Heringeriana
Jardim Botânico de Brasília
https://revistas.jardimbotanicodf.org/index.php/heringeriana
ISSN 2359-165X

Artigo Original

Effects of substrate amendment with biochar and arbuscular mycorrhizal fungi on the growth of *Theobroma speciosum* cultivated seedlings: a preliminary experiment

Karin Elisabeth von Schmalz[1] Ruan Santos de Farias[2] Vanessa Nascimento Brito[2] Isadora França[3] Rodolfo Salm[4] Emil José Hernández-Ruz[1,5]

[1]*Programa de Pós-Graduação em Biodiversidade e Biotecnologia da Rede Bionorte, Universidade Federal do Pará (UFPA), Faculdade de Ciências Biológicas. Rua Coronel José Porfirio, 2515. CEP 68372040. Altamira, Pará, Brazil*
[2]*Universidade Federal do Pará (UFPA), Faculdade de Ciências Biológicas, Laboratório de Microbiologia. Rua Coronel José Porfirio, 2515. CEP 68372040. Altamira, Pará, Brazil*
[3]*Universidade Federal do Pará (UFPA), Faculdade de Ciências Biológicas, Laboratório de Botânica. Rua Coronel José Porfirio, 2515. CEP 68372040. Altamira, Pará, Brazil*
[4]*Universidade Federal do Pará (UFPA), Faculdade de Ciências Biológicas, Laboratório de Ecologia. Rua Coronel José Porfirio, 2515. CEP 68372040. Altamira, Pará, Brazil*
[5]*Universidade Federal do Pará (UFPA), Faculdade de Ciências Biológicas, Laboratório de Zoologia. Rua Coronel José Porfirio, 2515. CEP 68372040. Altamira, Pará, Brazil*

*Corresponding author: karin.schmalz@gmail.com

Received 03 December 2024 | **Accepted** 13 November 2025 | **Published** 29 December 2025

How to cite: von Schmalz, K. E., Farias, R. S. de, Brito, V. N., França, I., Salm, R., & Hernández-Ruz, E. J. (2025). Efeitos da adição de biochar e fungos micorrízicos arbusculares ao substrato no crescimento de mudas cultivadas de *Theobroma speciosum*: um experimento preliminar. Heringeriana, 19(1), e918064. doi.org/10.70782/heringeriana.v19i1.918064

Abstract: *Theobroma speciosum* is an Amazonian species of high ecological and potential economic value, and has an untapped potential for agroforestry cultivation. The experiment, developed at the Experimental Shade House of the Federal University of Pará in Altamira, tested the effects of several types of substrates with different compositions, including varied proportions of biochar and inoculation with arbuscular mycorrhizal fungi (AMF), with the aim of testing the species' tolerance and growth under these conditions. The application of biochar alone did not improve growth of seedlings, with higher concentrations of 30% being detrimental for the plants. The composition of 15% biochar with AMF inoculation yielded larger seedlings after six months. Our results suggest that soil enrichment with a mixture of low proportions of biochar and the inoculation of AMF may improve productivity in *T. speciosum* and allow the inclusion of the species in soils needing biochar-mediated pollution remediation even under extreme temperatures, although further studies are needed to ascertain optimal soil amendment.

Keywords: Agroforestry; Amazonia; *cacauí*; forest restoration; soil amendment.

Resumo: Efeitos da adição de biochar e fungos micorrízicos arbusculares ao substrato no crescimento de mudas cultivadas de *Theobroma speciosum*: um experimento preliminar. *Theobroma speciosum* é uma espécie amazônica de alto valor ecológico e potencial econômico, com possibilidades ainda inexploradas para cultivo agroflorestal. Este experimento, desenvolvido nos Viveiros da Universidade Federal do Pará em Altamira, testou os efeitos de vários tipos de substratos com diferentes composições, incluindo proporções variadas de biochar e a inoculação com fungos micorrízicos arbusculares (FMA), com o objetivo de averiguar a tolerância e crescimento da espécie sob essas condições. A aplicação de biochar isoladamente não melhorou o crescimento das mudas, sendo que concentrações mais altas, como 30%, foram prejudiciais para as plantas. A composição com 15% de biochar e inoculação com FMA resultou em mudas maiores após seis meses. Nossos resultados sugerem que o enriquecimento do solo com uma mistura de baixas proporções de biochar e a inoculação de FMA pode melhorar a produtividade de *T. speciosum* e possibilitar a inclusão da espécie em solos que necessitam de remediação de poluição mediada por biochar, mesmo sob temperaturas extremas. No entanto, estudos adicionais são necessários para determinar a melhor forma de manejo do solo.

Palavras-chave: Agrofloresta; Amazônia; cacauí; manejo de solo; restauração florestal.

Introduction

Cacauí, cocoahy or *chocolatillo* (*Theobroma speciosum* Willd. ex Spreng) is an Amazonian wild species of cacao occurring in terra firme and várzea forests all throughout the biome. As one of the most broadly distributed species of the genus, it occurs as far east as the state of Maranhão, in Northeast Brazil, with distribution boundaries reaching Western Bolivia and Peru to the west, the Brazilian state of Tocantins to the south, and Venezuela to the north (Hokche et al., 2008; Jørgensen et al., 2014; Forzza et al., 2024). The species' edible fruits and flowers are an important source of food for local populations (Paz et al., 2021) and fauna (Barbosa et al., 2019), and has the potential both to be one of the substitutes of *T. cacao* as a commercial source of chocolate due to its high fat content (Martini & Tavares, 2005), and to serve as a reservoir of genetic variability, and source of resistant genes against common *Theobroma* diseases, to commercial cocoa cultivars (Dardengo et al., 2021; Siviero et al., 2022; Mar et al., 2024). Nevertheless, in many Amazonian regions, smallholder farmers are incentivised to increase density of traditionally commercial species of *Theobroma*, such as cocoa *T. cacao* and cupuazu *T. grandiflorum*, leading to increased shading and biodiversity loss, which can be mitigated by the agroforestry cultivation of rustic and wild species, such as *T. bicolor* and *T. speciosu*m (Lagneaux et al., 2021). The inclusion of *cacauí* in agroforestry systems, instead of monocultures or high-density commercial plantations, may provide better protection against climate change, incidence of plant diseases, loss of biodiversity and food system collapses, while ensuring the provision of ecosystem services such as the maintenance of pollinators and herbivorous insect control, and more efficient water cycling (Jaimes-Suárez et al., 2022).

In experimental settings, *T. speciosum* showed high sensitivity to contaminated soils from abandoned small-scale gold mining operations, although diameter growth and survivorship of seedlings was improved by the addition of pure and enriched biochar (Román-Dañobeytia et al., 2021). Biochar, the product of controlled pyrolysis of organic waste products from farms or industry, has a negative carbon output and has been hailed as a solution for carbon sequestration in soil (Roberts et al., 2010), as it can be stable in the soil for the long term (H. Wang et al., 2022). The use of biochar soil amendment as remediation has been proven efficient to immobilise contaminants in soils with excessive zinc (Kumar et al., 2018), lead (Yesto et al., 2024), arsenic (Frišták et al., 2024), nickel and chromium (Duwiejuah et al., 2024), perfluorooctanoic acid – PFOA (T. Wang et al., 2024) and microplastics (Debab et al., 2024). Biochar amendment has also been used to restore soil quality in disused landfills, and improved soil pH, organic matter, total organic carbon, water content, and bioavailable nitrogen and phosphorus, leading to an increase in species richness and diversity, and better plant growth, besides allowing for a change in the soil communities of bacteria and arbuscular mycorrhizal fungi – AMF (Chen et al., 2018; Kumar et al., 2018).

Amendments with both biochar and inoculation with AMF have been used to reduce root rot disease in asparagus *Asparagus officinalis* plantations (Elmer & Pignatello, 2011), to improve growth of kangkong *Ipomoea aquatica* and stonecrop *Sedum alfredii* in cadmium-contaminated soils (Hu et al., 2014), to suppress root disease in tomatoes *Solanum lycopersicum* (Akhter et al., 2015), to increase production and decrease cadmium transfer from contaminated soils in maize plantations (Liu et al., 2018), and to improve drought tolerance in chickpea *Cicer arietinum* (Hashem et al., 2019) and okra *Abelmoschus esculentus* plants (Jabborova, Annapurna, et al., 2021).

The synergetic actions of biochar and AMF in correcting soil quality, improving bioavailability of soil nutrients and reducing root disease, however, must be examined before applying the technique in different species of cultivated plants, to ascertain correct combinations and measure beneficial effects in plant growth (Figueira-Galán et al., 2023; T. Li et al., 2024; Wen et al., 2024; T. Zhao et al., 2024). To this end, and considering the ecological and potential economic importance of *T. speciosum*, we devised a preliminary experiment to verify the performance of soil amendments with different proportions of biochar and AMF, alone or in combination, on the growth of *T. speciosum* seedlings in a controlled environment. Given the results achieved in other agricultural species, our experiment aimed at ascertaining the performance of *T. speciosum* under soil amendment conditions with biochar and AMF, and we expected that seedling growth would benefit from the combined use of biochar and AMF, when compared to unaltered soil, in a shade house condition.

Methods

Location and environment

The experiment was conducted at the experimental shade house (3°12'44.16" S, 52°12'48.86" W) of the Altamira Campus of the Federal University of Pará (UFPA), city of Altamira, state of Pará, Brazil. The shade house is a plant nursery structure to regulate light exposure, made of garden shade mesh over pillars that filters 50% of sunlight (Fig. 1), were several species of Amazonian plants are grown. Luminosity, temperature and humidity at the shade house varied between months (Table 1), but only temperature varied significantly (Kruskal-Wallis: x^2 = 16.907, DF = 7, p = 0.02), with May and October being the only pair of months to show a significant difference (Dunn's test: z-score = 3.221, p = 0.018, Fig. 2). There was no significant difference of luminosity, temperature or humidity within the shade house at the same moment.

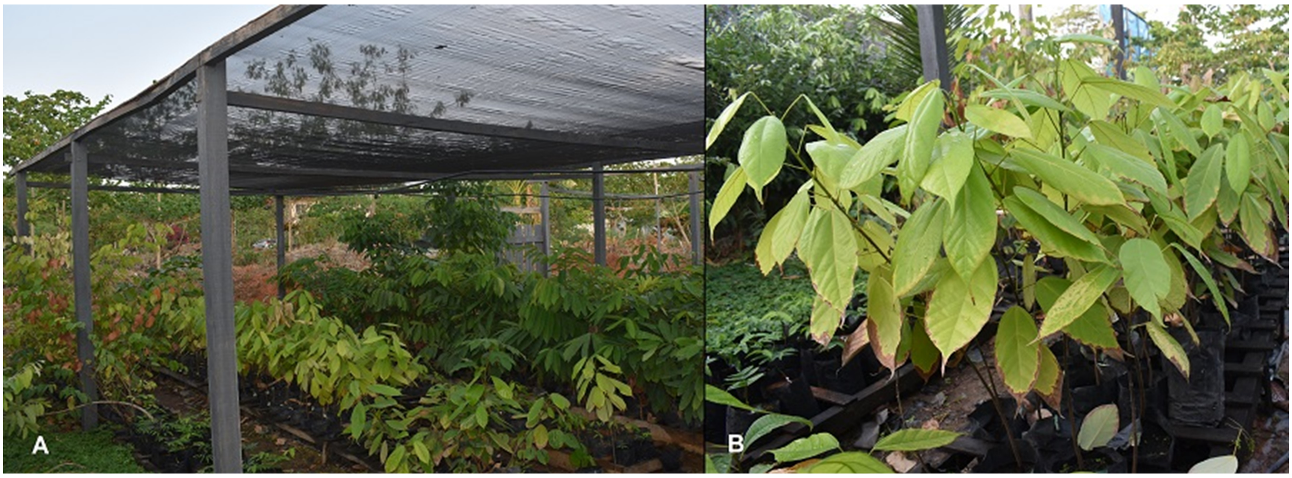

Figure 1: A - Experimental shade house at the Altamira Campus of the Federal University of Pará, in the city of Altamira, Pará, Brazil. B – *Theobroma speciosum* seedlings growing in seedling polyethylene bags, with different types of substrates.

Table 1. Minimum (Min), maximum (Max) and average plus standard deviation (Mean±SD) measurements by month of luminosity (in lux), temperature (in °C) and humidity (in %) of the experimental shade house at the Altamira Campus of the Federal University of Pará, in the city of Altamira, Pará, Brazil.

	Luminosity (Lux)			Temperature (°C)			Humidity (%)		
Month	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
April	465.7	861.3	616.3±161.1	30.5	35.9	34.4±2.3	44.7	71.7	58.3±9.9
May	373.7	1548.3	596.9±296.3	34.5	42.0	38.9±2.3	40.3	70.0	51.3±8.5
June	347.7	612.0	437.4±65.2	35.7	47.8	38.2±3.4	30.7	73.0	53.6±13.3
July	296.3	884.7	569.3±152.6	29.6	47.8	37.9±4.6	30.7	71.0	53.0±12.2
August	362.3	915.7	608.3±141.5	30.2	41.9	36.7±3.7	40.0	70.0	56.9±10.3
September	368.3	1002.3	737.1±237.3	30.7	39.8	35.5±3.2	42.3	70.3	55.6±9.0
October	351.0	1912.7	723.1±438.2	27.8	41.1	34.3±3.9	45.7	81.3	60.8±9.9
November	537.7	1064.7	738.3±212.5	33.5	41.6	37.4±3.1	42.3	284.3	77.6±83.7

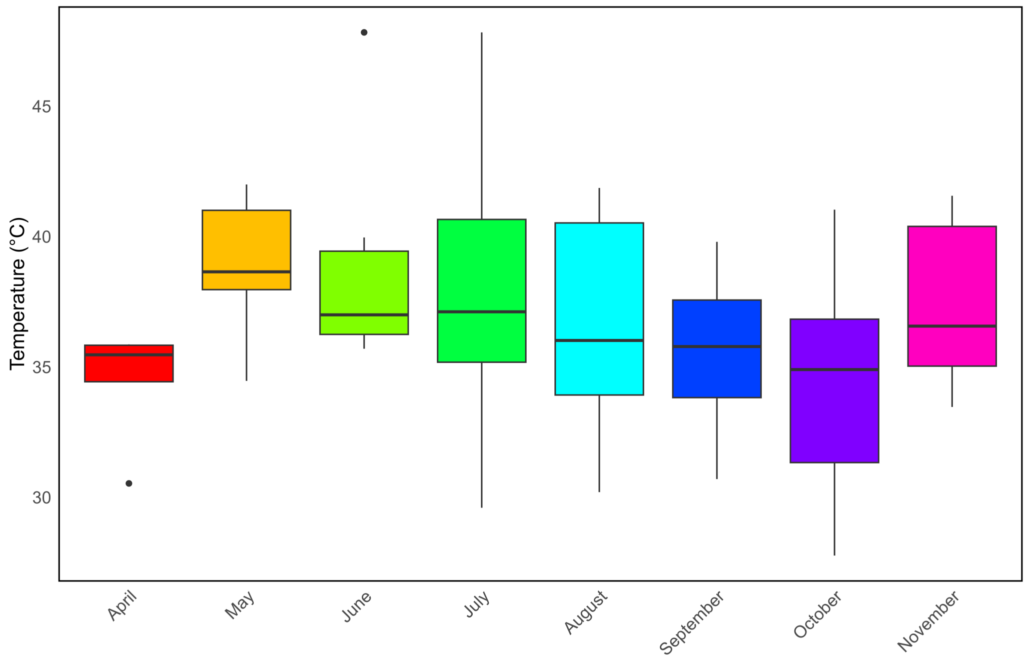

Figure 2: Boxplot of the differences in temperature (in °C) from April to November 2024 at the experimental shade house at the Altamira Campus of the Federal University of Pará, in the city of Altamira, Pará, Brazil. Boxes represent interquartile ranges (IQR), whiskers represent data range, thicker horizontal lines represent the median, and the circle represents an outlier > 1.5 IQR.

AMF cultivation

AMF spores were cultivated using a 1:1 (v/v) mixture of local black soil and sand, serving as a base for inoculum development. Maize seeds previously disinfected by 30-second immersion in 2.5% sodium hypochlorite were then planted in 3-litre pots containing the substrate mixed with spores of arbuscular mycorrhizal fungi (AMF), genera *Glomus* sp., *Acaulospora* sp. and *Rhizophagus* sp., acquired from the microbiology laboratory collection at UFPA, Altamira Campus. The pots were kept in a greenhouse for 60 days to encourage spore multiplication, after which period the aerial parts of the plants were removed, and the pots were sealed and maintained without irrigation for 30 days to stimulate fungal sporulation. Subsequently, the substrate containing the spores was stored under refrigerated conditions (4-8°C) prior to inclusion in the experiment.

Soil preparation and seedling acquisition

A 1:1(v/v) mixture of local black soil and construction-grade sand was prepared, according to modifications to Guerra et al. (2023), and served as control, being denominated Substrate T1. Five other substrates (T2 to T6) were prepared by incorporating enriched biochar and/or AMF inoculated substrate to the T1 mixture, with soils T2 and T3 without AMF addition and with, respectively, 15% and 30% of biochar, while soils T4, T5 and T6 received the addition of 20g of AMF inoculated substrate and, respectively, none, 15% and 30% of biochar (Table 2). The biochar used was of the commercial brand Biochar Brasil®, made by the pyrolysis of wood waste from reforested *Eucalyptus* sp. at 650°C for eight hours, with added chemical elements (Supplementary Table S1). Substrate mixtures were placed in 2.5-liter polyethylene seedling bags and received *Theobroma speciosum* seedlings provided by the Federal University of Pará (UFPA), Altamira Campus, which were transplanted at 30 days after germination. The pH of each substrate was tested at the end of the experiment (November 2024), and again ten months later (September 2025), with the help of a waterproof pH meter from Hanna Instruments®, model PH21.

Table 2. Types of substrates used in the experimental cultivation of *Theobroma speciosum* seedlings, with their respective proportion of biochar (in %), hydrogenionic potential (pH), and absence (N) or presence (Y) of arbuscular mycorrhizal fungi (AMF).

Substrate	Initial number of seedlings	Final number of seedlings	Proportion of biochar (%)	pH	Presence of AMF
T1 (Control)	10	10	0	7.45	N
T2	10	10	15	8.83	N
T3	10	10	30	9.40	N
T4	10	10	0	8.63	Y
T5	10	9	15	8.88	Y
T6	10	10	30	9.19	Y
Total	60	59			

Soil characteristics: field capacity and water retention in experimental substrates

To ascertain the characteristics of water retention in the six substrates used in the experiment, we measured the field capacity (FC) with the standard field method (Souza et al., 2013). Samples of 100 mL of each of the six types of substrates were taken from the cultivation bags, homogenised and sieved to remove large particles. The samples were placed in plastic funnels fitted with filter paper, positioned over graduated cylinders, and had 100 mL of distilled water slowly added to each sample, allowing complete infiltration. The material was left to rest for two hours to allow the drainable fraction to flow out. After this period, the volume of water retained by the substrates was quantified, obtained by the difference between the volume of water applied and the volume drained. Field capacity (FC) was then calculated using the formula:

$$FC = \frac{WR*100}{SV}$$

Where: FC = field capacity of the soil, in percentage of the soil volume tested; WR = water retained, calculated by subtracting the volume of water collected in the graduated cylinder from 100 mL; and SV = soil volume tested, measuring at 100 mL.

To estimate water retention capacity on a gravimetric basis, 100g samples of each one of the six substrates were weighed immediately after saturation with 100mL of distilled water (wet mass, or WM) and then dried at room temperature until a constant mass was reached (dry mass, or DM). From these values, the gravimetric water content was determined according to the equation (Teixeira et al., 2017):

$$GWC = \frac{WM-DM*100}{DM}$$

Where: GWC = Gravimetric water content (in %); WM = wet mass (g); DM = dry mass (in g).

The results of both tests can be verified on Table 3.

Experimental design

A randomised block design was employed, consisting of control (T1) and five treatments (T2 to T6) with ten replicates (R1 to R10) each, totalling 60 plants. Seedlings were measured before transplantation to the growing bags on 26 April 2024 (baseline time, henceforth called M0), and the total seedling height in centimetres (from the soil to the apical bud), diameter of the stem in millimetres and number of leaves in each individual were recorded. Measurements were repeated on 27 July, three months later (or M1), and again on 11 November 2024 (M2), a little over six months after transplantation. All seedlings were freely watered twice daily, in the morning and in the afternoon. Of the 60 seedlings transplanted, all but one (T5R10) survived the experiment. For further analysis, T5R10 was removed from the data.

Table 3. Results of tests of field capacity (FC) with drained volume water after application of 100 mL of distilled water in 100 mL soil samples, and wet mass (WM) and dry mass (DM) and gravimetric water content (GWC) after application of 100mL of distilled water in 100g soil samples of the six types of substrates used in the experimental cultivation of *Theobroma speciosum* seedlings at the Federal University of Pará, Altamira.

Substrate	Drained water volume (mL)	Field Capacity (FC) in %	Wet mass (WM) in g	Dry mass (DM) in g	Gravimetric Water Content (GWC) in %
T1 (Control)	58	42	137.04	89.33	53.40
T2	57	43	138.90	93.22	49.00
T3	77	23	121.93	93.56	30.32
T4	81	19	119.24	93.34	27.74
T5	76	24	123.56	94.49	30.76
T6	79	21	119.34	93.65	27.43

Data Analyses

All statistical analyses and graphs were produced using RStudio version 2024.09.1+394 (RStudio Team, 2024) and R-4.4.2 for Windows “Pile of Leaves” (R Core Team, 2024).

To select the measurements adequate to model the effects of substrates on plant growth, we tested the normality of distribution of height, diameter and number of leaves using the Anderson-Darling test (Anderson & Darling, 1954) on the R package *nortest* (Gross & Ligges, 2015), and checked for correlations within the measurements using the Spearman rank correlation coefficient (Zar, 2014) and a linear model with the *base* R package (R Core Team, 2024). To handle the random effects of individuals and repeated measures over time, and to account for the nested nature of substrate fixed effect, we fitted a linear mixed-effects model (LMM) using restricted maximum likelihood (REML) estimation (Harville, 1977). To test the significance of the fixed effect, we compared the LMM to a null model (containing only random effects) using a likelihood ratio test (LRT) via an analysis of variance (ANOVA) framework. The LRT was based on models refitted with maximum likelihood (ML) to ensure comparability of fixed effects. Both tests were performed with the R package *lme4* (Bates et al., 2015). The R^2 for the LLM was calculated with the package *performance* (Lüdecke et al., 2021) and, as *post hoc* tests, we applied an estimated marginal means (EMM) test using the package *emmeans* (Lenth, 2024). Kruskal-Wallis tests were used for the basic analysis of luminosity, temperature and humidity, and a Dunn’s test with Holm-Bonferroni correction was applied *post hoc* in the significant result (Dinno, 2015). Data was organised with the help of the package *dplyr* and graphs were constructed on *ggplot2*, both part of the package *tidyverse* (Wickham et al., 2019). All p-values were adjusted *post hoc* by the Holm-Bonferroni method (Holm, 1979).

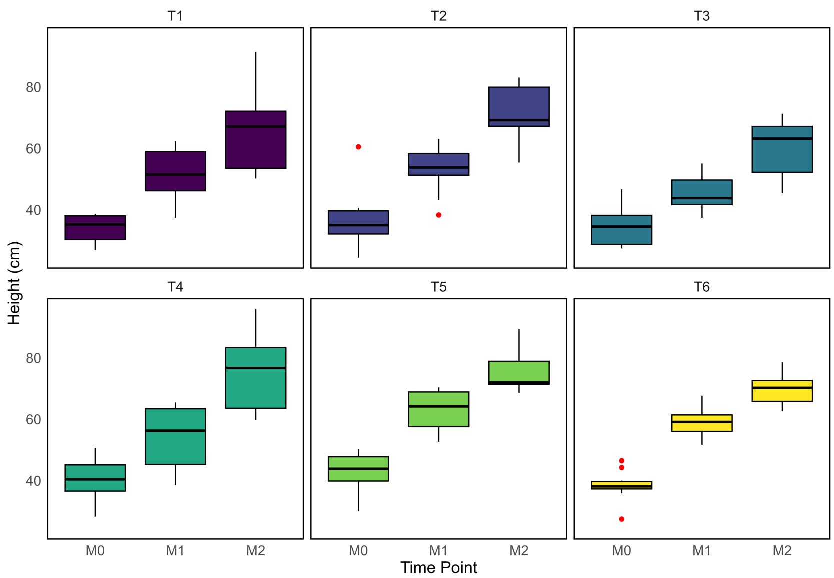

Figure 3: Boxplots of the height, in centimetres, of 59 seedlings of *Theobroma speciosum* planted in six different substrates and measured at baseline (M0), after three months (M1) and after approximately six months (M2). T1 is control. Thicker horizontal lines represent means; red dots represent outliers.

Results

The three measurements taken from the seedlings changed over time and presented diverse configurations in different substrates. Height increased in all substrates, with the spread appearing more restricted in substrate T6, although the initial measurements in M0 did not display a large spread, with very few outliers, while substrate T4 presented the larger range in variation (Fig. 3). Diameter also grew in all substrates, with seedlings in the control substrate T1 appearing to have the same restricted spread effect, with a few outliers, and T5 showing the larger mean after six months (Fig. 4). Number of leaves per seedling showed the least growth over time, with some individuals having fewer leaves after six months in the control substrate and T6, and averages remaining very close in all three points in time (Fig. 5).

To understand what drove the differences between substrate groups, we needed to choose a measure of growth using one or more of the measurements taken, as long as they met the assumptions for statistical modelling (Haase, 2008). Height (Anderson-Darling: A = 1.0545, p = 0.036) and number of leaves (Anderson-Darling: A = 0.9487, p = 0.048) did not present a normal distribution even after transformations by natural log and square root. Stem diameter, however, presented a normal distribution (Anderson-Darling: A = 0.57889, p = 0.262). As plant height and stem diameter were highly correlated (Spearman's correlation: *rho* = 0.870, p < 0.001), stem diameter was chosen as the measure of plant growth in our experiment. A linear regression model between height and diameter confirmed that stem diameter was a significant predictor of plant height ($F_{1,175}$ = 494.9, t = 22.25, p < 0.001, R^2 adj. = 73.7%). While there is some variability unexplained by diameter, the residuals are relatively small compared to the range of height values, further confirming the robustness of stem diameter as a proxy for plant growth (Fig. 6, Table 4).

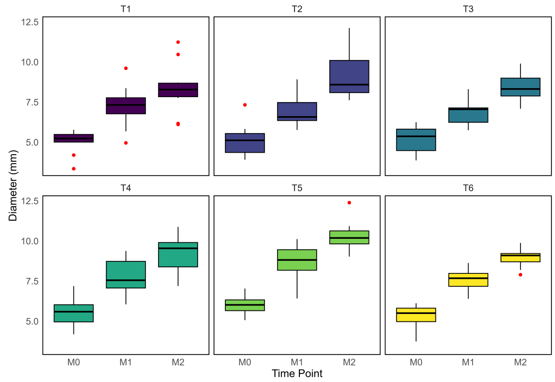

Figure 4: Boxplots of the diameter of the stem, in millimetres, of 59 seedlings of *Theobroma speciosum* planted in six different substrates and measured at baseline (M0), after three months (M1) and after approximately six months (M2). T1 is control. Thicker horizontal lines represent means; red dots represent outliers.

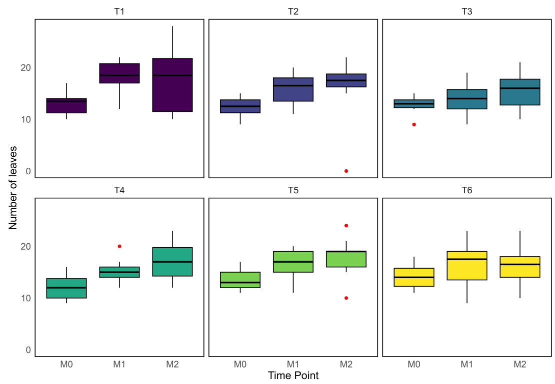

Figure 5: Boxplots of the number of leaves per individual of 59 seedlings of *Theobroma speciosum* planted in six different substrates and measured at baseline (M0), after three months (M1) and after approximately six months (M2). T1 is control. Thicker horizontal lines represent means; red dots represent outliers.

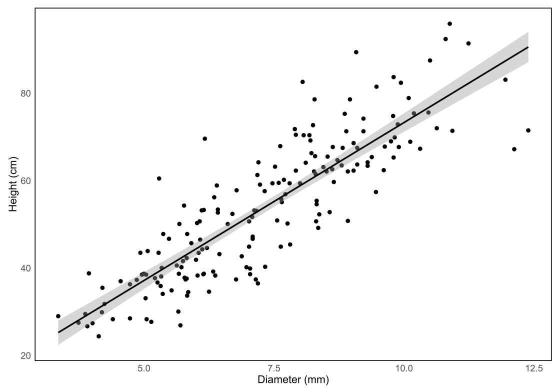

Figure 6: Scatterplot with regression line of the linear regression model of the total height (in centimetres) and stem diameter (in millimetres) of 59 seedlings of *Theobroma speciosum* planted in six types of substrates and measured in three moments in time. Shaded grey area represents CI = 95%.

Effect of Substrate on Seedling Growth

We fitted a linear mixed-effects model to explore the effect of substrate on the diameter of *T. speciosum* seedlings, while accounting for random variation by individual and through time. The results show a significant effect of Substrate T5, with a positive and substantial estimate (1.43), indicating a clear increase in diameter relative to the reference substrate. Other substrate types (T2, T3, T4, and T6) did not show significant effects on diameter, as their estimates had t-values near zero, suggesting no clear deviation from the control substrate, T1.

Model residuals had homogenous variance (Levene Test: F_{59} = 0.6358; p = 1), and did not show any patterns when plotted against fitted values, or when grouped by random factors (Supplementary Figs. S1, S2 and S3).

The likelihood ratio test comparing the full model (with substrate as a fixed effect) to the null model (random effects only) was significant (p = 0.035), indicating that including substrate in the model substantially improved the fit (Table 5). These results suggest that substrate type, particularly Substrate T5, plays an important role in influencing plant diameter growth. Random effect variance, however, indicated that the variance explained by the substrate only was about 5% (Marginal $R^2 = 0.049$), with most of the variation in diameter growth being explained by individual differences, when corrected for time (Conditional $R^2 = 0.914$). Albeit small, the effects of substrate do improve the model and may have significant impact in practical settings.

Table 4. Results of the linear regression model of the total height (in centimetres) and stem diameter (in millimetres) of 59 seedlings of *Theobroma speciosum* planted in six types of substrates and measured in three moments in time.

Coefficient	**Estimate**	**Std. Error**	**t-value**	**p**
(Intercept)	1.0246	2.4553	0.417	0.677
Diameter	7.2276	0.3249	22.246	<0.001
Statistic			Value	
Residual Standard Error			8.227	
Multiple R-squared			0.7388	
Adjusted R-squared			0.7373	
F-statistic			494.4	
p (F-statistic)			< 0.001	

Table 5. Summary of results of a linear mixed-effects model (LMM) using restricted maximum likelihood (REML) estimation, with confidence intervals (CI), of the effects of six types of substrates on 59 seedlings of *Theobroma speciosum*, and likelihood ratio test (ANOVA) of the model against a random-effects-only model (null model). Substrate is a fixed effect, individuals and time are random effects.

Effect	Estimate	Std. Error	t-value	2.5% CI	97.5% CI
sig01 (Ind.)				0.6327375	0.9958833
sig02 (Time)				0.8123784	4.5418840
sigma (Res.)				0.5739589	0.7427755
Intercept	6.88067	1.12223	6.131	4.3685562	9.3927725
Substrate T2	0.25667	0.40999	0.626	-.5242604	1.0375937
Substrate T3	-0.04733	0.40999	-0.115	-.8282604	0.7335937
Substrate T4	0.65667	0.40999	1.602	-0.1242604	1.4375937
Substrate T5	1.42859	0.42122	3.392	0.6262663	2.2309189
Substrate T6	0.40200	0.40999	0.981	-0.3789270	1.1829270
Conditional R2	0.914				
Marginal R2	0.049				
Random Effect Variance	Individual	0.6999			
	Time	3.5261			
Likelihood Ratio Test (p-value)					0.035

Table 6. Measurement of pH of the six types of substrates used in the experimental cultivation of *Theobroma speciosum* seedlings at the Federal University of Pará, Altamira, measured after the experiment and ten months later with a waterproof pH meter from Hanna Instruments®, model PH21, and the difference over time.

Substrate	**pH (28 Nov 2024)**	**pH (23 Sep 2025)**	**Difference in pH**
T1 (Control)	7.45	6.27	-1.18
T2	8.83	7.53	-1.3
T3	9.40	8.06	-1.34
T4	8.83	7.38	-1.45
T5	8.88	6.95	-1.93
T6	9.19	8.68	-0.51

Post hoc Estimated marginal means (EMMs) indicated that T5 had the largest average diameter (8.31 ± 1.13), while T3 had the smallest (6.83 ± 1.12). Pairwise comparisons showed T5 was significantly larger than T1 (estimate = -1.43, SE = 0.42, p = 0.0158) and T3 (estimate = -1.48, SE = 0.42, p = 0.0115). Substrate T5 also had the highest confidence interval bounds (Supplementary Table S2), despite still overlapping with the other substrates, indicating a visible effect (Fig. 7). The estimated diameter differences were most prominent between control T1 and T5, T3 and T5, and T5 and T6 with only the first two being significant (T1 – T5: t-ratio = -3.392, p = 0.016; T3 – T5: t-ratio = -3.504, p = 0.012). The substrate T5, with 15% biochar and AMF inoculum, performed better than both control T1 and the substrate T3, with 30% biochar (Fig. 8).

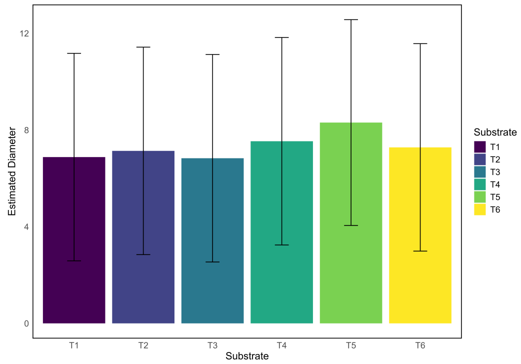

Figure 7: Estimated diameter of 59 seedlings of *Theobroma speciosum* planted in six types of substrates, after correcting for random factors of individual plants and three moments in time.

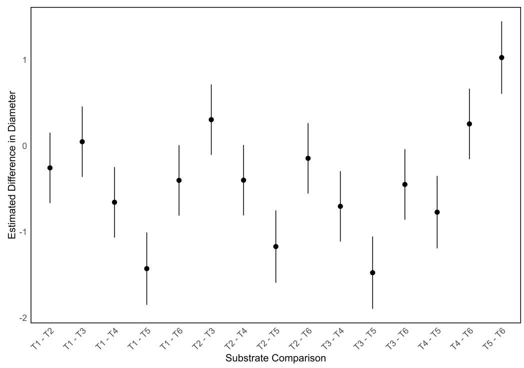

Figure 8: Estimated difference in diameter of 59 seedlings of *Theobroma speciosum* planted in six types of substrates, after correcting for random factors of individual plants and three moments in time.

Changes in substrate pH

The pH of the six experimental substrates changed from the end of the experiment to ten months later. Immediately after the end of the experiment, on 28 November 2024, all substrates were alkaline, with soil T1 (control) almost neutral (pH 7.45), and the others with increasing alkalinity corresponding to the amount of biochar added. After ten months, on 23 September 2025, all soils had a slight decrease in pH, with larger differences appearing in substrates T4 (no biochar and with addition of AMF) and T5 (15% biochar with addition of AMF). The measurements of pH and the different over time can be seen on Table 6.

Discussion

Theobroma speciosum is commonly found throughout the Amazonia biome within *terra firme* and dry *várzea* forests, where it is frequently collected by local populations, but it is also cultivated by smallholder farmers in spontaneous orchards and agroforests from seeds collected in the wild or exchanged between growers due to its high commercial value (Lagneaux et al., 2021). Besides being a potential substitute for *T. cacao* in chocolate production due to its fat seed reserves and phylogenetic proximity to the commercial variety (Martini & Tavares, 2005; Sousa-Silva & Figueira, 2005), the *cacaú* is hailed as a source of antioxidants and nutritional supplements (Mar et al. 2021), being the only species among 20 of the genus Theobroma to have significant levels of quercetin-3-*0*-glucoside (Mar et al., 2024), a flavonoid with significant anti-leishmanial effects (Iqbal et al., 2022). Furthermore, the species appears to be less affected by widespread *Theobroma* fungal diseases such as witches' broom, dry rot, cushion gall, pod rot, black pod and *Acremonium* brown spot, despite still being susceptible to leaf anthracnose cause by *Colletotrichum* sp. (Siviero et al., 2022). However, as a wild species with domestic cultivation restricted to rustic methods, there is a dearth of agricultural research on *T. speciosum*.

Our study, as the first in this topic, is pioneering the understanding of this important species, especially its interactions with new methods of soil amendment that will become increasingly necessary in the future. The role of biochar soil amendment in correcting pollutant contamination is crucial to the Amazon region due to the widespread soil pollution coming from urban settlements and industrial activities, especially mining, which deposits mercury in unsafe levels near waterways (Medeiros et al., 2017; Fernandes et al., 2018; Matos et al., 2023). Furthermore, biochar associated with AMF inoculation in soil appears to increase drought resistance in several agricultural species (Hashem et al., 2019; Jabborova, Annapurna, et al., 2021; Jabborova et al., 2022; Chauhan et al., 2024), and species with potential to be included in agroforestry and sustainable agricultural systems, such as the *cacaú*, need urgent research to combat threats to local and global food systems (Gomez-Zavaglia et al., 2020; Wijerathna-Yapa & Pathirana, 2022; Zurek et al., 2022).

In our study, *T. speciosum* seedlings grew as expected in the six-month interval under a 50% shading net, both in height and diameter, showing no signs of etiolation, thus confirming the adequacy of luminosity conditions (Armarego-Marriott et al., 2020). Despite very high peaks of temperature in the period, reaching an average maximum of 47.8°C in June and July, continuous growth confirmed the resistance of the species to high temperatures.

Only one specimen died during the period, again showing the species' resilience for agricultural purposes. As climate change increases temperatures worldwide, crop losses of traditional agricultural species can reach over 50%, as very few can resist temperatures above 40°C without major physiological changes (Saeed et al., 2023). Diversifying food systems to include species naturally resistant to higher temperatures can ensure food security in extreme weather (Hertel et al., 2021).

All experimental substrates were well-tolerated by the seedlings, despite a large increase in soil pH where biochar was added. Biochar is well-known for ameliorating pH in acidic soils (Chintala et al., 2014; Mosharrof et al., 2021; Zhang et al., 2025), with even stronger effects in controlled environments (Singh et al., 2022), but the alkalinisation of the already slightly-alkaline local soil (pH 7.45) did not appear to have an overall detrimental effect in seedling growth, except in the extremely alkalinised T3 substrate (pH 9.40), amended with 30% of biochar and lacking AMF inoculation, which had the smallest growth of all experimental substrates. Detrimental effects of excessive biochar amendment were reported in several species, including reduction of yield, alkalinisation, nutrient precipitation, excess nitrogen and sodium, and changes in soil microbiota (Mukherjee & Lal, 2014; Sun et al., 2020; Brtnicky et al., 2021). Many of these problems can be corrected with the concomitant use of fertilisers, as shown in ornamental *Viola* plants (Regmi et al., 2022), but it is sensible to examine each soil, and species requirements, before amending soils with biochar, given its very long permanence in the ground (Joseph et al., 2021). The inoculation of AMF is also known to increase pH and is often used as remediation to soil acidity (S. Zhao et al., 2024; T. Zhao et al., 2024), a result repeated in our study in substrate T4, which, even without biochar, was more alkaline than the control substrate T1.

When the pH of the substrates was rechecked after ten months, there was a decline in the pH, especially in the substrate T5, with AMF inoculation and 15% biochar, and T4, with AMF and no biochar. This decline in pH can be explained by the watering regime of the experimental cultivation, and the natural decomposition of organic matter. As the plants received free watering twice a day, it could have led to leaching of base cations, as it happens in areas with heavy rainfall, like the Amazon basin, where soils are generally acidic due to this effect (Tusar et al., 2023). This indicates that the synergies between biochar, water availability and nutrients potentially affect soil pH in the long term, and frequent monitoring is needed to avoid excessive soil acidification.

The amendment with biochar increased slightly the field capacity of substrate T2 (15% biochar without AMF) in relation to substrate T1 (control), while all others had smaller FC percentages than T1. Also, the gravimetric water content (GWC) of all amended substrates was lower than the control, with substrate T6, with 30% biochar and AMF inoculation, presenting the lower percentage of GWC, at 27.43%. Three recent reviews of the relation between biochar amendment and water retention in soils, mainly in agricultural settings, showed a large variation between several water-related measurements and biochar application. For example, a meta-analysis of 37 papers on biochar and water soil retention in both experimental and field conditions showed a strong effect of soil texture, with an increase in water retention and availability in coarse, sandy soils in experimental conditions and negligible effects in soils with more clay, as these have fewer spaces between particles in which biochar can lodge and retain water (Edeh et al., 2020). A similar result was found in another systematic review of 176 distinct experiments, with higher increase in water availability and retention of loosely-compacted, coarse and sandy soils and negligible results, or even decreased percentages, in soils with higher compaction (Razzaghi et al., 2020). A study on sandy soils repeated the results, with an increase in water retention percentages in loosely-compacted soils, which benefitted from biochar amendment to create resilience to droughts (L. Li et al., 2021). Similar soil-dependent effects on water retention and hydraulic conductivity was found in a study with maize: AMF inoculation increased water retention in sandy soils, while improving drainage in more compacted substrates (Pauwels et al., 2023). In our research, the substrates were a 1:1 mixture of fine sand and black, fine soil high in organic matter, which, as found in the reviews, do not appear to have significant increases in FC and GWC after biochar amendment, due to fewer spaces in which biochar particles can be lodged, and may have a decrease in FC and GWC after AMF inoculation, due to higher evaporation and water use by the plants.

In our experiment, a significant diameter growth increase was found in seedlings planted on substrate T5, with 15% biochar and AMF inoculum. Positive results were found with the use of moderate biochar amendment (1-15%) and AMF inoculation (Mickan et al., 2016; Hashem et al., 2019; Solaiman et al., 2019; Meng et al., 2023). A study in the tropics found optimum concentrations of biochar to increase leafy vegetables production to be 20-30% (Shen et al., 2020), and concentrations as high as 100% were tested on the germination of agricultural seeds, with concentrations between 10% and 25% showing positive effects for different species (Carril et al., 2023). In our study, the lack of positive effects in the soil treated with higher concentrations of biochar may indicate that these are not adequate for this species, although these effects may be offset by the presence of AMF in the soil, including in soils that appear to have lower water retention and availability, such as substrate T5 (Kakouridis et al., 2022). Further research is necessary to ascertain the best concentrations and combinations of biochar and AMF to increase growth in *T. speciosum* seedlings.

There were no significant differences in the number of leaves in the *T. speciosum* seedlings, although an increase of leaf number in biochar-amended soils was found in ginger *Zingiber officinale* (Jabborova, Wirth, et al., 2021), groundnut *Arachis hypogaea* (Yusif et al., 2016) and tobacco *Nicotiana tabacum* (Yang et al., 2024); these effects were found only in concentrations $< 5\%$ and ceased in higher values. Two scenarios may explain the lack of increase in the number of leaves in our experiment: the first is that *T. speciosum* is a slow growth species (Barbosa et al., 2019), needing a longer time than our experiment to stabilise under 50% shading conditions, as found in slow-growing maple *Acer saccharum* seedlings cultivated under low luminosity (Bonser & Aarssen, 1994); the second is that biochar concentrations were too high for the species, and the number of leaves were registered still during the initial phase of biochar amendment (up to six months), as biochar ages in the soil and changes both its composition and microbiota, with better long-term results in several crops after one biochar application (Joseph et al., 2021).

Conclusion

Overall, our study showed that soil amendment with biochar is well tolerated by *T. speciosum* seedlings even in high concentrations, and there are synergies between moderate concentrations of biochar and arbuscular mycorrhizal fungi that enhance growth of seedlings in controlled conditions. This opens the possibility of using the *cacauí* as an agroforestry species, diversifying food production even in soils that need remediation against pollutants and in the event of higher temperatures and less water availability.

Acknowledgements

KEVS would like to thank Alexandra Elbakyan, Ijad Madisch, Sören Hofmayer and Horst Fickenscher for their contributions to science, and to the community at Stack Overflow for helping with R coding. EJHR would like to thank for the partial funding generously provided by the Brazilian Biodiversity Fund (FUNBIO) through the *Floresta Viva* initiative, and for financial support from the Brazilian National Bank for Economic and Social Development (BNDES) and Energisa, Fundo Vale, Norte Energia, KFW and Reset Assurini Project.

References

Akhter, A., Hage-Ahmed, K., Soja, G., & Steinkellner, S. (2015). Compost and biochar alter mycorrhization, tomato root exudation, and development of *Fusarium oxysporum* f. sp. *lycopersici*. *Frontiers in Plant Science*, *6*, 529. https://doi.org/10.3389/fpls.2015.00529

Anderson, T. W., & Darling, D. A. (1954). A test of goodness of fit. *Journal of the American Statistical Association*, *49*(268), 765–769. https://doi.org/10.1080/01621459.1954.10501232

Armarego-Marriott, T., Sandoval-Ibañez, O., & Kowalewska, Ł. (2020). Beyond the darkness: recent lessons from etiolation and de-etiolation studies. *Journal of Experimental Botany*, *71*(4), 1215–1225. https://doi.org/10.1093/jxb/erz496

Barbosa, L., França, I., & Ruz, E. H. (2019). Primer registro de la dispersión de frutos de *Theobroma speciosum*. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, *43*(168), 518–520. https://doi.org/10.18257/raccefyn.891

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Bonser, S. P., & Aarssen, L. W. (1994). Plastic allometry in young sugar maple (*Acer saccharum*): adaptive responses to light availability. *American Journal of Botany*, *81*(4), 400–406. https://doi.org/10.1002/j.1537-2197.1994.tb15463.x

Brtnicky, M., Datta, R., Holatko, J., Bielska, L., Gusiatin, Z. M., Kucerik, J., Hammerschmiedt, T., Danish, S., Radziemska, M., & Mravcova, L. (2021). A critical review of the possible adverse effects of biochar in the soil environment. *Science of the Total Environment*, *796*, 148756. https://doi.org/10.1016/j.scitotenv.2021.148756

Carril, P., Ghorbani, M., Loppi, S., & Celletti, S. (2023). Effect of biochar type, concentration and washing conditions on the germination parameters of three model crops. *Plants*, *12*, 2235. https://doi.org/10.3390/plants12122235

Chauhan, P. K., Upadhyay, S. K., Rajput, V. D., Dwivedi, P., Minkina, T., & Wong, M. H. (2024). Fostering plant growth performance under drought stress using rhizospheric microbes, their gene editing, and biochar. *Environ Geochem Health*, *46*(2), 41. https://doi.org/10.1007/s10653-023-01823-1

Chen, X. W., Wong, J. T. F., Chen, Z. T., Tang, T. W. L., Guo, H. W., Leung, A. O. W., Ng, C. W. W., & Wong, M. H. (2018). Effects of biochar on the ecological performance of a subtropical landfill. *Science of the Total Environment*, *644*, 963–975. https://doi.org/10.1016/j.scitotenv.2018.06.379

Chintala, R., Mollinedo, J., Schumacher, T. E., Malo, D. D., & Julson, J. L. (2014). Effect of biochar on chemical properties of acidic soil. *Archives of Agronomy and Soil Science*, *60*(3), 393–404. https://doi.org/10.1080/03650340.2013.789870

Dardengo, J. d. F. E., Rossi, A. A. B., Oliveira, L. O. d., Pena, G. F., Rivas, L. H., Silva, C. J. d., & Rufatto, F. P. (2021). Structure and genetic diversity of *Theobroma speciosum* (Malvaceae) and implications for Brazilian Amazon conservation. *Rodriguésia*, *72*, e02022018. https://doi.org/10.1590/2175-7860202172023

Debab, A., Boudjabi, S., Chenchouni, H., Ababsa, N., & Brahimi, A. (2024). Effects of incorporating biochar on soil quality and barley yield in microplastics-contaminated soils. *Chemosphere*, *368*, 143760. https://doi.org/10.1016/j.chemosphere.2024.143760

Dinno, A. (2015). Nonparametric pairwise multiple comparisons in independent groups using Dunn's test. *The Stata Journal*, *15*(1), 292–300. https://doi.org/10.1177/1536867X1501500117

Duwiejuah, A. B., Mutawakil, Z., & Oyelude, E. O. (2024). Eco-friendly banana peel biochar for adsorption of toxic metals from landfill treatment pond leachate. *International Journal of Phytoremediation*, *27*(5), 596–605. https://doi.org/10.1080/15226514.2024.2428434

Edeh, I. G., Mašek, O., & Buss, W. (2020). A meta-analysis on biochar's effects on soil water properties–New insights and future research challenges. *Science of the Total Environment*, *714*, 136857. https://doi.org/10.1016/j.scitotenv.2020.136857

Elmer, W. H., & Pignatello, J. J. (2011). Effect of biochar amendments on mycorrhizal associations and *Fusarium* crown and root rot of *Asparagus* in replant soils. *Plant Disease*, *95*(8), 960–966. https://doi.org/10.1094/PDIS-10-10-0741

Fernandes, A. R., Souza, E. S., Souza Braz, A. M., Birani, S. M., & Alleoni, L. R. F. (2018). Quality reference values and background concentrations of potentially toxic elements in soils from the Eastern Amazon, Brazil. *Journal of Geochemical Exploration*, *190*, 453–463. https://doi.org/10.1016/j.gexplo.2018.04.012

Figueira-Galán, D., Heupel, S., Duelli, G., Morgano, M. T., Stapf, D., & Requena, N. (2023). Exploring the synergistic effects of biochar and arbuscular mycorrhizal fungi on phosphorus acquisition in tomato plants by using gene expression analyses. *Science of the Total Environment*, *884*, 163506. https://doi.org/10.1016/j.scitotenv.2023.163506

Forzza, R., Zappi, D., & Souza, V. (2024). *Theobroma speciosum* Willd. ex Spreng. *In*: Flora e Funga do Brasil. Jardim Botânico do Rio de Janeiro. http://servicos.jbrj.gov.br/flora/search/Theobroma_speciosum

Frišták, V., Beliančínová, K., Polt'áková, L., Moreno-Jimenéz, E., Zimmerman, A. R., Ďuriška, L., Černičková, I., Laughinghouse IV, H. D., & Pipíška, M. (2024). Engineered Mg-modified biochar-based sorbent for arsenic separation and pre-concentration. *Scientific Reports*, *14*(1), 28680. https://doi.org/10.1038/s41598-024-79446-4

Gomez-Zavaglia, A., Mejuto, J. C., & Simal-Gandara, J. (2020). Mitigation of emerging implications of climate change on food production systems. *Food Research International*, *134*, 109256. https://doi.org/10.1016/j.foodres.2020.109256

Gross, J., & Ligges, U. (2015). *Nortest: Tests for normality* [R package version 1.0-4]. https://CRAN.R-project.org/package=nortest

Guerra, T., Hamada, M., Serra, A., Hernández-Ruz, E., Leão, F., Costa, W., Ferreira, T., Stehlin, A., Costa, G., Nascimento, N., Sousa, N., Lima, A., Lima, K., Pinto, R., & Araújo, E. (2023). *Manual de Boas Práticas para a Restauração Ecológica da Volta Grande do Xingu*. Biocev Projetos Inteligentes, Belo Horizonte.

Haase, D. L. (2008). Understanding forest seedling quality: measurements and interpretation. *Tree Planters' Notes*, *52*(2), 24–30.

Harville, D. A. (1977). Maximum likelihood approaches to variance component estimation and to related problems. *Journal of the American Statistical Association*, *72*(358), 320–338. https://doi.org/10.1080/01621459.1977.10480998

Hashem, A., Kumar, A., Al-Dbass, A. M., Alqarawi, A. A., Al-Arjani, A.-B. F., Singh, G., Farooq, M., & Abd_Allah, E. F. (2019). Arbuscular mycorrhizal fungi and biochar improves drought tolerance in chickpea. *Saudi Journal of Biological Sciences*, *26*(3), 614–624. https://doi.org/10.1016/j.sjbs.2018.11.005

Hertel, T., Elouafi, I., Tanticharoen, M., & Ewert, F. (2021). Diversification for enhanced food systems resilience. *Nat Food*, 832–834. https://doi.org/10.1038/s43016-021-00403-9

Hokche, O., Berry, P. E., & Huber, O. (2008). *Nuevo catálogo de la flora vascular de Venezuela* (Vol. 1). Fundación Instituto Botánico de Venezuela Caracas.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, 65–70. https://www.jstor.org/stable/4615733

Hu, J., Wu, F., Wu, S., Lam, C. L., Lin, X., & Wong, M. H. (2014). Biochar and Glomus caledonium influence Cd accumulation of upland kangkong (*Ipomoea aquatic* Forsk.) intercropped with Alfred stonecrop (*Sedum alfredii* Hance). *Sci Rep*, *4*(1), 4671. https://doi.org/10.1038/srep04671

Iqbal, K., Noor, S., Shah, A., & Amin, A. (2022). Assessment of *in vitro* and *in vivo* effect of Quercetin 3-Glucoside, Oxyresveratrol and Quercetin O-Hexoside against *Leishmania tropica*. *Brazilian Journal of Pharmaceutical Sciences*, *58*, e21306. https://doi.org/10.1590/s2175-97902022e21306

Jabborova, D., Annapurna, K., Al-Sadi, A. M., Alharbi, S. A., Datta, R., & Zuan, A. T. K. (2021). Biochar and Arbuscular mycorrhizal fungi mediated enhanced drought tolerance in Okra (*Abelmoschus esculentus*) plant growth, root morphological traits and physiological properties. *Saudi Journal of Biological Sciences*, *28*(10), 5490–5499. https://doi.org/10.1016/j.sjbs.2021.08.016

Jabborova, D., Annapurna, K., Azimov, A., Tyagi, S., Pengani, K. R., Sharma, P., Vikram, K., Poczai, P., Nasif, O., & Ansari, M. J. (2022). Co-inoculation of biochar and arbuscular mycorrhizae for growth promotion and nutrient fortification in soybean under drought conditions. *Frontiers in Plant Science*, *13*, 947547. https://doi.org/10.3389/fpls.2022.947547

Jabborova, D., Wirth, S., Halwani, M., Ibrahim, M. F., Azab, I. H. E., El-Mogy, M. M., & Elkelish, A. (2021). Growth response of ginger (*Zingiber officinale*), its physiological properties and soil enzyme activities after biochar application under greenhouse conditions. *Horticulturae*, *7*(8), 250. https://doi.org/10.3390/horticulturae7080250

Jaimes-Suárez, Y. Y., Carvajal-Rivera, A. S., Galvis-Neira, D. A., Carvalho, F. E., & Rojas-Molina, J. (2022). Cacao agroforestry systems beyond the stigmas: Biotic and abiotic stress incidence impact. *Frontiers in Plant Science*, *13*, 921469. https://doi.org/10.3389/fpls.2022.921469

Jørgensen, P., Nee, M., Beck, S., Garden, M. B., de Bolivia, H. N., Cárdenas, H. N. F. M., del Oriente Boliviano, H., & Garden, N. Y. B. (2014). *Catálogo de las plantas vasculares de Bolivia*. Missouri Botanical Garden Press, St. Louis, Missouri.

Joseph, S., Cowie, A. L., Van Zwieten, L., Bolan, N., Budai, A., Buss, W., Cayuela, M. L., Graber, E. R., Ippolito, J. A., & Kuzyakov, Y. (2021). How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. *GCB Bioenergy*, *13*(11), 1731–1764. https://doi.org/10.1111/gcbb.12885

Kakouridis, A., Hagen, J. A., Kan, M. P., Mambelli, S., Feldman, L. J., Herman, D. J., Weber, P. K., Pett-Ridge, J., & Firestone, M. K. (2022). Routes to roots: direct evidence of water transport by arbuscular mycorrhizal fungi to host plants. *New Phytologist*, *236*(1), 210–221. https://doi.org/10.1111/nph.18281

Kumar, A., Joseph, S., Tsechansky, L., Privat, K., Schreiter, I. J., Schüth, C., & Graber, E. R. (2018). Biochar aging in contaminated soil promotes Zn immobilization due to changes in biochar surface structural and chemical properties. *Science of the Total Environment*, *626*, 953–961. https://doi.org/10.1016/j.scitotenv.2018.01.157

Lagneaux, E., Andreotti, F., & Neher, C. M. (2021). Cacao, copoazu and macambo: Exploring *Theobroma* diversity in smallholder agroforestry systems of the Peruvian Amazon. *Agroforestry Systems*, *95*(7), 1359–1368. https://doi.org/10.1007/s10457-021-00610-0

Lenth, R. (2024). emmeans: Estimated Marginal Means, aka Least-Squares Means. *R package version 1.8. 5*.

Li, L., Zhang, Y.-J., Novak, A., Yang, Y., & Wang, J. (2021). Role of biochar in improving sandy soil water retention and resilience to drought. *Water*, *13*(4), 407. https://doi.org/10.3390/w13040407

Li, T., Yang, H., Zhang, N., Dong, L., Wu, A., Wu, Q., Zhao, M., Liu, H., Li, Y., & Wang, Y. (2024). Synergistic effects of arbuscular mycorrhizal fungi and biochar are highly beneficial to *Ligustrum lucidum* seedlings in Cd-contaminated soil. *Environmental Science and Pollution Research*, *31*(7), 11214–11227. https://doi.org/10.1007/s11356-024-31870-9

Liu, L., Li, J., Yue, F., Yan, X., Wang, F., Bloszies, S., & Wang, Y. (2018). Effects of arbuscular mycorrhizal inoculation and biochar amendment on maize growth, cadmium uptake and soil cadmium speciation in Cd-contaminated soil. *Chemosphere*, *194*, 495–503. https://doi.org/10.1016/j.chemosphere.2017.12.025

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software*, *6*(60), 3139. https://doi.org/10.21105/joss.03139

Mar, J. M., Fonseca Júnior, E. Q., Corrêa, R. F., Campelo, P. H., Sanches, E. A., & Araújo Bezerra, J. (2024). *Theobroma* spp.: a review of it's chemical and innovation potential for the food industry. *Food Chemistry Advances*, *4*, 100683. https://doi.org/10.1016/j.focha.2024.100683

Martini, M., & Tavares, D. (2005). Seed reserves from seven species of the genus *Theobroma*: a review. *Rev. Inst. Adolfo Lutz*, *64*, 10–19.

Matos, G. S. B., Neto, A. B. B., Gama, M. A. P., Gonçalves, D. A. M., Cardoso, D. F. S. R., & Ramos, H. M. N. (2023). Soil potentially toxic element contents in an area under different land uses in the Brazilian Amazon. *Heliyon*, *9*(6), e17108. https://doi.org/10.1016/j.heliyon.2023.e17108

Medeiros, A. C., Faial, K. R. F., Faial, K. C. F., Silva Lopes, I. D., Oliveira Lima, M., Guimarães, R. M., & Mendonça, N. M. (2017). Quality index of the surface water of Amazonian rivers in industrial areas in Pará, Brazil. *Marine Pollution Bulletin*, *123*, 156–164. https://doi.org/10.1016/j.marpolbul.2017.09.002

Meng, L., Wu, Y., Mu, M., Wang, Z., Chen, Z., Wang, L., Ma, Z., Cui, G., & Yin, X. (2023). Effects of different concentrations of biochar amendments and Pb toxicity on rhizosphere soil characteristics and bacterial community of red clover (*Trifolium pretense* L.) *Frontiers in Plant Science*, *14*, 1112002. https://doi.org/10.3389/fpls.2023.1112002

Mickan, B. S., Abbott, L. K., Stefanova, K., & Solaiman, Z. M. (2016). Interactions between biochar and mycorrhizal fungi in a water-stressed agricultural soil. *Mycorrhiza*, *26*(6), 565–574. https://doi.org/10.1007/s00572-016-0693-4

Mosharrof, M., Uddin, M. K., Jusop, S., Sulaiman, M. F., Shamsuzzaman, S., & Haque, A. N. A. (2021). Changes in acidic soil chemical properties and carbon dioxide emission due to biochar and lime treatments. *Agriculture*, *11*(3), 219. https://doi.org/10.3390/agriculture11030219

Mukherjee, A., & Lal, R. (2014). The biochar dilemma. *Soil Research*, *52*(3), 217–230. https://doi.org/10.1071/SR13359

Pauwels, R., Graefe, J., & Bitterlich, M. (2023). An arbuscular mycorrhizal fungus alters soil water retention and hydraulic conductivity in a soil texture specific way. *Mycorrhiza*, *33*(3), 165–179. https://doi.org/10.1007/s00572-023-01106-8

Paz, F. S., Pinto, C. E., Brito, R. M., Imperatriz-Fonseca, V. L., & Giannini, T. C. (2021). Edible fruit plant species in the Amazon forest rely mostly on bees and beetles as pollinators. *Journal of Economic Entomology*, *114*(2), 710–722. https://doi.org/10.1093/jee/toaa284

R Core Team. (2024). *R: A language and environment for statistical computing*. https://www.r-project.org/

Razzaghi, F., Obour, P. B., & Arthur, E. (2020). Does biochar improve soil water retention? A systematic review and meta-analysis. *Geoderma*, *361*, 114055. https://doi.org/10.1016/j.geoderma.2019.114055

Regmi, A., Singh, S., Moustaid-Moussa, N., Coldren, C., & Simpson, C. (2022). The negative effects of high rates of biochar on violas can be counteracted with fertilizer. *Plants*, *11*(4), 491. https://doi.org/10.3390/plants11040491

Roberts, K. G., Gloy, B. A., Joseph, S., Scott, N. R., & Lehmann, J. (2010). Life cycle assessment of biochar systems: estimating the energetic, economic, and climate change potential. *Environmental Science & Technology*, *44*(2), 827–833. https://doi.org/10.1021/es902266r

Román-Dañobeytia, F., Cabanillas, F., Lefebvre, D., Farfan, J., Alferez, J., Polo-Villanueva, F., Llacsahuanga, J., Vega, C. M., Velasquez, M., & Corvera, R. (2021). Survival and early growth of 51 tropical tree species in areas degraded by artisanal gold mining in the Peruvian Amazon. *Ecological Engineering*, *159*, 106097. https://doi.org/10.1016/j.ecoleng.2020.106097

RStudio Team. (2024). Rstudio: Integrated development environment for r. *Boston, MA*, *770*(394), 165–171.

Saeed, F., Chaudhry, U. K., Raza, A., Charagh, S., Bakhsh, A., Bohra, A., Ali, S., Chitikineni, A., Saeed, Y., & Visser, R. G. (2023). Developing future heat-resilient vegetable crops. *Functional & Integrative Genomics*, *23*(1), 47. https://doi.org/10.1007/s10142-023-00967-8

Shen, Y., Song, S., Thian, B. W. Y., Fong, S. L., Ee, A. W. L., Arora, S., Ghosh, S., Li, S. F. Y., Tan, H. T. W., & Dai, Y. (2020). Impacts of biochar concentration on the growth performance of a leafy vegetable in a tropical city and its global warming potential. *Journal of Cleaner Production*, *264*, 121678. https://doi.org/10.1016/j.jclepro.2020.121678

Singh, H., Northup, B. K., Rice, C. W., & Prasad, P. V. (2022). Biochar applications influence soil physical and chemical properties, microbial diversity, and crop productivity: a meta-analysis. *Biochar*, *4*(1), 8. https://doi.org/10.1007/s42773-022-00138-1

Siviero, A., Macedo, P. E. F., & Moreira, G. T. S. (2022). Doenças em cacaueiro e cupuaçuzeiro no Acre. *Agrotrópica*, *34*(2), 159–164. https://doi.org/10.21757/0103-3816.2022v34n2p159-164

Solaiman, Z. M., Abbott, L. K., & Murphy, D. V. (2019). Biochar phosphorus concentration dictates mycorrhizal colonisation, plant growth and soil phosphorus cycling. *Scientific Reports*, *9*(1), 5062. https://doi.org/10.1038/s41598-019-41671-7

Sousa-Silva, C., & Figueira, A. (2005). Phylogenetic analysis of *Theobroma* (Sterculiaceae) based on Kunitz-like trypsin inhibitor sequences. *Plant Systematics and Evolution*, *250*(1), 93–104. https://doi.org/10.1007/s00606-004-0223-2

Souza, E. J., Cunha, F. F., Magalhães, F. F., Silva, T. R., Borges, M. R. Z., & Roque, C. G. (2013). Métodos para estimativa da umidade do solo na capacidade de campo. *Revista de Ciências Agro-Ambientais, Alta Floresta-MT*, *11*(1), 43–50.

Sun, J., Li, W., Li, C., Chang, W., Zhang, S., Zeng, Y., Zeng, C., & Peng, M. (2020). Effect of different rates of nitrogen fertilization on crop yield, soil properties and leaf physiological attributes in banana under subtropical regions of China. *Frontiers in Plant Science*, *11*, 613760. https://doi.org/10.3389/fpls.2020.613760

Teixeira, P. C., Donagemma, G. K., Fontana, A., & Teixeira, W. G. (2017). Manual de métodos de análise de solo.

Tusar, H. M., Uddin, M. K., Mia, S., Suhi, A. A., Wahid, S. B. A., Kasim, S., Sairi, N. A., Alam, Z., & Anwar, F. (2023). Biochar-acid soil interactions—A review. *Sustainability*, *15*(18), 13366. https://doi.org/10.3390/su151813366

Wang, H., Nan, Q., Waqas, M., & Wu, W. (2022). Stability of biochar in mineral soils: Assessment methods, influencing factors and potential problems. *Science of the Total Environment*, *806*, 150789. https://doi.org/10.1016/j.scitotenv.2021.150789

Wang, T., Wu, J., Hu, T., Wang, C., Li, S., Li, Z., & Chen, J. (2024). Mechanistic insights into adsorption-desorption of PFOA on biochars: Effects of biomass feedstock and pyrolysis temperature, and implication of desorption hysteresis. *Science of The Total Environment*, *957*, 177668. https://doi.org/10.1016/j.scitotenv.2024.177668

Wen, Y., Shi, F., Zhang, B., Li, K., Chang, W., Fan, X., Dai, C. L., & Song, F. (2024). Rhizophagus irregularis and biochar can synergistically improve the physiological characteristics of saline-alkali resistance of switchgrass. *Physiologia Plantarum*, *176*(3), e14367. https://doi.org/10.1111/ppl.14367

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wijerathna-Yapa, A., & Pathirana, R. (2022). Sustainable agro-food systems for addressing climate change and food security. *Agriculture*, *12*(10), 1554. https://doi.org/10.3390/agriculture12101554

Yang, Y., Ahmed, W., Ye, C., Yang, L., Wu, L., Dai, Z., Khan, K. A., Hu, X., Zhu, X., & Zhao, Z. (2024). Exploring the effect of different application rates of biochar on the accumulation of nutrients and growth of flue-cured tobacco (*Nico-*

tiana tabacum). *Frontiers in Plant Science*, *15*, 1225031. https://doi.org/10.3389/fpls.2024.1225031

Yesto, S. J. K., Shang, H., Lv, X., Abdalla, J. T., Wang, T., & Yu, Y. (2024). Effect of inorganic component of biochar on lead adsorption performance and the enhancement by MgO modification. *Environmental Science and Pollution Research*, *31*, 65427–65445. https://doi.org/10.1007/s11356-024-35556-0

Yusif, S., Muhammad, I., Hayatu, N., Sauwa, M., Tafinta, I., Mohammed, M., Lukman, S., Abubakar, G., & Hussain, A. (2016). Effects of biochar and rhizobium inoculation on nodulation and growth of groundnut in Sokoto State, Nigeria. *Journal of Applied Life Sciences International*, *9*(2), 1–9. https://doi.org/10.9734/JALSI/2016/27297

Zar, J. (2014). Spearman Rank Correlation: Overview. In R. S. Kenett, N. T. Longford, W. W. Piegorsch, & F. Ruggeri (Eds.), *Wiley statsref: Statistics reference online*. Wiley. https://doi.org/10.1002/9781118445112.stat05964

Zhang, N., Xing, J., Wei, L., Liu, C., Zhao, W., Liu, Z., Wang, Y., Liu, E., Ren, X., Jia, Z., et al. (2025). The potential of biochar to mitigate soil acidification: a global meta-analysis. *Biochar*, *7*(1), 49. https://doi.org/10.1007/s42773-025-00451-5

Zhao, S., Yan, L., Kamran, M., Liu, S., & Riaz, M. (2024). Arbuscular mycorrhizal fungi-assisted phytoremediation: A promising strategy for cadmium-contaminated soils. *Plants*, *13*(23), 3289. https://doi.org/10.3390/plants13233289

Zhao, T., Wang, L., & Yang, J. (2024). Synergistic effects of combined application of biochar and arbuscular mycorrhizal fungi on the safe production of rice in cadmium contaminated soil. *Science of The Total Environment*, *951*, 175499. https://doi.org/10.1016/j.scitotenv.2024.175499

Zurek, M., Hebinck, A., & Selomane, O. (2022). Climate change and the urgency to transform food systems. *Science*, *376*(6600), 1416–1421. https://doi.org/10.1126/science.abo2364