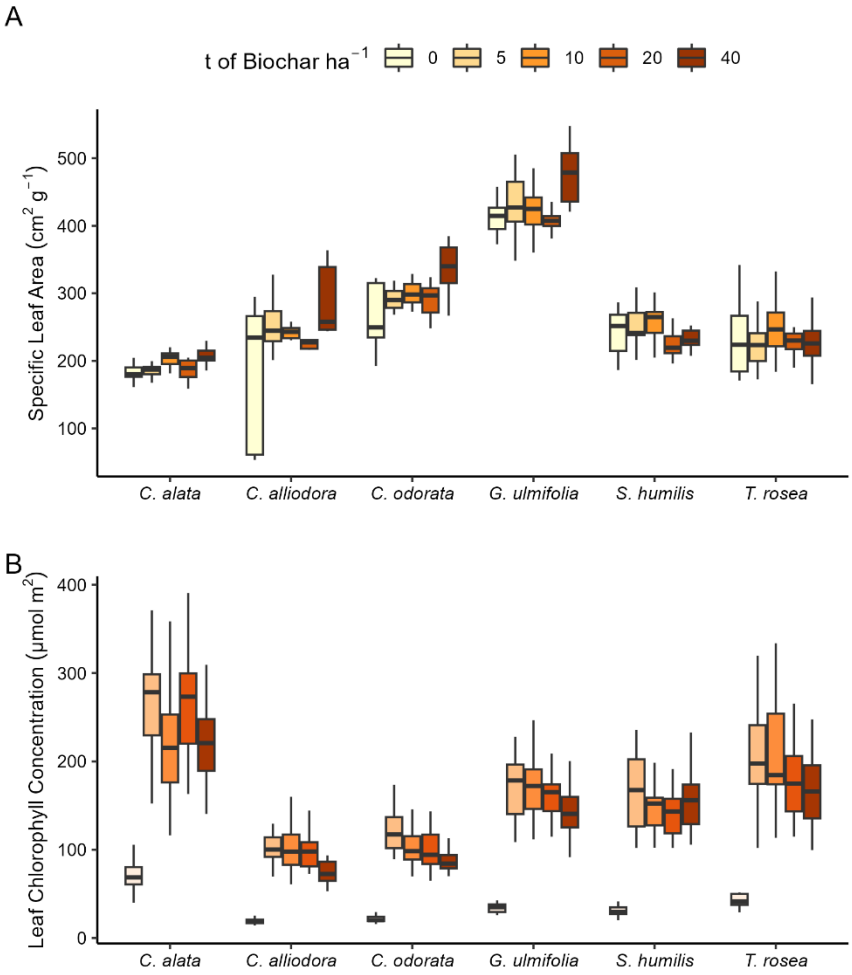


Figure 1 Effects of rates of biochar addition on total dry mass (A) and masses of leaf (B), stem (C), and root (D) of seedlings of six tropical dry forest tree species.



570 **Figure 2.** Effects of rate of biochar addition on specific leaf area (A) and leaf chlorophyll
571 concentrations (B) of seedlings of six tropical dry forest tree species.

572

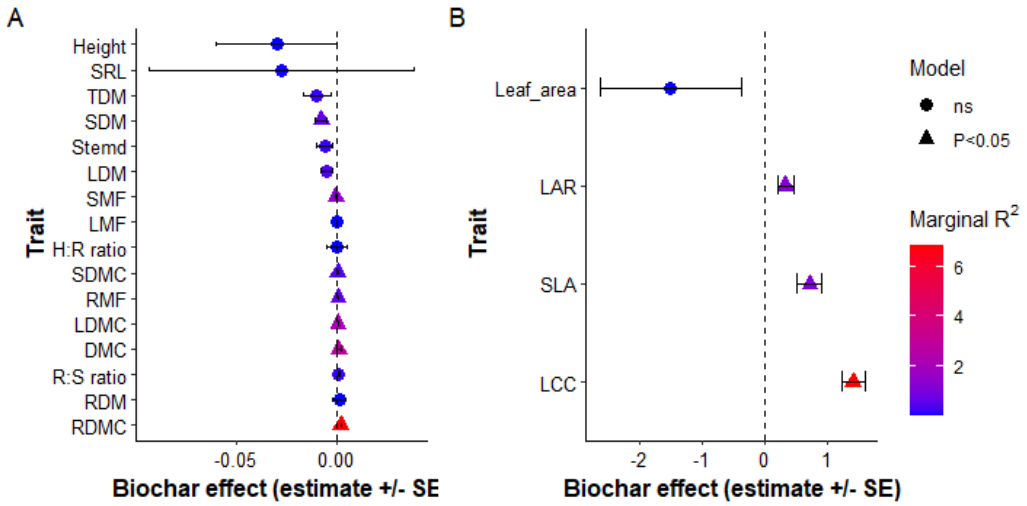
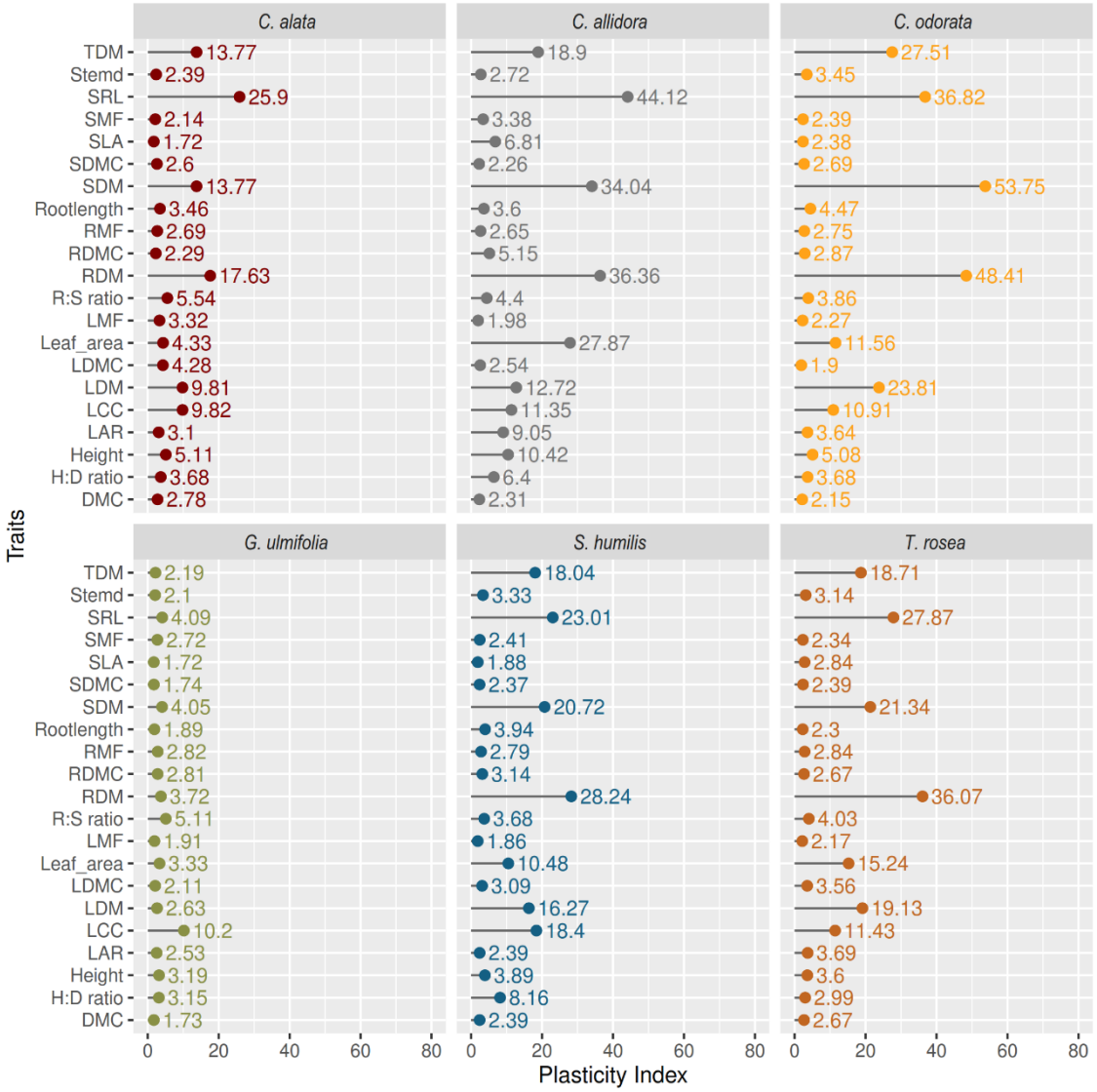


Figure 3. Linear mixed-effects model estimates of overall effects of biochar addition on seedling functional traits, averaged across six tropical dry forest tree species. Triangles and circles indicate biochar effects at $P < 0.05$ and $P > 0.05$, respectively. Color gradients of symbols indicate variance explained by the addition of biochar (marginal R^2). Data are presented in two panels for clarity, due to contrasting ranges of effect estimates. See Table 1 for trait descriptions.

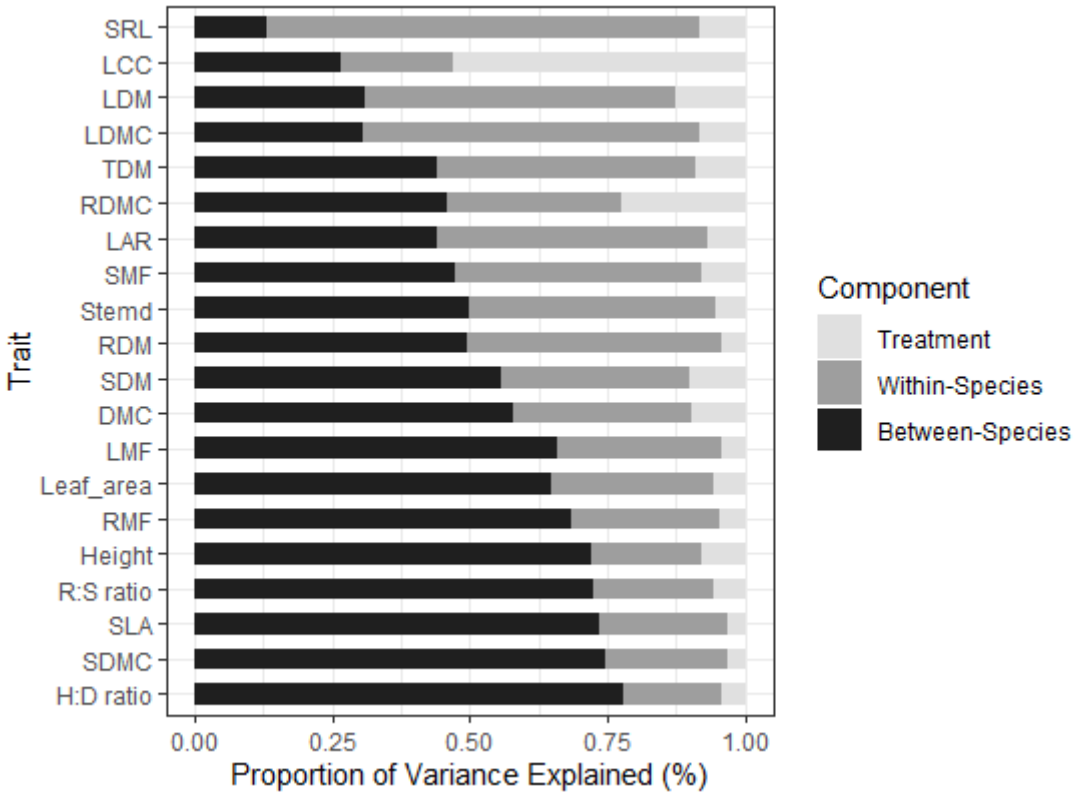


582

583 **Figure 4.** Seedling trait plasticity indexes for six tropical dry forest tree species in response to

584 overall effects of biochar addition. See Table 1 for trait descriptions.

585



586

587 **Figure 5.** Linear mixed-effects model analysis of inter-specific (black), intra-specific (dark
588 gray), and a cross-species (light gray) variation in seedling trait responses to biochar addition.
589 See Table 1 for trait descriptions.

SUPPLEMENTARY MATERIAL

Table S1. Mixed-effects model estimates of overall effects of biochar addition on seedling traits in six tropical dry forest tree species. See Table 1 for trait descriptions.

Trait	**Estimate**	**SE**	***P*-value**	$\mathbf{R^2_{conditional}}$	$\mathbf{R^2_{marginal}}$
Height	-0.03	0.03	0.31	75.6	<0.1
Stemd	-0.006	0.004	0.13	50.7	0.2
LCC	1.3988	0.1849	<0.001	41.0	5.9
SDM	-0.008	0.003	0.004	58.9	0.6
RDM	0.00097	0.0032	0.76	50.6	<0.1
LDM	-0.005	0.003	0.08	34.8	0.4
TDM	-0.012	0.007	0.11	46.6	0.2
DMC	0.00088	0.001318	<0.001	63.9	2.8
RMF	0.000641	0.000186	0.001	70.0	0.6
SMF	-0.000585	0.000147	<0.001	50.8	1.4
LMF	-0.0000566	0.000183	0.76	66.9	<0.1
LDMC	0.00068	0.000159	<0.001	34.8	2.1
SDMC	0.000378	0.0001258	0.004	76.2	0.4
RDMC	0.001924	0.0001845	<0.001	63.9	6.9
Leaf_area	-1.513	1.135	0.19	65.9	0.2
LAR	0.3226	0.1276	0.012	46.5	1.2
SLA	0.7089	0.198	<0.001	75.2	1.1
SRL	-0.0273	0.0659	0.68	15.4	<0.1
R:S ratio	0.0009596	0.0005034	0.06	67.7	0.2
H:R ratio	-0.0000397	0.00485	0.99	80.1	<0.1