**Fronteiras: Journal of Social, Technological and Environmental Science**
http://periodicos.unievangelica.edu.br/fronteiras/
ISSN 2238-8869

*Article*

# Symbiotic Potential of Soil Microorganisms in Promoting the Growth of Atlantic Forest Tree Species

**Thainá Alves dos Santos [1], Felipe Ferreira da Silva [2], Sergio Miana de Faria [3], Guilherme Montandon Chaer[4]**

[1] Doctoral student in Environmental and Forest Sciences from Federal Rural University of Rio de Janeiro, Seropédica, RJ, Brasil. ORCID: 0000-0003-4059-7852. E-mail: thaina.232@gmail.com

[2] Ph.D. in Environmental Sciences and Conservation from Federal University of Rio de Janeiro. Macaé, RJ, Brazil. ORCID: 0000-0003-2839-5350. E-mail: felipe.agrobiologia@gmail.com

[3] Ph.D. in Biological Sciences from University of Dundee. Reseacher of Embrapa Agrobiologia, Seropédica, RJ, Brazil. ORCID: 0000-0001-5066-046X. E-mail: sergio.defaria@embrapa.br

[4] Ph.D. in Crop and Soil Science from Oregon State University. Reseacher of Embrapa Agrobiologia, Seropédica, RJ, Brazil. ORCID: 0000-0003-0734-2866. E-mail: guilherme.chaer@embrapa.br

**ABSTRACT**

The objective of this study was to evaluate the potential of topsoil obtained from a natural forest fragment in the Brazilian Atlantic Forest to serve as a source of symbiotic microorganisms capable of promoting the growth of native tree species in ecological restoration projects. In greenhouse conditions, 14 experiments in a completely randomized design were conducted in 1,700 cm³ pots filled with sterilized substrate. The effect of adding 80 g of topsoil, either in natura or sterilized, to the substrate was tested on the growth of 14 tree species. Plant height, stem diameter, mycorrhizal colonization and dry masses of shoot, roots, and nodules of tree species were measured. Applying the topsoil to the pots generally resulted in significant increases in height and stem diameter of seedlings compared to the control group during the three to six-month evaluation period. This effect was particularly greater in the nodulating species *Plathymenia reticulata*, *Dalbergia nigra* and *Mimosa bimucronata*, with increases in height and stem diameter of up to 328% and 484%, respectively. Forest topsoil also had a positive impact on the growth of shoot, roots, and nodules of the plants, significantly differing from the control groups. Only the plants that received the topsoil *in natura* exhibited mycorrhizal colonization and the formation of nodules in nitrogen-fixing species. These plants that established mycorrhizas and nodules presented higher concentrations of phosphorous and nitrogen in their biomass, respectively. Under controlled conditions, the use of forest topsoil proved to be a promising strategy for the introduction of microorganisms that can enhance the growth of tree species, thereby holding potential for implementation in nurseries and field settings.
**Keywords:** ecological restoration; topsoil; root symbionts; nitrogen-fixing bacteria; arbuscular mycorrhizal fungi.

**RESUMO**

O objetivo deste estudo foi avaliar o potencial do *topsoil* obtido de um fragmento florestal natural da Mata Atlântica brasileira para servir como fonte de microrganismos simbióticos capazes de promover o crescimento de espécies arbóreas nativas em projetos de restauração ecológica. Em casa de vegetação, foram conduzidos 14 experimentos em delineamento inteiramente casualizado, em vasos de 1.700 cm³ preenchidos com substrato esterilizado. O efeito da adição de 80 g de solo superficial, in natura ou esterilizado, ao substrato foi testado no crescimento de 14 espécies arbóreas. Foram mensuradas a altura das plantas, o diâmetro do caule, a colonização micorrízica e as massas secas da parte aérea, das raízes e dos nódulos das espécies arbóreas. A aplicação do *topsoil* nos vasos geralmente resultou em aumentos significativos na altura e no diâmetro do caule das mudas em comparação com o grupo controle durante o período de avaliação de três a seis meses. Este efeito foi particularmente maior nas espécies nodulantes *Plathymenia reticulata*, *Dalbergia nigra* e *Mimosa bimucronata*, com aumentos na altura e no diâmetro do caule de até 328% e 484%, respectivamente. O *topsoil* florestal também teve um impacto positivo no crescimento da parte aérea, das raízes e dos nódulos das plantas, diferindo significativamente do grupo controle. Apenas as plantas que receberam a camada superficial do solo *in natura* apresentaram

Submissão: 26/10/2023 Aceite: 28/12/2023 Publicação: 05/04/2024

v.13, n.1, 2024 • p. 105-129. • DOI http://dx.doi.org/10.21664/2238-8869.2024v13i1p.105-129.

colonização micorrízica e formação de nódulos nas espécies fixadoras de nitrogênio. Essas plantas que estabeleceram micorrizas e nódulos apresentaram maiores concentrações de fósforo e nitrogênio em sua biomassa, respectivamente. Sob condições controladas, o uso do *topsoil* florestal provou ser uma estratégia promissora para a introdução de microrganismos que podem aumentar o crescimento de espécies arbóreas, mantendo assim potencial para implementação em viveiros e ambientes de campo.
**Palavras-chave:** restauração ecológica; topsoil; simbiontes radiculares; bactérias fixadoras de nitrogênio; fungos micorrízicos arbusculares.

## Introduction

Tropical forests are important reservoirs of biodiversity (Sun et al. 2020) and provide various ecosystem services, such as carbon sequestration, nutrient cycling, and water regulation (Jakovac et al. 2021). The first 10 centimeters of the forest topsoil contain a high abundance of microbial biomass (Barros et al. 2019), which plays a critical role in maintaining important ecological functions of the soil (Ivanova et al. 2018), such as the decomposition and the mineralization of organic matter, the cycling of nutrients, as well as plant resistance to biotic and abiotic stresses, impacting the productivity and vegetal composition (Vandenkoornhuyse et al. 2015; Pedone-Bonfim et al. 2018; Berkelmann et al. 2020). However, the conversion of tropical forests to alternative land uses, primarily agriculture and pasture, persists as a significant threat to these critical ecosystems worldwide (Jakovac et al. 2021).

Microorganisms represent the most sensitive fraction to changes in soil use and management (Barros et al. 2019). Management practices in forest areas converted to pastures and agriculture, such as intensive soil preparation, fertilization, and herbicide application, lead to reduced microbial biomass and biotic homogenization (Gossner et al. 2016; Berkelmann et al. 2020), causing impacts on the structure and functional potential of communities (Berkelmann et al. 2020).

In the Brazilian Atlantic Forest biome, planting native tree seedlings is the most common technique used in restoration projects of degraded areas (Brancalion et al. 2019). However, focusing on reintroducing plants without restoring associated microbiomes may limit restoration success (Koziol et al. 2018; Redford 2023). In this sense, the use of natural forest topsoil in the production of seedlings of native tree species or using it as a planting amendment in areas designated for restoration can serve as an effective nucleation strategy. This approach may facilitate the reintroduction of native microorganisms and promotes beneficial ecological interactions between plants and associated microorganisms, favoring the growth and establishment of plants in the field.

Averill et al. (2022) analyzed several experiments showing that restoring the soil microbiome can help in the recovery of degraded ecosystems. They point out that, on average, transposition of the native soil microbiome using topsoil samples resulted in a 64% increase in plant biomass in all ecosystems studied. In addition, these authors showed that soil microbiome recovery can improve carbon uptake and nutrient cycling, as well as increase plant resistance to abiotic and biotic stresses.

In another study, Silva (2018) demonstrated the beneficial effects of using two topsoils originated from remnants of natural vegetation in the Caatinga biome in Brazil on the growth of *Mimosa caesalpiniifolia* Benth., a native leguminous tree species. The experiment, conducted under greenhouse conditions, showed that adding only 10 g of topsoil in the planting furrow of *M. caesalpiniifolia* seeds resulted in a remarkable increase in biomass production, reaching up to 256% when compared to the control (which received sterilized topsoil). In addition, the growth of the species in the presence of topsoil was similar to treatments that received inoculants composed of arbuscular mycorrhizal fungi (AMF) spores and strains of rhizobia previously selected for that particular plant species.

This study aimed to evaluate the impact of using topsoil collected from an Atlantic Forest fragment on the growth and establishment of symbioses with nitrogen-fixing bacteria (NFB) and arbuscular mycorrhizal fungi in 14 tree species commonly used in forest restoration projects within the biome. The findings from this study have the potential to enhance our understanding of the influence exerted by native soil microbiota on the growth of native tree species of the biome, thereby contributing to more effective forest restoration efforts.

## 2 Material and methods

### *2.1 Collection of the forest topsoil*

Topsoil samples were obtained from a fragment of native vegetation in the Atlantic Forest situated in Lídice, Rio Claro municipality, Rio de Janeiro state, Brazil (22º50'18"S, 44º12'52"W; 550 m to 650 m asl). The predominant vegetation of the site is characterized as Dense Montane Rainforest (Brazilian Institute of Geography and Statistics [IBGE] 2012). The climate of the region falls under the Cwa category according to the Köppen classification (Alvares et al. 2013), which is characterized by cold and dry winters, and hot and rainy summers. The region has an annual average rainfall of 1,300 mm from January 2017 to December 2020, with monthly average temperatures ranging from 15.2 ºC to 25.7 ºC and an annual average of 21.3 ºC (National Institute of Meteorology [INMET] 2021).

In March 2021, topsoil samples were collected at 20 points following a zigzagging gradient, from the forest interior to the forest edge, covering a distance of 500 m. At each sampling point, soil from the 0-2.5 cm depth was collected within a 0.04 m² area using a hoe. The raw litter layer above the topsoil was discarded before sampling. The collected samples were homogenized to create a composite sample. In the laboratory, the composite sample was air-dried, crushed, and sieved through a 2 mm mesh. To maintain the integrity of the samples, they were kept refrigerated at 5 ºC until analysis and experimental installation were carried out.

### *2.2 Physical and chemical analyses of topsoil*

Physical and chemical analyses of the topsoil were carried out using established methodologies (Teixeira et al. 2017). Granulometric analysis was performed using the densitometer method to determine the contents of the clay and sand + silt fractions (Table 1). Chemical analyses included determining the organic matter using the Walkley and Black method, pH in water (1:2.5 soil/water) measured by potentiometry, calcium ($Ca^{2+}$) and magnesium ($Mg^{2+}$) by atomic absorption spectrometry, and aluminum ($Al^{3+}$) by titration after extraction with 1 mol $L^{-1}$ KCl. Phosphorus (P) was determined by spectrophotometry, potassium ($K^{+}$) by flame photometry, and potential acidity (H+Al) by titration after extraction with calcium acetate. Sum of bases (S), potential cation exchange capacity (CEC at pH 7.0), and percent of base saturation (V) were calculated. Table 1 presents the characterization of fertility and percentages of sand, clay, and silt in topsoil, *in natura* and after sterilization (see section 2.4).

**Table 1** Chemical and physical characterization of the forest topsoil, in natura and sterilized (control treatment), collected from an Atlantic Forest fragment at Rio Claro, RJ, Brazil, at a depth of 0-2.5 cm

| | MO | pH | N | P | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $Al^{3+}$ | H + Al | S[a] | CEC pH 7[b] | BS[c] | Sand+Silt | Clay |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | $dag/dm^3$ | | ------$mg/dm^3$------ | | | ----------$cmol_c/dm^3$---------- | | | | | | % | -----g/kg----- | |
| Topsoil *in natura* | 4,1 | 5,1 | 1,0 | 11,2 | 53 | 2,1 | 0,9 | 0,6 | 5,8 | 3,14 | 8,94 | 35 | 700 | 300 |
| Topsoil sterilized | 4,0 | 5,2 | 1,0 | 11,2 | 53 | 2,2 | 0,8 | 0,4 | 6,0 | 3,14 | 9,14 | 34 | 705 | 295 |

[a]Sum of bases; [b]cation exchange capacity at pH 7; [c]base saturation. Source: elaborated by the authors.

## 2.3 *Biological analyses*

The biological analyses consisted of quantifying the density of nitrogen-fixing nodular bacteria (rhizobia) and arbuscular mycorrhizal fungi spores present in the topsoil to determine if the soil provided viable symbiotic microorganisms to the plants.

### *2.3.1 Determination of rhizobia density*

The rhizobia density in the topsoil was determined by the most probable number method (MNP) (Jarvis et al. 2010), using *Mimosa pudica* L. (sensitive plant) and *Phaseolus vulgaris* L. (common bean) as bait plants. To break the dormancy of the *M. pudica* seeds, they were immersed in 98% $H_2SO_4$ for 10 minutes (Rahman et al. 2018). Both species seeds were disinfested by immersing then in 70% (w/v) alcohol for 1 minute and then in 2% (w/v) NaClO for 3 minutes. The seeds were then washed ten times with autoclaved distilled water and placed in sterile Petri dishes containing cotton and filter paper soaked in sterile distilled water. Petri dishes with seeds were then incubated in a B.O.D. type germination chamber at 30°C with a 12-hour photoperiod until the radicles emerged.

For the MNP analysis, 10 grams of the topsoil sample were transferred to a 250 mL Erlenmeyer flask containing 90 mL of autoclaved sterile saline solution (0.85% NaCl), and successive dilutions were made, obtaining dilutions of $10^{-1}$ to $10^{-6}$. In the greenhouse, the plants were grown in Leonard pots containing 1.5 kg of substrate composed of sand and vermiculite (1:1), which had previously been sterilized in an autoclave at 121°C for 60 minutes. Three Leonard pots were used for each dilution, and three for the control without inoculation. Three seeds were arranged per pot, and only one individual was kept two days after sowing. Then, an aliquot of 1 mL was applied to the radicle region of the plant, and the pots were weekly supplied with Norris's nutrient solution, free of nitrogen (Guzmán & Döbereiner 1968).

After 30 days of inoculation, the plants were harvested and evaluated for nodulation, and the nodular rhizobia density estimates were made by the MPN package in R 4.0.5 software at a 95% confidence level (Ferguson & Ihrie 2019).

### *2.3.2 Determination of density and identification of AMF spores*

The extraction of arbuscular mycorrhizal fungi spores was performed from a sample containing 50 $cm^3$ of the forest topsoil using the wet sieving (Gerdermann & Nicolson 1963) and density gradient centrifugation techniques (Jenkins 1964). Subsequently, the recovered spores were placed in a Petri dish and counted under a stereoscopic microscope. Different morphotypes were separated according to their color, size, and shape .and were arranged on a slide with polyvinyl alcohol-lactic glycerol (PVLG) under a cover slip. On the same slide, another group of AMF spores was fixed with PVLG supplemented with Melzer's reagent (1:1), and gently

broken under the cover slip to highlight the inner walls of the spores (when present) to aid in species identification.

Species identification was performed under an optical microscope with bright-field illumination and an immersion objective, using the Identification Manual of AMF developed by Schenck and Perez (1988) and the descriptions of the species available on the International Culture Collection of Arbuscular Mycorrhizal Fungi website (http://invam.caf.wvu.edu/).

### *2.4 Experimental design and conduction*

To assess the impact of topsoil on the growth of native tree species of the Brazilian Atlantic Forest biome, 14 greenhouse experiments were conducted at Embrapa Agrobiologia in Seropédica, Rio de Janeiro state. Each experiment involved one of 14 tree species commonly used in Atlantic Forest reforestation efforts (Table 2), selected based on seed availability at the time of the study.

**Table 2.** Names and botanical families of tree species from the Atlantic Forest selected for the greenhouse study conducted in Seropédica, RJ

| Species | Popular name | Family |
|---|---|---|
| *Anadenanthera peregrina* (L.) Speg. | Angico-vermelho[a] | Leguminosae |
| *Apuleia leiocarpa* (Vogel) J.F.Macbr. | Garapa | Leguminosae |
| *Ceiba speciosa* (A.St.-Hil.) Ravenna | Paineira-rosa | Malvaceae |
| *Cenostigma pluviosum* (DC.) Gagnon & G.P.Lewis | Sibipiruna | Leguminosae |
| *Dalbergia nigra* (Vell.) Allemão ex Benth. | Jacarandá-da-bahia[a] | Leguminosae |
| *Guazuma ulmifolia* Lam. | Mutambo | Malvaceae |
| *Handroanthus serratifolius* (Vahl) S.Grose | Ipê-amarelo | Bignoniaceae |
| *Mimosa bimucronata* (DC.) Kuntze | Maricá[a] | Leguminosae |
| *Plathymenia reticulata* Benth. | Vinhático[a] | Leguminosae |
| *Pseudobombax grandiflorum* (Cav.) A.Robyns | Embiruçu | Malvaceae |
| *Psidium cattleyanum* Sabine | Araçá | Myrtaceae |
| *Schinus terebinthifolius* Raddi | Aroeira-pimenteira | Anacardiaceae |
| *Senna macranthera* (DC. ex Collad.) H.S.Irwin & Barneby | Fedegoso | Leguminosae |
| *Senna multijuga* (Rich.) H.S.Irwin & Barneby | Aleluia | Leguminosae |

[a]Species that perform symbiotic association with noduliferous nitrogen-fixing bacteria. Source: elaborated by the authors.

Seeds of *S. multijuga*, *P. reticulata*, *S. macranthera*, *A. leiocarpa*, *G. ulmifolia*, *D. nigra*, and *P. grandiflorum* were sown in March 2021, while *H. serratifolius* and *P. cattleyanum* were sown in April, *S. terebinthifolius* in May, *M. bimucronatha* in June, and *A. peregrina*, *C. speciosa*, and *C. pluviosum* in August of the same year. This occurred due to the availability of seeds from these species throughout the year.

Each experiment was designed with a completely randomized layout with two treatments: (1) application of forest topsoil and (2) a control treatment, where the same topsoil was applied after undergoing sterilization. The treatments had eight replicates, totaling 16 experimental units per experiment. The topsoil used in the control treatment was autoclaved at 121 °C for 60 minutes, with the procedure repeated after two days.

To break seed dormancy, particular treatments were implemented for seven of the 14 species (Table 3). After the immersion period, the seeds were washed in running water. In a biological safety cabinet, the seeds were disinfected by immersion in 70% (v/v) ethanol for 1 minute and then in 2% (v/v) NaClO for 15 minutes, and then washed ten times with autoclaved distilled water. The seeds were then placed in sterile Petri dishes containing a layer of cotton soaked in autoclaved distilled water and lined with filter paper. The dishes were

then incubated in a B.O.D. germination chamber at 30 °C with a 12-hour photoperiod until radicle emergence occurred.

**Table 3** Methods for overcoming seed dormancy of tree species from the Atlantic Forest used in experiments to evaluate the effect of topsoil addition

| Species | Overcoming dormancy by immersion | Reference |
|---|---|---|
| *S. multijuga* | $H_2SO_4$ 98%/10 min | Piveta et al. 2010 |
| *P. reticulata* | $H_2SO_4$ 98%/10 min | Silva et al. 2013 |
| *S. macranthera* | $H_2SO_4$ 98%/15 min | Cipriani et al. 2007 |
| *A. leiocarpa* | $H_2SO_4$ 98%/5 min | Castro et al. 2019 |
| *G. ulmifolia* | $H_2SO_4$ 98%/8 min | Paiva Sobrinho et al. 2012 |
| *M. bimucronata* | $H_2SO_4$ 98%/10 min | Kestring et al. 2009 |
| *C. speciosa* | $H_2O$ 25ºC/24h | Carvalho and Nakagawa 2000 |

Source: elaborated by the authors.

The experimental units consisted of pots with a capacity of 1,700 cm³, filled with a substrate composed of sand and vermiculite in a 1:1 (v/v) ratio that was autoclaved prior to use. An opening of 5 cm in depth was made in the substrate, where 80 g of topsoil and three pre-germinated seeds of the target forest species were sown in each pot (Figure 1). After sowing, the pots were irrigated with filtered water and subsequently irrigated weekly. Thinning was conducted at 30 days, leaving only one seedling per experimental unit.

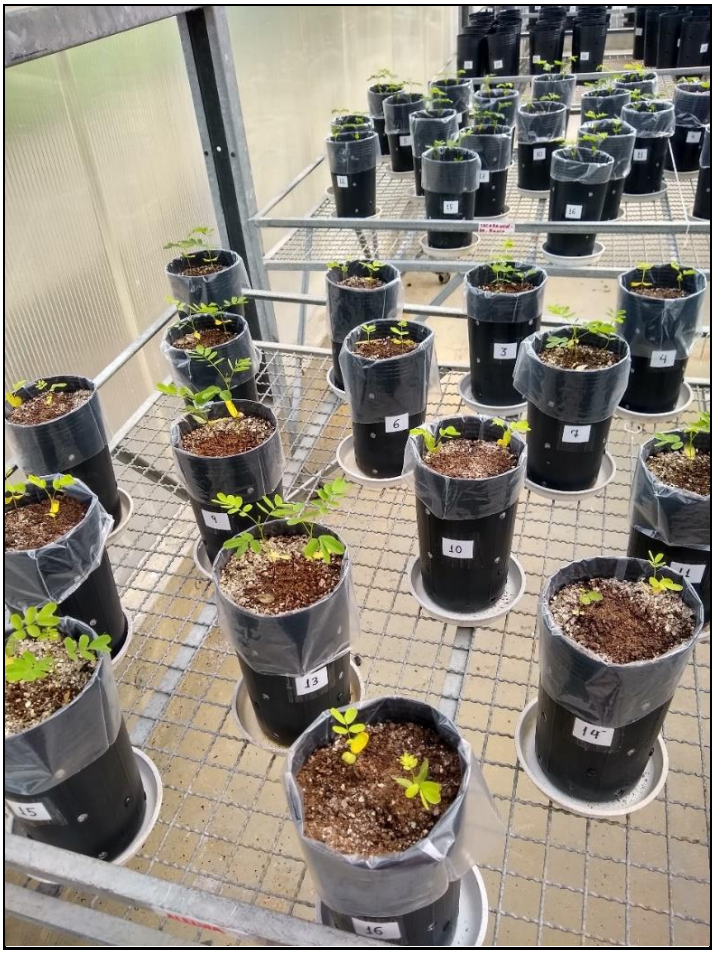


**Figure 1.** View of the experimental units containing in natura or sterilized forest topsoil, arranged in a greenhouse. Source: elaborated by the authors.

After cotyledon fall, each experimental unit received 100 mL of Norris modified nutrient solution at half the salt concentration (Guzmán & Döbereiner 1968) every two weeks. Except for *A. peregrina*, *D. nigra*, *M. bimucronata*, and *P. reticulata* (legumes that associate with nitrogen-fixing bacteria), the species received mineral nitrogen through the application of ammonium nitrate ($NH_4NO_3$) solution in increasing doses (5mg N to 30mg N per plant). This procedure was used because nitrate in the soil limits root infection, nodule development, and nitrogenase activity, the enzyme responsible for biological nitrogen fixation (Dwivedi et al. 2015).

### *2.5 Assessments*

The shoot growth of each plant was measured monthly, starting in September 2021. The height of each individual was measured using a graduated tape, while the stem diameter was measured using a digital caliper. The duration of the 14 experiments ranged from three to six months after the start of assessments, depending on the growth rate exhibited by each species.

Upon completion of the growth phase, the shoot and root parts of the plants were collected separately. The roots were washed in running water under a sieve to remove remaining soil particles. Then, nodules of nodulating legume species were collected. All materials were placed in identified paper bags and dried in an oven at 65°C for 72 hours. The dry mass of the shoot part (SDM), root (RDM), and nodules (NDM) were determined separately using an analytical balance. For the SDM variable, eight repetitions per treatment were used; while for RDM and NDM, only five repetitions were employed, the rest were reserved for additional analysis not covered in the scope of this study. Thus, the SDM/RDM ratio was calculated with five replications, using the shoot part corresponding to the root of the same experimental unit.

For the determination of the nutrient concentrations of the dry mass of shoots, the material was ground in a benchtop stainless steel mill, and N, P, K, Ca, and Mg were analyzed. N was determined by mass spectrometry, P by the colorimetric method, $K^+$ by flame photometry, and $Ca^{2+}$ and $Mg^{2+}$ by atomic absorption. Chemical analyses were performed according to the methodologies described by Nogueira and Souza 2005.

To determine the mycorrhizal colonization, 0.5 g samples of fresh fine roots from three experimental units per treatment were washed, cleared with 2.5% KOH (Koske & Gemma 1989), and stained with 0.05% methyl blue (Grace & Stribley 1991). Root colonization percentage by AMF was assessed using the intersection method on a quadrat grid (Giovannetti & Mosse 1980) with the aid of a stereoscopic microscope. Based on the percentage obtained, mycorrhizal colonization was qualitatively evaluated using the methodology of Carneiro et al. (1998) and categorized as high (>50%), medium (>20% and <50%), low (<20%), or absent (0%).

### *2.6 Data Analysis*

The statistical assumptions of normality of residuals and homogeneity of variances were verified by performing the Shapiro-Wilk and Bartlett tests, respectively, both at a 5% probability level. Once the assumptions were met, the variance analysis was carried out using the F test at a 5% probability level. The analyses were performed using the "easyanova" package in the R software (Arnhold 2021). However, as the variables of nodules dry mass and mycorrhizal colonization did not meet the statistical assumptions, they were analyzed using the Kruskal-Wallis test, also at a 5% probability level.

Statistical analysis of nutrient concentrations within the dry mass of shoots could not be carried out due to limited sample replicates. This limitation arose from the insufficient sample mass available for individual analyses, necessitating the pooling of samples to assess the plant tissue composition.

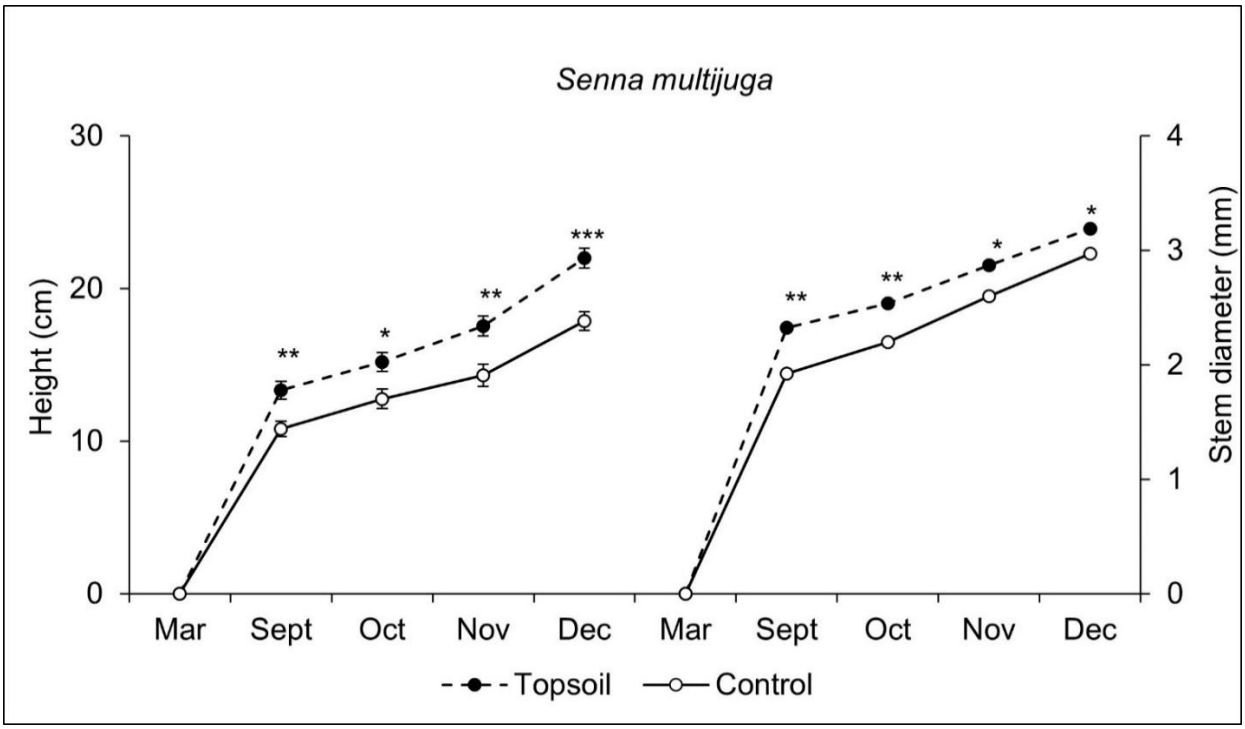
## 3 Results

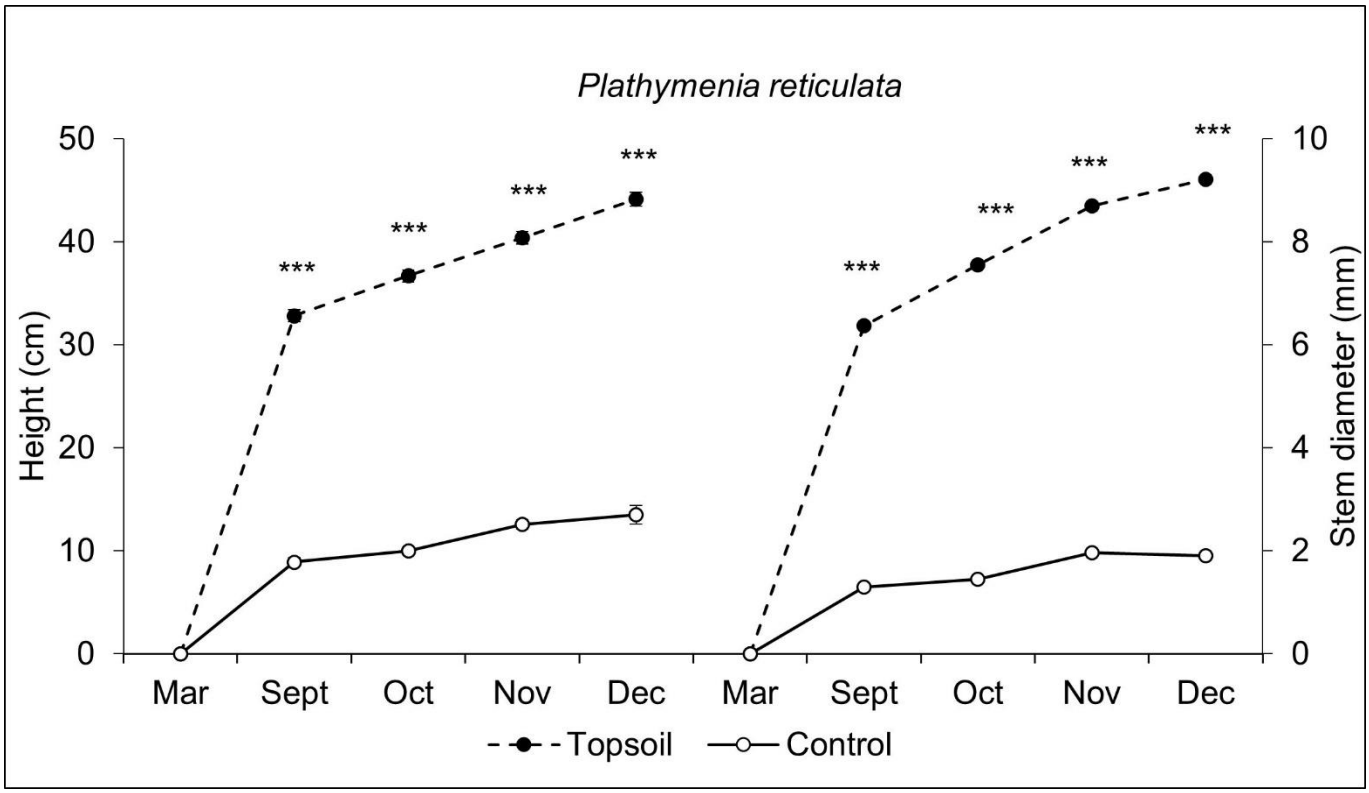
The topsoil showed a density of 93 (CI = 23-388) and 3.57 (CI = 0.5-26) rhizobia cells per gram of soil when *M. pudica* and *P. vulgaris* legumes were used as bait plants, respectively.

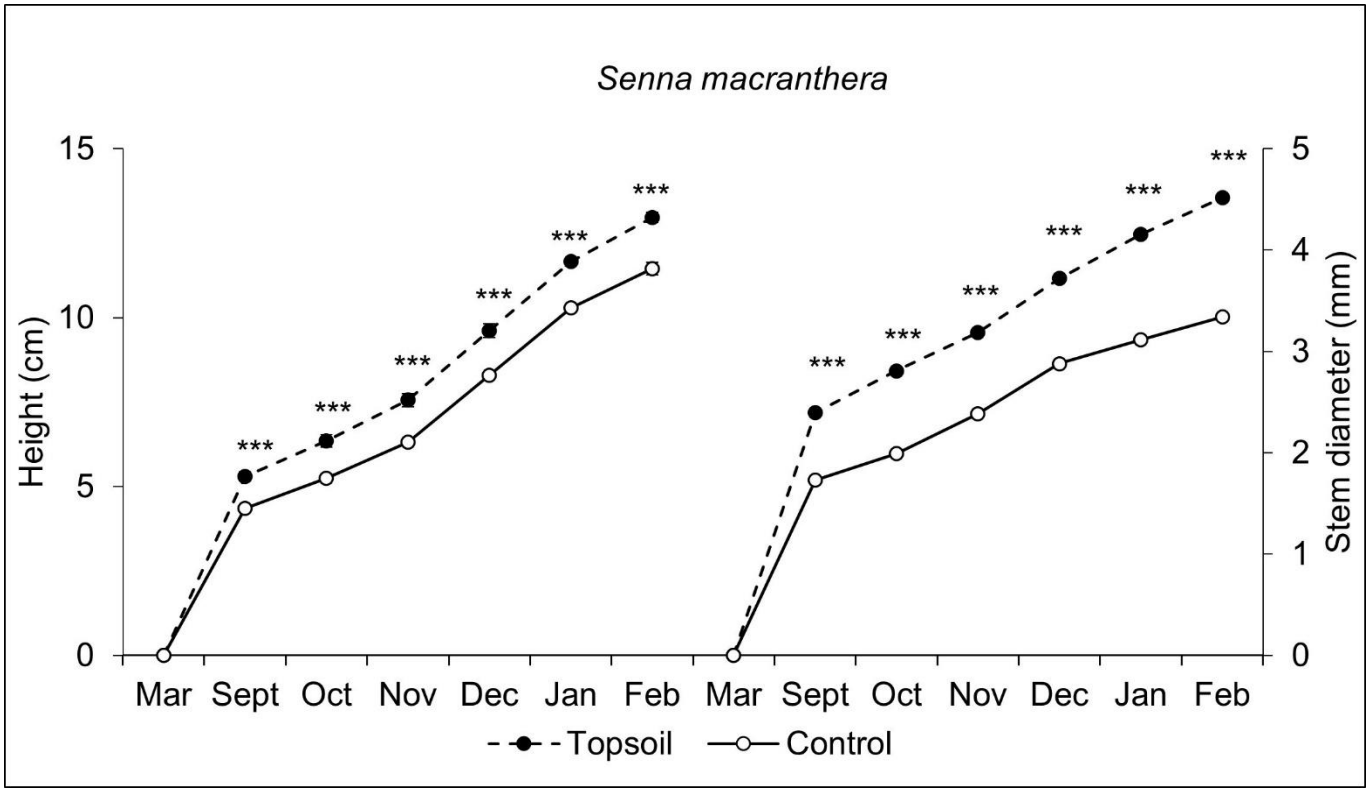
A total of 400 AMF spores were recovered from 50 cm³ of topsoil. The following species of arbuscular mycorrhizal fungi were present in the sample: *Acaulospora foveata* Trappe & Janos; *Acaulospora mellea* Spain & Schenck; *Acaulospora rehmii* Sieverding & Toro; *Acaulospora scrobiculata* Trappe; *Acaulospora tuberculata* Janos & Trappe; *Glomus clavisporum* (Trappe) Almeida & Schenck; *Glomus macrocarpum* Tul. & C. Tul.; and *Racocetra verrucosa* (Koske & C. Walker) Oehl, FA Souza & Sieverd.

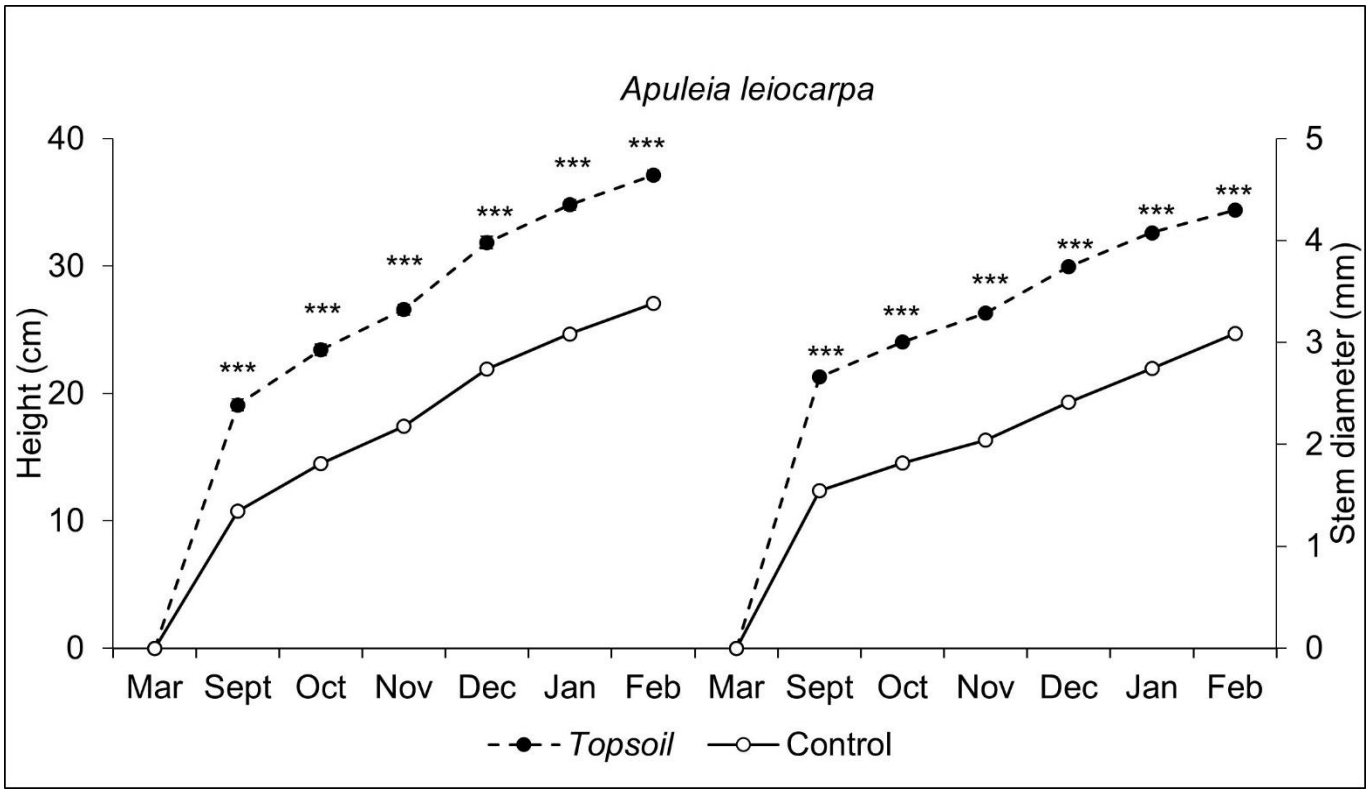
The addition of natural forest topsoil significantly increased the mean height growth of all 14 tree species evaluated, in comparison to the control treatment. This effect was observed from the first assessment, conducted one month after sowing, and persisted throughout the following months (Figure 2). However, two species, *A. peregrina* and *C. speciosa*, exhibited a deviation from this pattern. In these cases, plants treated with topsoil did not show significant differences from the control groups in the last month and from the fourth month after planting, respectively. Similarly, the mean values of stem diameter of the studied species were positively affected when applied the topsoil *in natura* during the study period. However, in the first month of evaluation, *C. pluviosum* did not show a significant difference between treatments, while *A. peregrina* did not show differences in the last two months of evaluation, that is, January and February 2022.

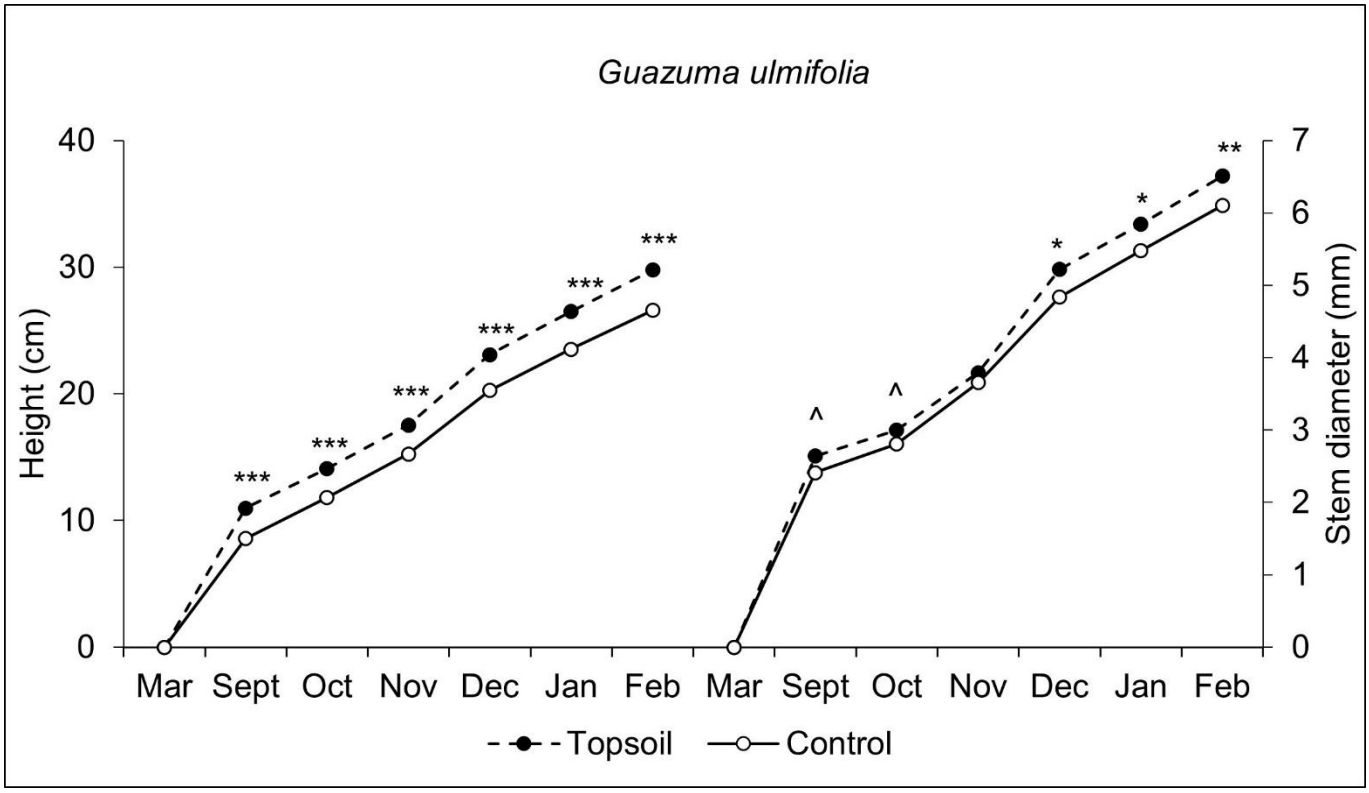
The species *P. reticulata*, *D. nigra* and *M. bimucronata*, known to associate with nitrogen-fixing bacteria, showed the highest monthly gains in height and stem diameter with the use of topsoil, compared to the control treatment. Although *A. peregrina* also has the ability to associate with rhizobia, which could result in greater growth gains, significant increases in height and stem diameter were not observed over the evaluated period, except in the first months.

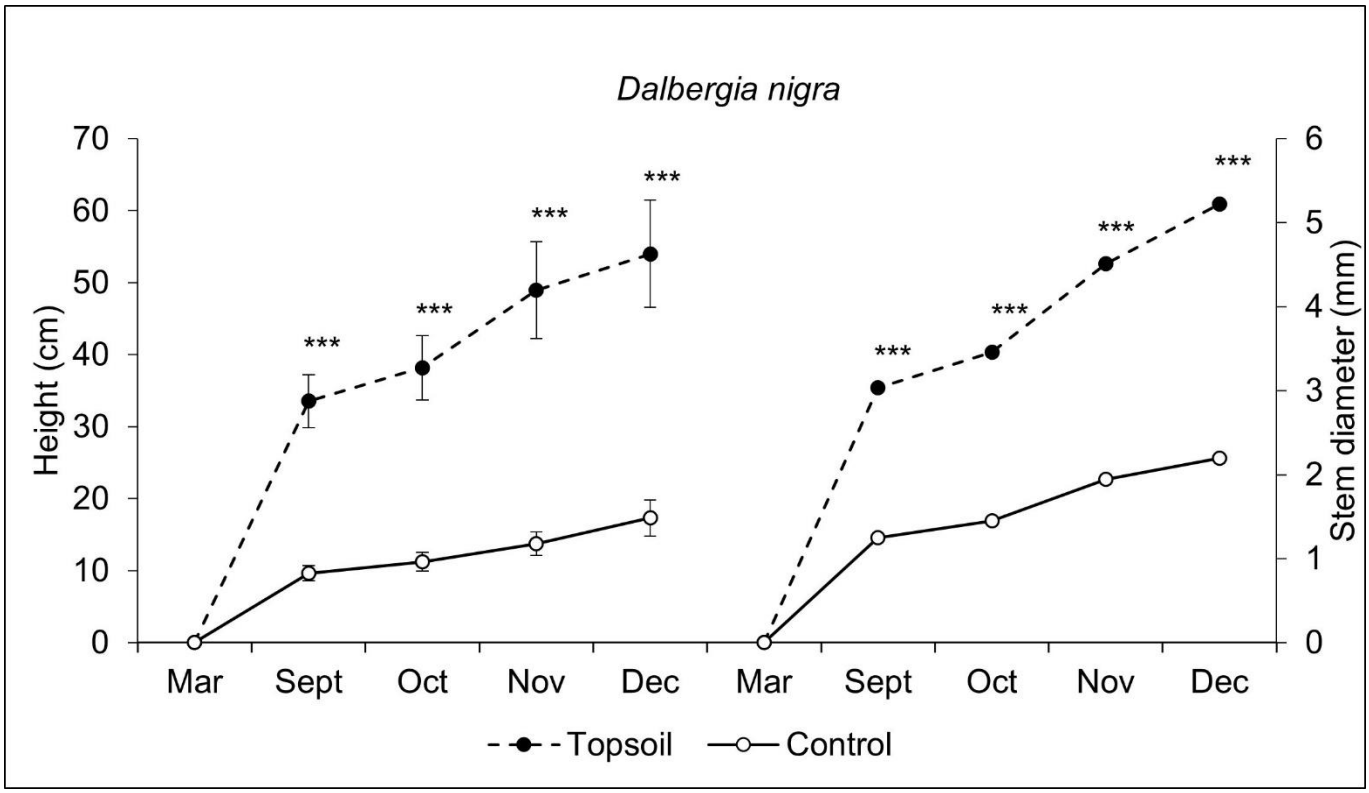


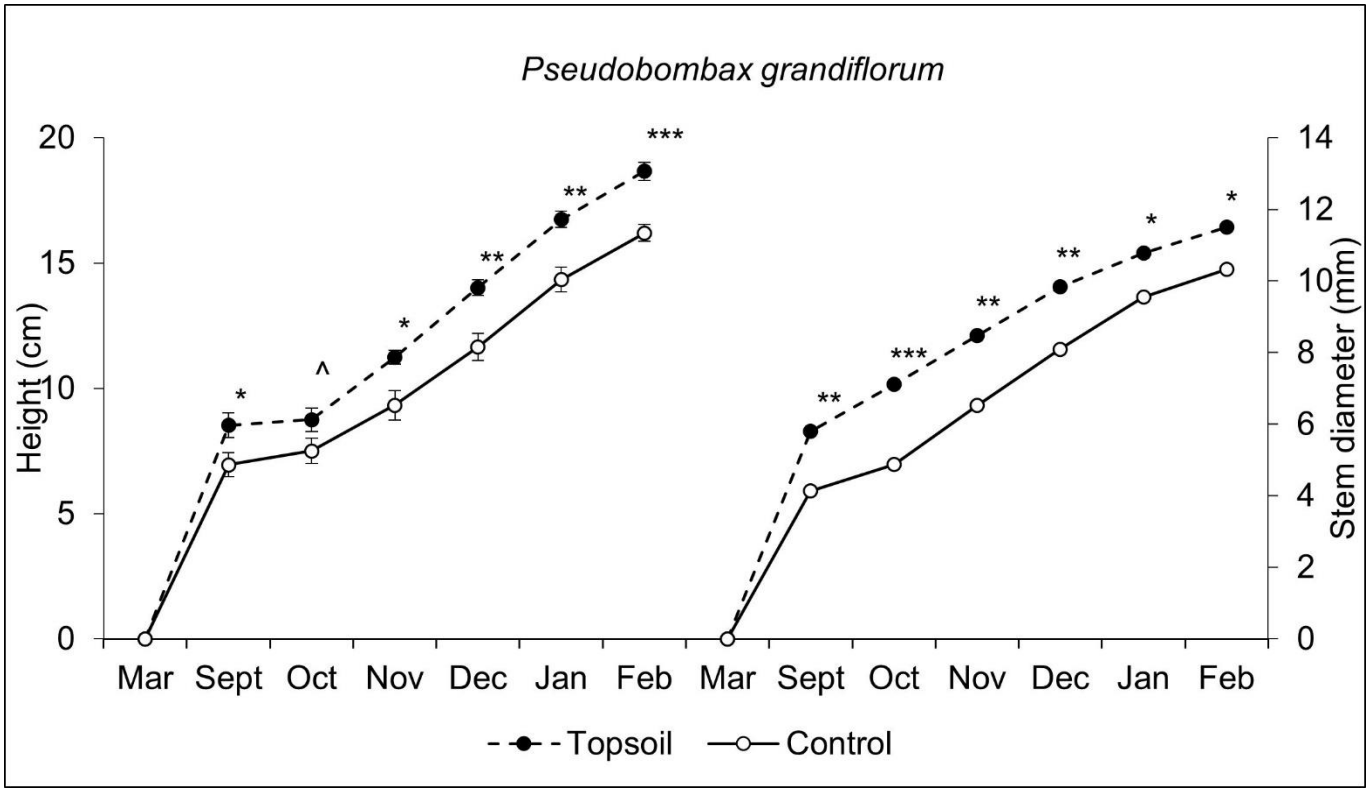

Plathymenia reticulata
Height (cm)
Stem diameter (mm)
0
10
20
30
40
50
0
2
4
6
8
10
***
Mar
Sept
Oct
Nov
Dec
Topsoil
Control

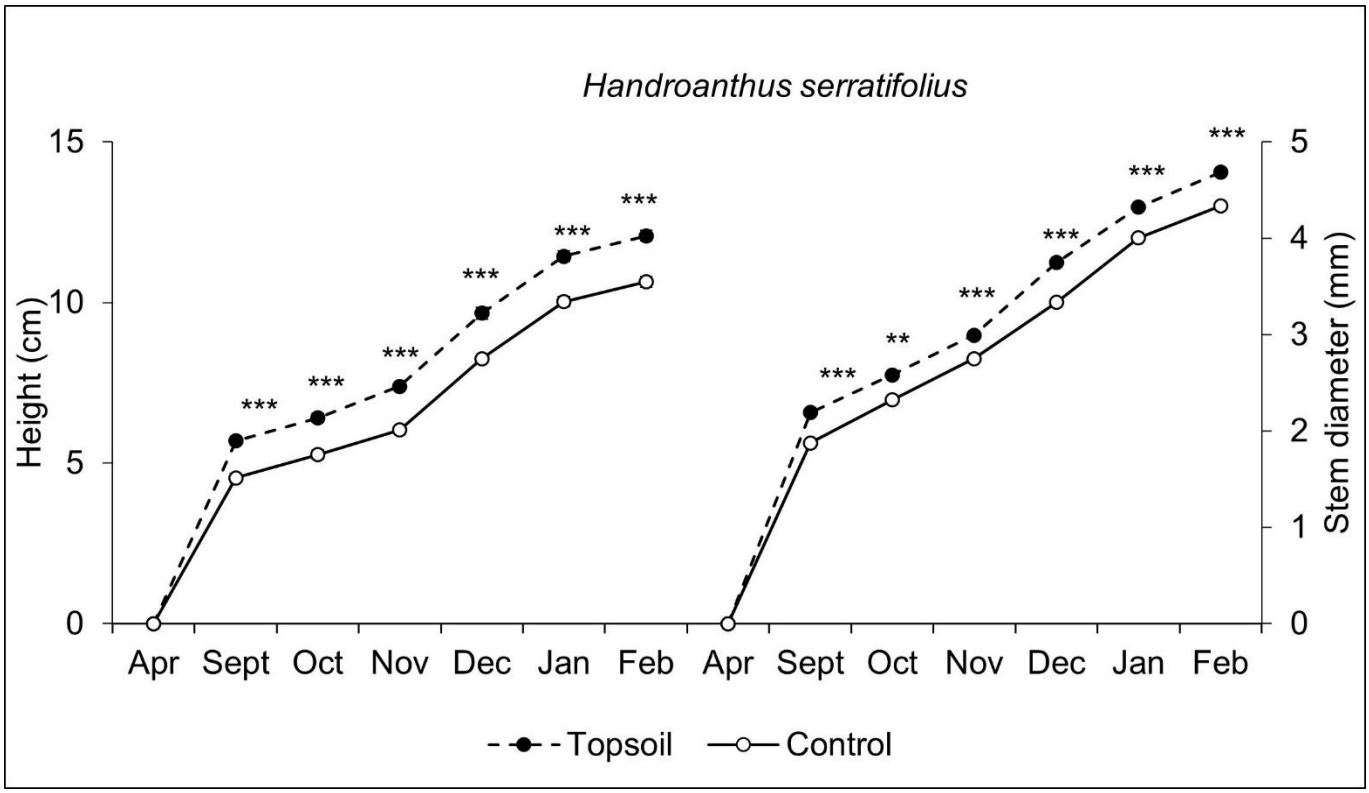

Senna macranthera
Height (cm)
Stem diameter (mm)
0
5
10
15
0
1
2
3
4
5
***
Mar
Sept
Oct
Nov
Dec
Jan
Feb
Topsoil
Control

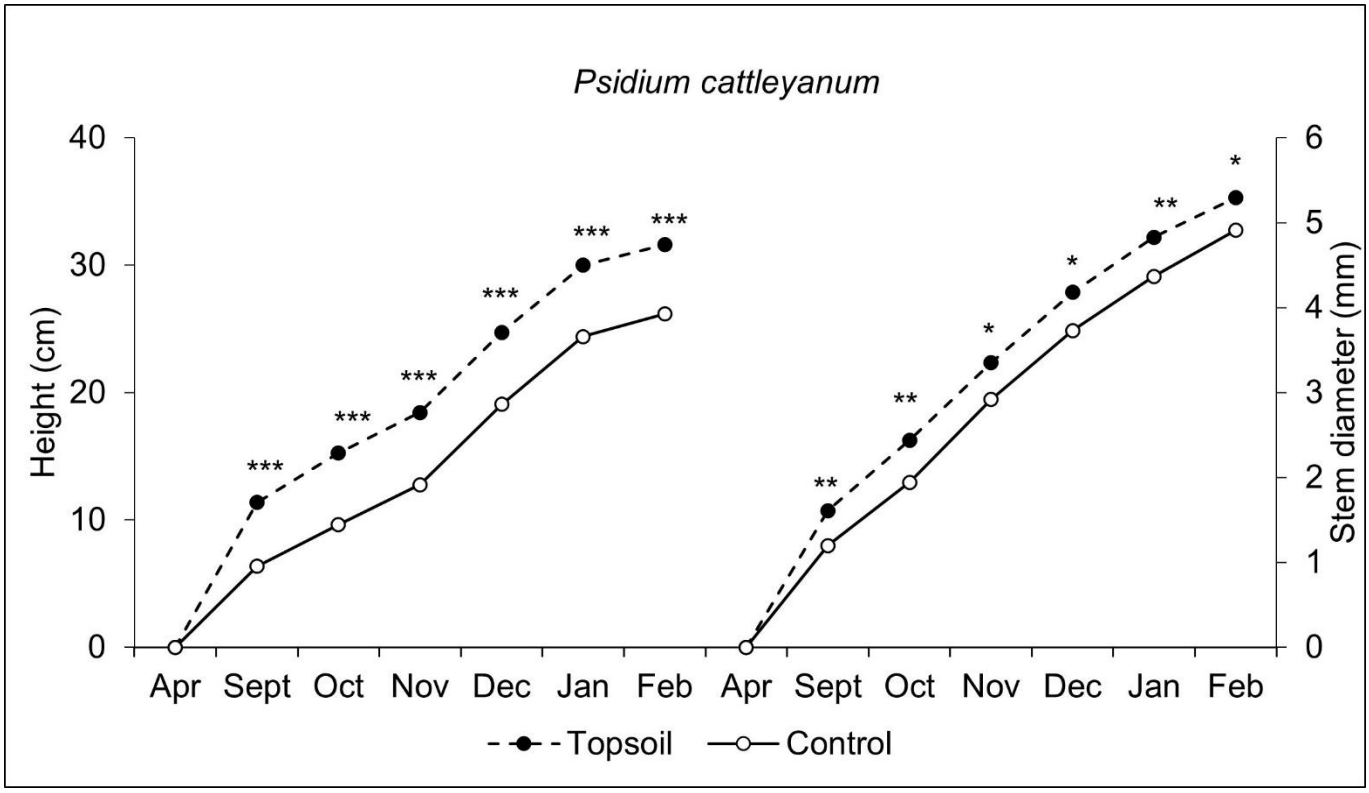

Apuleia leiocarpa
Height (cm)
Stem diameter (mm)
Mar Sept Oct Nov Dec Jan Feb
Topsoil
Control

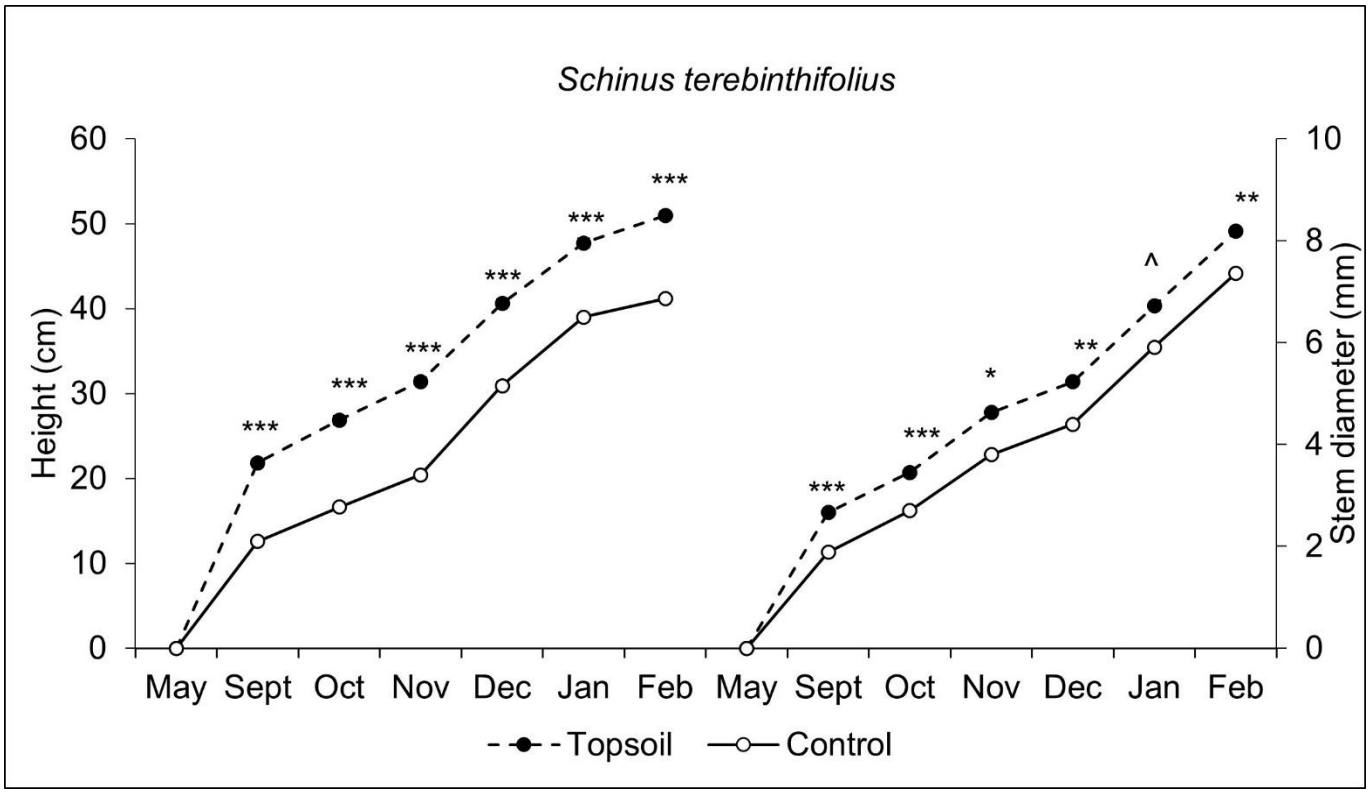

Guazuma ulmifolia
Height (cm)
Stem diameter (mm)
Mar Sept Oct Nov Dec Jan Feb
Topsoil
Control

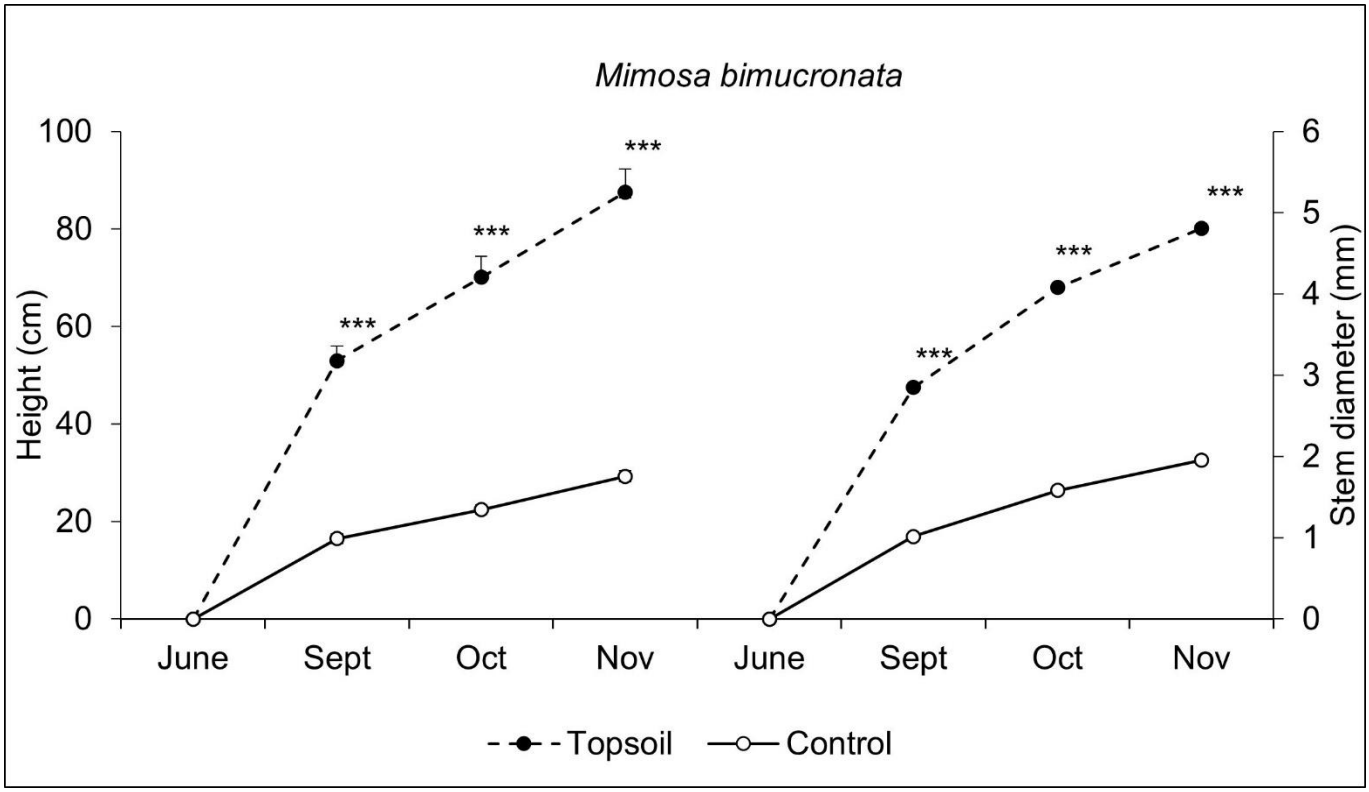

Dalbergia nigra
Height (cm)
Stem diameter (mm)
Mar
Sept
Oct
Nov
Dec
Topsoil
Control

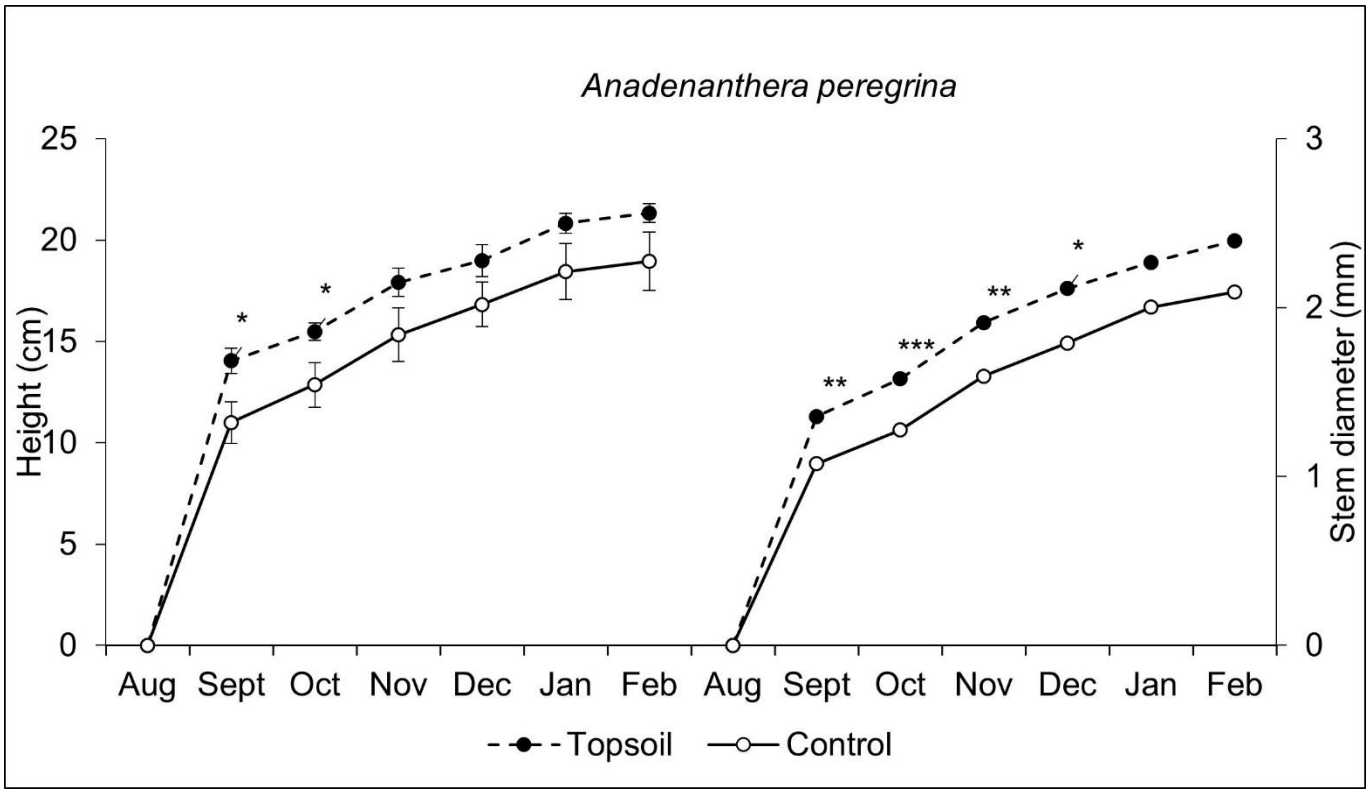

Pseudobombax grandiflorum
Height (cm)
Stem diameter (mm)
Mar
Sept
Oct
Nov
Dec
Jan
Feb
Topsoil
Control

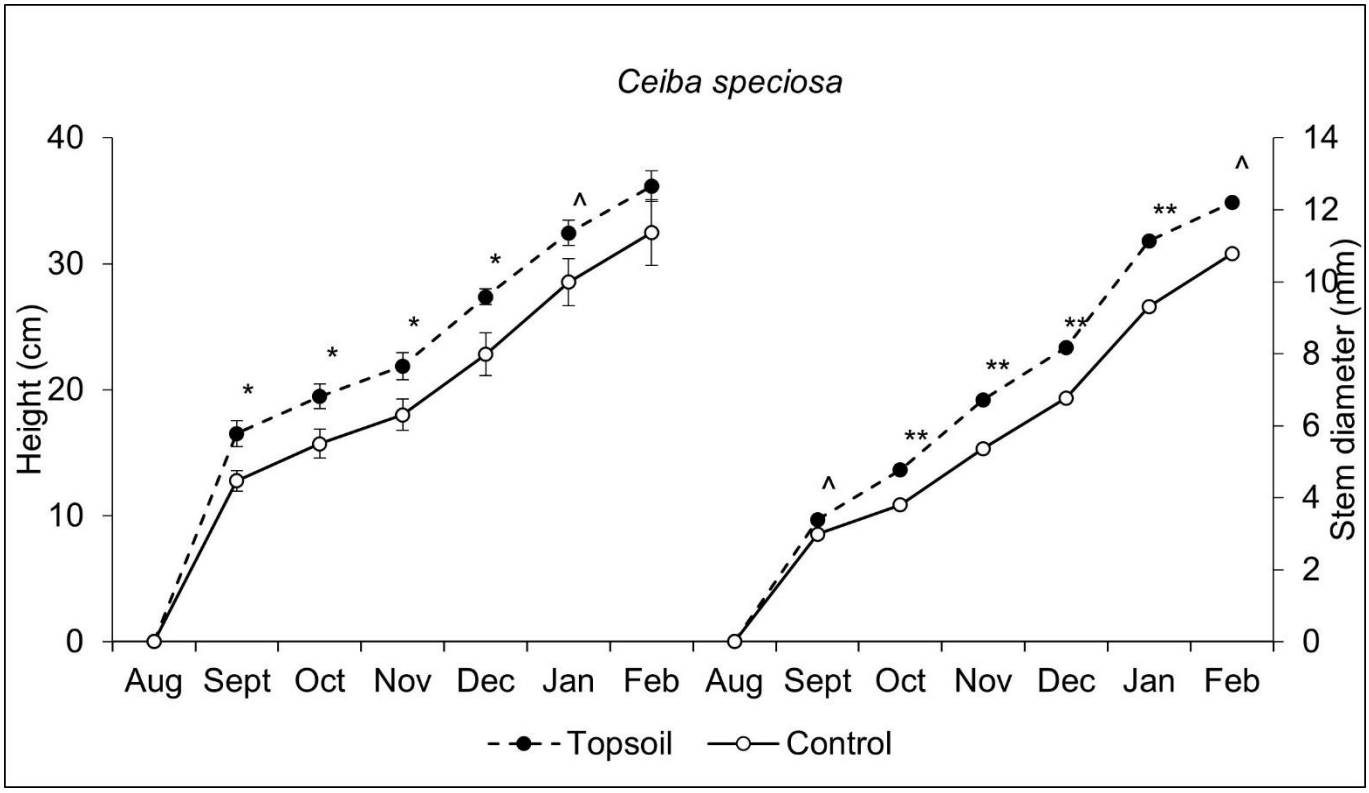

Handroanthus serratifolius
Height (cm)
Stem diameter (mm)
0
5
10
15
0
1
2
3
4
5
Apr
Sept
Oct
Nov
Dec
Jan
Feb
Topsoil
Control


Psidium cattleyanum
Height (cm)
Stem diameter (mm)
0
10
20
30
40
0
1
2
3
4
5
6
Apr
Sept
Oct
Nov
Dec
Jan
Feb
Topsoil
Control


Schinus terebinthifolius
Height (cm)
Stem diameter (mm)
0
10
20
30
40
50
60
0
2
4
6
8
10
***
***
***
***
***
***
***
***
*
**
^
**
May Sept Oct Nov Dec Jan Feb May Sept Oct Nov Dec Jan Feb
Topsoil
Control


Mimosa bimucronata
Height (cm)
Stem diameter (mm)
0
20
40
60
80
100
0
1
2
3
4
5
6
***
***
***
***
***
***
June Sept Oct Nov June Sept Oct Nov
Topsoil
Control


Anadenanthera peregrina
Height (cm)
Stem diameter (mm)
0
5
10
15
20
25
0
1
2
3
Aug
Sept
Oct
Nov
Dec
Jan
Feb
*
*
**
***
**
*
Topsoil
Control


Ceiba speciosa
Height (cm)
Stem diameter (mm)
0
10
20
30
40
0
2
4
6
8
10
12
14
Aug
Sept
Oct
Nov
Dec
Jan
Feb
*
*
*
*
^
^
**
**
**
**
Topsoil
Control

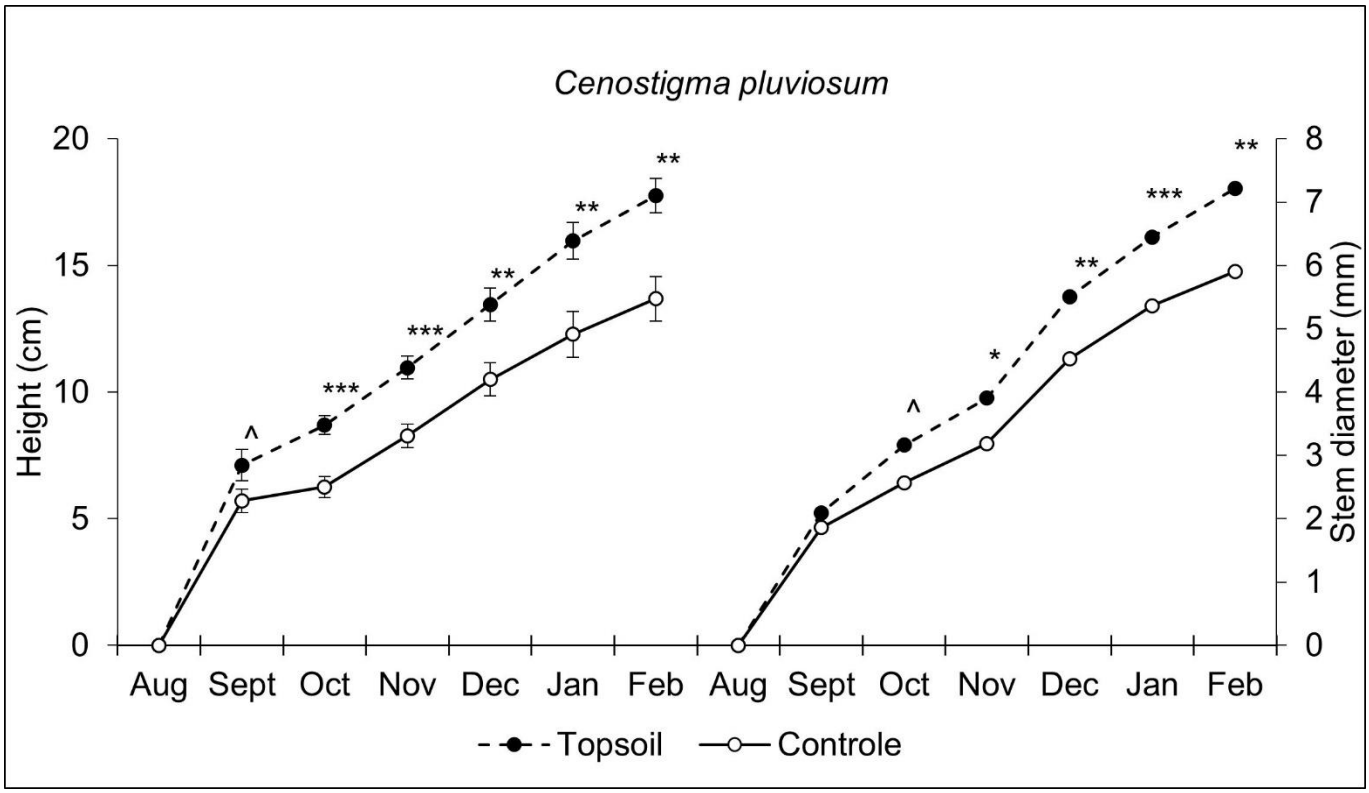



**Figure 2.** Average height (cm) and stem diameter (mm) growth curves of S. multijuga, P. reticulata, S. macranthera, A. leiocarpa, G. ulmifolia, D. nigra, P. grandiflorum, H. serratifolius, P. cattleyanum, S. terebinthifolius, M. bimucronata, A. peregrina, C. speciosa, and C. pluviosum in response to the addition of in natura or sterilized forest topsoil (control) under greenhouse cultivation conditions. Where: * ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), ^ ($p<0.1$), and absence of a graphical symbol indicates that there is no significant difference between treatments by F-test. Source: elaborated by the authors.

The positive effect of topsoil was also observed in the biomass production (Table 4). At the end of the study, plants that received topsoil *in natura* mostly showed superior performance in terms of shoot, root, and nodule development, demonstrating statistically significant differences when compared to the control treatment. There were only two exceptions, as no significant differences were discerned between treatments in the shoot dry mass of *P. grandiflorum* and the root dry mass of *G. ulmifolia*.

In the presence of topsoil in natura, the greatest gains in shoot biomass were found in the nodulating species *P. reticulata* and *M. bimucronata*, while the greatest gains in root biomass were found in species *P. reticulata* and *H. serratifolius*.

The addition of forest topsoil had varying effects on the shoot dry mass to root dry mass ratio (SDM/RDM) of the tree species under study. Among the tested species, *A. leiocarpa*, *D. nigra*, and *H. serratifolius* displayed a significantly lower SDM/RDM ratio in plants treated with topsoil *in natura*. In contrast, *P. grandiflorum*, *M. bimucronata*, and *C. pluviosum* exhibited an opposite pattern, with a higher SDM/RDM ratio in the presence of the sterilized topsoil (Table 4).

Plants grown in pots with sterilized topsoil presented no mycorrhizal colonization. However, when using topsoil *in natura*, all tree species showed mycorrhizal colonization, although in low levels, ranging from 1% to 20%, except *C. pluviosum*, where AMF colonization was classified as medium, according to the methodology proposed by Carneiro et al. (1998).

**Table 4** Shoot dry mass (SDM), root dry mass (RDM), SDM/RDM ratio, nodule dry mass (NDM), and percentage of mycorrhizal colonization (MC) of the evaluated tree species in response to the addition of forest topsoil (in natura) or sterilized topsoil (control) in greenhouse experiments

| Tree species | Treatment | SDM (g) | RDM (g) | SDM/RDM | NDM (g) | MC (%) |
|---|---|---|---|---|---|---|
| *S. multijuga* | topsoil | 1.59* | 1.32* | 1.14$^{ns}$ | - | 2.44* |
| | control | 1.14 | 0.99 | 1.08 | - | 0 |
| *P. reticulata* | topsoil | 9.58* | 5.84* | 1.75 $^{ns}$ | 0.31* | 2.23* |
| | control | 0.24 | 0.16 | 1.44 | 0 | 0 |
| *S. macranthera* | topsoil | 3.91* | 3.30* | 1.32 $^{ns}$ | - | 2* |
| | control | 2.94 | 2.34 | 1.42 | - | 0 |
| *A. leiocarpa* | topsoil | 4.69* | 5.13* | 0.9 | - | 14.45* |
| | control | 2.35 | 1.84 | 1.21* | - | 0 |
| *G. ulmifolia* | topsoil | 4.54* | 5.32 $^{ns}$ | 0,91 $^{ns}$ | - | 18.23* |
| | control | 3.73 | 4.63 | 0,81 | - | 0 |
| *D. nigra* | topsoil | 2.25* | 2.23* | 1.23 | 0.16* | 9.56* |
| | control | 0.21 | 0.06 | 3.30* | 0 | 0 |
| *P. grandiflorum* | topsoil | 5.65$^{(0.11)}$ | 12.36* | 0.45$^{(0.06)}$ | - | 12.78* |
| | control | 5.05 | 8.69 | 0.59 | - | 0 |
| *H. serratifolius* | topsoil | 2.72* | 7.19* | 0.35 | - | 4.43* |
| | control | 1.99 | 2.79 | 0.74* | - | 0 |
| *P. cattleyanum* | topsoil | 7.90* | 5.35* | 1.59 | - | 8.97* |
| | control | 6.04 | 3.07 | 1.98$^{(0.10)}$ | - | 0 |
| *S. terebinthifolius* | topsoil | 9.83* | 6.91* | 1.47 | - | 8.98* |
| | control | 6.97 | 4.28 | 1.55 $^{ns}$ | - | 0 |
| *M. bimucronata* | topsoil | 6.54* | 2.54* | 3.06* | 0.36* | 1.00* |
| | control | 0.36 | 0.23 | 1.57 | 0 | 0 |
| *A. peregrina* | topsoil | 0.33* | 1.81* | 0.18 $^{ns}$ | 0.06* | 1.65* |
| | control | 0.22 | 1.14 | 0.15 | 0 | 0 |
| *C. speciosa* | topsoil | 7.23* | 9.52* | 0.55 | - | 2.74* |
| | control | 4.91 | 7.42 | 0.68 $^{ns}$ | - | 0 |
| *C. pluviosum* | topsoil | 6.97* | 6.59* | 1.09* | - | 40.50* |
| | control | 4.71 | 5.35 | 0.81 | - | 0 |

Mean followed by an asterisk indicates that the treatments differed from each other by the F test ($p<0.05$) in the SDM and RDM variables for the given species. For the NDM and MC variables, the mean followed by an asterisk indicates that the treatments differed according to the Kruskal-Wallis test ($p<0.05$). P values between 0.05 and 0.11 are displayed within parentheses.

ns: no statistically significant differences between the treatments.

-: unexpected nodulation.

Source: elaborated by the authors.

The nutrient concentrations in the shoot dry mass varied from species to species, but as a general trend, the highest macronutrient concentrations were obtained when topsoil in natura was incorporated into the substrate (Table 5). Among nodulating tree species that received topsoil, and consequently formed root nodules, there was an overall increase in nitrogen concentration in the dry mass, with the exception of P. reticulata. Likewise, plants that received the topsoil and were colonized by arbuscular mycorrhizal fungi tended to exhibit higher phosphorus concentration in their biomass.

**Table 5** Nutrient concentration in the shoot dry mass of 14 forest tree species in response to the addition of in natura or sterilized forest topsoil (control) in planting pots in a greenhouse

| Tree species | Treatment | N (%) | Ca (g/kg) | K (g/kg) | Mg (g/kg) | P (g/kg) |
|---|---|---|---|---|---|---|
| *S. multijuga* | Topsoil | 2.47 | 13.54 | 15.55 | 2.32 | 4.50 |
| | Control | 2.04 | 17.05 | 11.15 | 2.16 | 3.66 |
| *P. reticulata* | Topsoil | 1.43 | 11.96 | 16.39 | 2.76 | 9.52 |
| | Control | 1.55 | 7.89 | 6.53 | 2.13 | 1.39 |
| *S. macranthera* | Topsoil | 0.93 | 15.67 | 12.27 | 1.80 | 3.30 |
| | Control | 0.85 | 11.08 | 10.05 | 1.70 | 2.94 |
| *A. leiocarpa* | Topsoil | 0.84 | 9.41 | 11.24 | 1.95 | 2.56 |
| | Control | 1.11 | 7.77 | 9.83 | 1.57 | 2.00 |
| *G. ulmifolia* | Topsoil | 2.54 | 8.02 | 21.96 | 2.65 | 2.25 |
| | Control | 0.76 | 9.92 | 25.14 | 2.88 | 1.91 |
| *D. nigra* | Topsoil | 1.70 | 15.69 | 16.00 | 7.32 | 8.63 |
| | Control | 1.53 | 6.98 | 15.49 | 3.71 | 3.21 |
| *P. grandiflorum* | Topsoil | 0.66 | 9.98 | 20.39 | 4.19 | 1.55 |
| | Control | 0.64 | 10.72 | 17.57 | 4.29 | 1.26 |
| *H. serratifolius* | Topsoil | 1.23 | 10.42 | 16.11 | 3.47 | 1.71 |
| | Control | 1.07 | 10.48 | 15.91 | 3.41 | 1.50 |
| *P. cattleyanum* | Topsoil | 0.51 | 8.41 | 11.46 | 2.45 | 1.17 |
| | Control | 0.51 | 10.18 | 11.08 | 2.75 | 1.15 |
| *S. terebinthifolius* | Topsoil | 0.50 | 5.26 | 18.23 | 0.98 | 1.19 |
| | Control | 0.49 | 4.77 | 15.57 | 0.89 | 1.17 |
| *M. bimucronata* | Topsoil | 2.39 | 21.82 | 12.61 | 3.05 | 2.74 |
| | Control | 1.93 | 10.82 | 10.73 | 2.17 | 1.21 |
| *A. peregrina* | Topsoil | 1.13 | 17.28 | 12.90 | 2.47 | 8.72 |
| | Control | 0. 55 | 17.62 | 14.11 | 2.65 | 8.51 |
| *C. speciosa* | Topsoil | 0.66 | 12.14 | 23.59 | 3.83 | 1.53 |
| | Control | 0.62 | 11.18 | 23.52 | 3.60 | 1.12 |
| *C. pluviosum* | Topsoil | 0.61 | 11.34 | 9.67 | 0.72 | 1.91 |
| | Control | 0.79 | 8.88 | 8.00 | 0.56 | 1.30 |

Source: elaborated by the authors.

## 4. Discussion

The plant microbiome is critical to plant health, but it is rarely considered in ecological restoration. The focus on reintroducing plants without restoring the associated microbiomes may be limiting restoration success (Koziol et al. 2018, 2022), because the symbiotic relationships and processes facilitated by microbiomes play a crucial role in transforming plantings into thriving forests (Busby et al. 2022).

The present study supports the hypothesis that a small volume of forest topsoil applied to the substrate of seedlings destined for reforestation can provide a microbiota that boosts plant growth. In general, plant species

showed better performance in all attributes analyzed when the topsoil *in natura* was added to the pots. Remarkably, among the species, the highest monthly averages in height and stem diameter were achieved by three leguminous species (*P. reticulata*, *D. nigra*, *M. bimucronata*) which form symbiotic associations with both arbuscular mycorrhizal fungi and nitrogen-fixing bacteria, a unique tripartite association. These legumes can be indicated as facilitators of the succession process, as they provide conditions for the establishment of more demanding species, such as rapid growth, increased shading and the supply of nutrients to the soil due to the high production of litter (Martins et al. 2018, Lima et al. 2021).

Several other studies show how the synergistic effect of tripartite association can maximize nutrient acquisition, thus favoring the development and growth of tree legumes (Gross et al. 2004; Borges et al. 2016; Oliveira Júnior et al. 2017; Bournaud et al. 2018; Silva 2018; Afkhami et al. 2020; Freire et al. 2020; Silva 2022). For example, Gross et al. (2004) found significantly higher nodulation in *A. peregrina* plants when inoculated with both NFB and AMF, compared to the treatment containing only NFB. Therefore, the concomitant inoculation of both microsymbionts led to a 60% increase in plant biomass at 10 months, compared to the control. Inoculation with only NFB or AMF did not provide any difference in plant biomass production. In this tripartite association, in addition to mycorrhizae providing plants with greater phosphorus uptake, they also allow for greater dispersal of rhizobia through their hyphae, allowing for contact with the rhizosphere and subsequently promoting nodulation (Novais et al. 2020; Zhang et al. 2020). AMF can also increase nodule abundance and mass, and in return, rhizobia can promote mycorrhizal colonization. Indirect effects occur when microorganisms that provide complementary rewards to host plants cause an increase in plant performance, resulting in an increase in quality/quantity of resources available for the supply of one or more microorganisms (Afkhami et al. 2020).

The amount of spores present in the inoculum added to the substrate (around 640 spores) was probably not a limiting factor that would result in low mycorrhizal colonization, since in the studies mentioned at least 100 to 150 spores are commonly added. In this study, mycorrhizal colonization levels were relatively low in plants that received the fresh topsoil. A possible explanation is the amount of phosphorus present in the nutrient solution (3.2 mg of P per plant every two weeks). In general, plants show less responsiveness to mycorrhization in soils with high levels of P (Pedone-Bonfim et al. 2018), as showed by Freire et al. (2020) in the mycorrhizal colonization of the forest tree species *Tachigali vulgaris* L.F. Gomes da Silva & H.C. Lima when using the same nutrient solution as in the current research, both under greenhouse conditions. In another study, Silva et al. (2016) showed a decrease in mycorrhizal colonization in *Cecropia pachystachya* Trécul with increasing P levels in the soil, but without affecting the height, stem diameter, biomass, and phosphorus levels in the aboveground parts of the plants. On the contrary, the maintenance of a high root colonization rate at high P doses is indicative of high mycorrhizal dependence of the tree species (Santos et al. 2016).

Despite the low levels of mycorrhizal colonization, all tree species that received topsoil consistently exhibited higher phosphorus levels in their shoot mass. This observation suggests a potential influence of strong mycorrhizal efficiency combined with an overall increase in root mass across all tree species. The elevated phosphate nutrition levels may have also caused a positive impact on nodulation and nitrogen fixation in the legume species like *P. reticulata*, *D. nigra*, and *M. bimucronata*. The increased phosphorous supply resulting from topsoil application could also potentially benefit rhizobia associated to these species, as they require a significant amount of ATP for the biological nitrogen fixation process. This phosphorous supply to the plant not only facilitates its growth but also enhances the availability of photosynthates, thereby potentially amplifying biological nitrogen fixation (Nasto et al. 2014).

Several studies have shown that the use of native soil as a source of microorganisms is a powerful tool to accelerate ecological restoration. In degraded grassland areas, reintroduction of soil microbiome from preserved native grasslands has improved survival and productivity of reintroduced native grasses (Wubs et al. 2016; Koziol et al. 2022). In a study developed by Machado et al. (2004), they found that native rhizobia present in unsterilized substrate in pots showed good efficacy in the growth of *Calliandra macrocalyx* Harms compared to selected strains.

In a greenhouse study conducted by Silva (2018), it was found that applying 10 grams of native Caatinga biome topsoil to pots containing pre-germinated seeds of *Mimosa caesalpiniifolia* Benth. resulted in similar growth gains in this tree legume compared to the use of laboratory-produced inoculants containing nitrogen-fixing bacteria (NFB) and arbuscular mycorrhizal fungi (AMF) specifically recommended for this plant species. The author suggests that the high efficiency of topsoil inoculants may result from the adaptation of native NFB and AMF to the conditions of the biome and compatibility with the host plant species.

In another recent greenhouse study conducted by McMahen et al. (2022), additions of forest topsoil, corresponding to 5% (38 mL) and 25% (188 mL) of 750 mL pots containing copper and gold mining rejects or mine sterile soil, increased the survival and growth of seedlings of temperate tree species *Salix scouleriana* (willow), *Alnus viridis* (alder), and *Picea engelmannii* (fir). The greatest ectomycorrhizal fungal colonization was obtained with the highest proportion of forest soil. However, the growth of *A. viridis* was minimally affected by the addition of topsoil, possibly due to the limited presence of necessary nitrogen fixing bacteria and the incompatibility with the fungi present in the inoculum. This highlights the critical importance of selecting a donor soil that not only contains the target plants but also other plant species that form associations with compatible microorganisms. Furthermore, the study yielded distinct results in sterilized topsoil among plant species. It was evident that plants with greater dependence on symbiotic microorganisms, such as *P. engelmannii* and *A. viridis*, benefited from the biological component of the topsoil. In contrast, *S. scouleriana*, a species with lower mycorrhizal dependence, appeared to primarily benefit from the physical and chemical attributes of the forest topsoil.

Interestingly, one study has failed to demonstrate a positive impact of topsoil on the growth of native tree species. According to Silva (2022), the addition of 5 g of topsoil collected from remnants of native vegetation of Caatinga vegetation did not lead to growth enhancement in leguminous trees like *Anadenanthera colubrina* (Vell.) Brenan, *Enterolobium contortisiliquum* (Vell.) Morong, *Mimosa caesalpiniifolia* Benth., *Piptadenia retusa* P.G.Ribeiro, Seigler & Ebinger and *Mimosa ophthalmocentra* Mart. ex Benth. However, the author proposed that the lack of response to the topsoil might be attributed to several factors, including the low density of rhizobia in the topsoil, the small quantity of topsoil utilized, and the presence of competitive rhizobia with limited symbiotic efficiency. However, in the same study, the author demonstrated that inoculants produced with autochthonous rhizobial strains isolated from the same topsoil proved to be efficient in nodulating and promoting the growth of the evaluated tree species.

New studies may elucidate whether topsoil has similar efficiency to inoculants of selected strains of nitrogen-fixing bacteria and arbuscular mycorrhizal fungi. If this approach proves successful in these conditions, it could serve as a simple and cost-effective alternative recommended for the production of high-quality seedlings in nurseries located near planting sites, thereby increasing the chances of success in establishing and growing seedlings in reforestation projects.

Further, field research is essential to investigate the impact of topsoil microbiota on plant growth when applied directly to planting holes. Different application methods should be explored, including placement at the base of the planting hole, around the seedlings, or on the soil surface, among others. Currently, field

experiments using topsoil as inoculum are underway in Wales, UK, and the Yucatán Peninsula, México, aiming to assess whether the planting of native seedlings alongside the introduction of soil microbiota from nearby undisturbed forests can enhance the growth and survival of tree species (Busby et al. 2022).

A single gram of soil can house thousands of individual microbial taxa, including bacteria, fungi, archaea, protists and viruses (Fierer 2017). When these microorganisms interact with the host plant, they are able to exert beneficial, neutral, or detrimental effects on plant growth and development (Lobo et al. 2019). The successful restoration of forests, with their intricated web of organisms, processes and interactions, fundamentally depends on the comprehensive restoration of the entire forest microbiome, including even the presence of pathogens (Busby et al. 2022).

A final crucial highlight is that neither this study nor any of the other studies mentioned here, focusing on the use of topsoil as a source of microorganisms, directly assessed the presence and impact of pathogenic microorganisms. Furthermore, while our study conclusively established the significant influence of NFB and AMF on the growth rate of the assessed tree species, it is essential to acknowledge that the potential contribution of other plant growth-promoting microorganisms should not be overlooked.

## 5. Conclusion

In the present study, the dose of 80 g of forest topsoil proved to be effective in promoting the growth of the 14 tree species found in the Brazilian Atlantic Forest biome under greenhouse conditions. The observed growth stimulation can be attributed to the presence of arbuscular mycorrhizal fungi spores and nodulating nitrogen-fixing bacteria, which established beneficial associations with the trees. This highlights the significant potential of topsoil as a valuable biotechnological tool, whether for seedling production or direct application as a natural inoculant for plants used in restoration projects targeting degraded areas.

## Acknowledgments

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES)-Finance Code 001.

## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

Afkhami ME, Almeida BK, Hernandez DJ, Kiesewetter KN, Revillini DP 2020. Tripartite mutualisms as models for understanding plant–microbial interactions. Curr Opin Plant Biol 56:28-36. https://doi.org/10.1016/j.pbi.2020.02.003

Alvares AC, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G 2013. Koppen's climate classification map for Brazil. Meteorol Z 22(6):711-728. http://dx.doi.org/10.1127/0941-2948/2013/0507

Arnhold E 2022. Easyanova: Analysis of Variance and Other Important Complementary Analyses. Version 4.0.0, https://cran.r-project.org/web/packages/easyanova/easyanova

Averill C, Anthony MA, Baldrian P, Finkbeiner F, Van den Hoogen J, Kiers T, Kohout P, Hirt E, Smith GR, Crowther TW 2022. Defending Earth's terrestrial microbiome. Nat Microbiol 7(11):1717-1725. http://dx.doi.org/10.1038/s41564-022-01228-3

Barros JA, Medeiros EV, Costa DP, Duda GP, Lima JRS, Santos UJ, Antonino ACD, Hammecker, C 2019. Human disturbance affects enzyme activity, microbial biomass and organic carbon in tropical dry sub-humid pasture and forest soils. Arch Agron Soil Sci 66:458-472. https://doi.org/10.1080/03650340.2019.1622095

Berkelmann D, Schneider D, Meryandini A, Daniel R 2020. Unravelling the effects of tropical land use conversion on the soil microbiome. Environmental Microbiome 15(5). https://doi.org/10.1186/s40793-020-0353-3

Borges WL, Prin Y, Ducousso M, Le Roux C, Faria SM 2016. Rhizobial characterization in revegetated areas after bauxite mining. Braz J Microbiol 47(2):314-321. https://doi.org/10.1016/j.bjm.2016.01.009

Bournaud C, James EK, Faria, SM, Lebrun M, Melkonian R, Duponnois R, Tisseyre P, Moulin L, Prin Y 2018. Interdependency of efficient nodulation and arbuscular mycorrhization in *Piptadenia gonoacantha*, a Brazilian legume tree. Plant Cell Environ 41(9). https://doi.org/10.1111/pce.13095

Brancalion, PHS, Meli P, Tymus JRC, Lenti FEB, Benini RM, Silva APM, Isernhagen I, Holl KD 2019 What makes ecosystem restoration expensive? A systematic cost assessment of projects in Brazil. Biol Conserv 240:108274. https://doi.org/10.1016/j.biocon.2019.108274

Busby PE, Newcombe G, Neat AS, Averill C 2022. Facilitating reforestation through the plant microbiome: Perspectives from the phyllosphere. Annu. Rev. Phytopathol. 60:337-356. https://doi.org/10.1146/annurev-phyto-021320-010717

Carneiro MAC, Siqueira JO, Moreira FMS, Carvalho D, Botelho SA, Saggin Júnior, OJ 1998. Micorriza arbuscular em espécies arbóreas e arbustivas nativas de ocorrência no sudoeste do Brasil. Cerne 4(1):129-145

Carvalho NM, Nakagawa J 2000. Sementes: Ciência, tecnologia e produção. Funep, Jaboticabal

Castro DS, Amaro HTR, Araujo EF, Borges EEL, Martins Filho S 2019. Superação de dormência em sementes de garapa ((*Apuleia leiocarpa* (VOGEL)) JF MACBR. Colloq Agrariae 15(4):51-59. https://doi.org/10.5747/ca.2019.v15.n4.a311

Cipriani VB, Garlet J, Lima BM 2019. Quebra de dormência em sementes de *Chloroleucon acacioides* e *Senna macranthera*. Revista de Ciências Agrárias 42(1):49-54. https://doi.org/10.19084/RCA18238

Dwivedi SL, Sahrawat KL, Upadhyaya HD, Mengoni A, Galardini M, Bazzicalupo M, Biondi EG, Hungria M, Kaschuk G, Blair MW, Ortiz R 2015. Advances in host plant and rhizobium genomics to enhance symbiotic nitrogen fixation in grain legumes. Adv Agron 129:1-116. https://doi.org/10.1016/bs.agron.2014.09.001

Ferguson M, Ihrie J. Cran.r-project.org [homepage on the Internet]. MPN: Most Probable Number and Other Microbial Enumeration Techniques; 2019 [updated 2022 Oct 12; cited 2022 Nov 19]. Available from: https://cran.r-project.org/web/packages/MPN.

Fierer N 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol 15(10):579-590. https://doi.org/10.1038/nrmicro.2017.87

Freire JM, Faria SMD, Zilli JE, Saggin Júnior OJ, Camargo IS, Rouws JRC, Jesus EC 2020. Symbiotic efficiency of inoculation with nitrogen-fixing bacteria and arbuscular mycorrhizal fungi in *Tachigali vulgaris* seedlings. Rev Árvore 44. https://doi.org/10.1590/1806-908820200000024

Gerdermann JN, Nicolson TH 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 46(2):235-244. https://doi.org/10.1016/S0007-1536(63)80079-0

Giovannetti M, Mosse B 1980. An evaluation of techniques to measure vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84(3):484-500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Gossner MM, Lewinsohn TM, Kahl T, Grassein F, Boch S, Prati D, Birkhofer K, Renner SC, Sikorski J, Wubet T, Arndt H, Baumgartner V, Blaser S, Blüthgen N, Börschig C, Buscot F, Diekötter T, Jorge LR, Jung K, Keyel AC, Klein AM, Klemmer S, Krauss J, Lange M, Müller J, Overmann J, Pašalić E, Penone C, Perović DJ, Purschke O, Schall P, Socher SA, Sonnemann I, Tschapka M, Tscharntke T, Türke M, Venter PC, Weiner CN, Werner M, Wolter V, Wurst S, Westphal C, Fischer M, Weisser WW, Allan E 2016. Land-use intensification causes multitrophic homogenization of grassland communities. Nature 540(7632):266-269. https://doi.org/10.1038/nature20575

Grace C, Stribley DP 1991. A safer procedure for routine staining of vesicular-arbuscular mycorrhizal fungi. Mycol. Res.95:11160-1162. https://doi.org/10.1016/S0953-7562(09)80005-1

Gross E, Cordeiro L, Caetano FH 2004. Nodulação e micorrização em *Anadenanthera peregrina* var. falcata em solo de cerrado autoclavado e não autoclavado**.** Rev Bras Cienc Solo 28:95-101. https://doi.org/10.1590/S0100-06832004000100010

Guzmán I, Döbereiner J 1968. Effectiveness and efficiency in the symbiosis of your cross inoculated tropical legumes. In: Anais da 4º Reunião Latino Americana sobre inoculantes para leguminosas, Universidade Federal do Rio Grande do Sul, Porto Alegre 81-91.

Instituto Brasileiro de Geografia e Estatística – IBGE 2012. Manual técnico da vegetação brasileira, 2nd ed. IBGE, Rio de Janeiro (Manuais Técnicos em Geociências, 1)

Instituto Nacional de Meteorologia - INMET 2021. *Dados históricos anuais*. bdmep.inmet.gov.br Accessed 25 March 2021

Ivanova EA, Pershinaa EV, Kutovayab OV, Sergalieva NK, Nagievad AG, Zhiengalievd AT, Provorova N A, Andronova EE 2018. Comparative analysis of microbial communities of contrasting soil types in different plant communities. Russ J Ecol 49(1):30-39. https://doi.org/10.1134/S106741361801006X

Jakovac CC, Junqueira AB, Crouzeilles R, Peña-Claros M, Mesquita RC, Bongers F 2021. The role of land-use history in driving successional pathways and its implications for the restoration of tropical forests. Biol Rev 96(4):1114-1134. https://doi.org/10.1111/brv.12694

Jarvis B, Wilrich C, Wilrich PT 2010. Reconsideration of the derivation of Most Probable Numbers, their standard deviations, confidence bounds and rarity values. J Appl Microbiol 109(5):1660-1667. https://doi.org/10.1111/j.1365-2672.2010.04792.x

Jenkins WR 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis Rep 48:692.

Kestring D, Klein J, de Menezes LCCR, Rossi MN 2009. Imbibition phases and germination response of Mimosa bimucronata (Fabaceae: Mimosoideae) to water submersion. Aquatic Botany 91(2):105-109. https://doi.org/10.1016/j.aquabot.2009.03.004

Koske RE, Gemma JN 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res 92(4):486-488. https://doi.org/10.1016/S0953-7562(89)80195-9

Koziol L, Schultz PA, House GL, Bauer JT, Middleton EL, Bever JD 2018. The plant microbiome and native plant restoration: the example of native mycorrhizal fungi. BioScience 68(12):996-1006. https://doi.org/10.1093/biosci/biy125

Koziol L, Bauer JT, Duell EB, Hickman K, House GL, Schultz PA, Tipton AG, Wilson GWT, Bever JD 2022. Manipulating plant microbiomes in the field: Native mycorrhizae advance plant succession and improve native plant restoration. J Appl Ecol 59:1976-1985. https://doi.org/10.1111/1365-2664.14036

Lima KDRD, Correia MEF, Campello EFC, Resende AS 2021. Regeneração natural após 13 anos de plantio com espécies arbóreas fixadoras de nitrogênio em Valença, no estado do Rio de Janeiro. Ciênc Florest 31:830-845. https://doi.org/10.5902/1980509844460

Lobo CB, Tomás MSJ, Viruel E, Ferrero MA, Lucca ME 2019. Development of low-cost formulations of plant growth-promoting bacteria to be used as inoculants in beneficial agricultural technologies. Microbiological research 219:12-25. https://doi.org/10.1016/j.micres.2018.10.012

Machado RL, Resende AS, Pitard R, Faria SM 2004. Obtenção e seleção de estirpes de rizóbio para leguminosas florestais com potencial de uso em áreas degradadas**.** Embrapa Agrobiologia, Seropédica

Martins EM, Silva ERD, Campello EFC, Lima SSD, Nobre CP, Correia MEF, Resende AS 2019. O uso de sistemas agroflorestais diversificados na restauração florestal na Mata Atlântica. Ciênc Florest 29:632-648. https://doi.org/10.5902/1980509829050

McMahen K, Lavkulich LM, Grayston SJ, Simard SW 2022. Small-volume additions of forest topsoil improve root symbiont colonization and seedling growth in mine reclamation. Appl Soil Ecol 180:104622, 2022. https://doi.org/10.1016/j.apsoil.2022.104622

Nasto MK, Alvarez-Clare S, Lekberg Y, Sullivan BW, Townsend AR, Cleveland CC 2014. Interactions among nitrogen fixation and soil phosphorus acquisition strategies in lowland tropical rain forests. Ecol Lett 17(10):1282-1289. https://doi.org/10.1111/ele.12335

Nogueira ARA, Souza GB 2005. Manual de laboratórios: solo, água, nutrição vegetal, nutrição animal e alimentos. Embrapa Pecuária Sudeste, São Carlos

Novais CB, Sbrana C, Jesus EC, Rouws LFM, Giovannetti M, Avio L, Siqueira JO, Saggin Júnior OJ, Silva EMR, Faria SM 2020. Mycorrhizal networks facilitate the colonization of legume roots by a symbiotic nitrogen-fixing bacterium. Mycorrhiza 30:389-396. https://doi.org/10.1007/s00572-020-00948-w

Oliveira Júnior JQ, Jesus EC, Lisboa FJ, Berbara RLL, Faria SM 2017. Nitrogen-fixing bacteria and arbuscular mycorrhizal fungi in *Piptadenia gonoacantha* (Mart.) Macbr. Braz J Microbiol 48(1):95-100. https://doi.org/10.1016/j.bjm.2016.10.013

Paiva Sobrinho SD, Siqueira AG, Morais PDB, Silva SJD 2012. Superação da dormência em sementes de mutamba (*Guazuma ulmifolia* Lam. -Sterculiaceae). Rev Árvore 36(5):797-802. https://doi.org/10.1590/S0100-67622012000500001

Pedone-Bonfim MVL, Silva DKA, Maia LC 2018. Mycorrhizal benefits on native plants of the Caatinga, a Brazilian dry tropical forest. Symbiosis 74:79-88. https://doi.org/10.1007/s13199-017-0510-7

Piveta G, Menezes VO, Pedroso DC, Muniz MFB, Blume E, Wielewicki AP 2010. Superação de dormência na qualidade de sementes e mudas: influência na produção de *Senna multijuga* (LC Rich.) Irwin & Barneby. Acta Amaz 40(2):281-288. https://doi.org/10.1590/S0044-59672010000200006

Rahman MH, Khatun S, Ali SR, Yasmin S, Kamruzzaman M, Rashid MH 2018. Morpho-physiological diversity of root nodule rhizobia from Mimosa (*Mimosa pudica* L.) and water Mimosa (*Neptunia oleracea* L.). J Bacteriol Mycol 5(1):1061, 2018

Redford KH 2023. Extending conservation to include Earth's microbiome. Conserv Biol e14088. https://doi.org/10.1111/cobi.14088

Santos RS, Scoriza RN, Silva EMR, Saggin Júnior OJS 2016. Selection of mycorrhizal fungi for the initial growth of *Albizia polycephala*. Rev Bras Cienc Agrar 11(2):98-103. https://doi.org/10.5039/agraria.v11i2a5362

Schenck NC, Perez Y 1988. A manual for identification of vesicular-arbuscular mycorrhizal fungi, 2nd ed. University of Florida, Gainesville

Silva CF, Pereira MG, Santos VL, Miguel DL, Silva EMR 2016. Arbuscular mycorrhizal fungi: Composition, length extraradical mycelium and glomalin in areas of Atlantic Forest, Rio de Janeiro. Cienc Florest 26(2):419-433. https://doi.org/10.5902/1980509822743

Silva MM, Ataíde GM, Ribeiro LP, Catarina A, Cunha MC, Silva LBJ 2013. Superação da dormência e germinação de sementes de *Plathymenia reticulata* (Fabaceae) em diferentes temperaturas. In: III Semana de Engenharia Florestal da Bahia, Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista 1214-1219

Silva FF 2018. Uso de *topsoil* como fonte de inóculo de microrganismos simbiontes para leguminosas florestais usadas na recuperação de áreas degradadas na Caatinga. Dissertation, Universidade Federal Rural do Rio de Janeiro

Silva, FF 2022. Avaliação da associação simbiótica entre bactérias fixadoras de nitrogênio, fungos micorrízicos arbusculares e leguminosas arbóreas nativas da Caatinga. Thesis, Universidade Federal do Rio de Janeiro

Sun Y, Luo C, Jiang L, Song M, Zhang D, LI J, Ostle NJ, Zhang G 2020. Land-use changes alter soil bacterial composition and diversity in tropical forest soil in China. Sci Total Environ 712. https://doi.org/10.1016/j.scitotenv.2020.136526

Teixeira PC, Donagemma GK, Fontana A, Teixeira WG 2017. Manual de métodos de análise de solo, 3rd ed. Embrapa, Brasília, DF

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A 2015. The importance of the microbiome of the plant holobiont. New Phytol. 206(4):1196-1206. https://doi.org/10.1111/nph.13312

Wubs ERJ, van der Putten WH, Bosch M, Bezemer TM 2016. Soil inoculation steers restoration of terrestrial ecosystems. Nature Plants 2:16107. https://doi.org/10.1038/nplants.2016.107

Zhang W, Li XG, Sun K, Tang MJ, Xu FJ, Zhang M, Dai CC 2020. Mycelial network-mediated rhizobial dispersal enhances legume nodulation. ISME J 14(4):1015-1029. https://doi.org/10.1038/s41396-020-0587-5