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Urochloa brizantha and Amazonian Dark Earths reshape soil microbiota without affecting tree growth in degraded Central Amazon Oxisols

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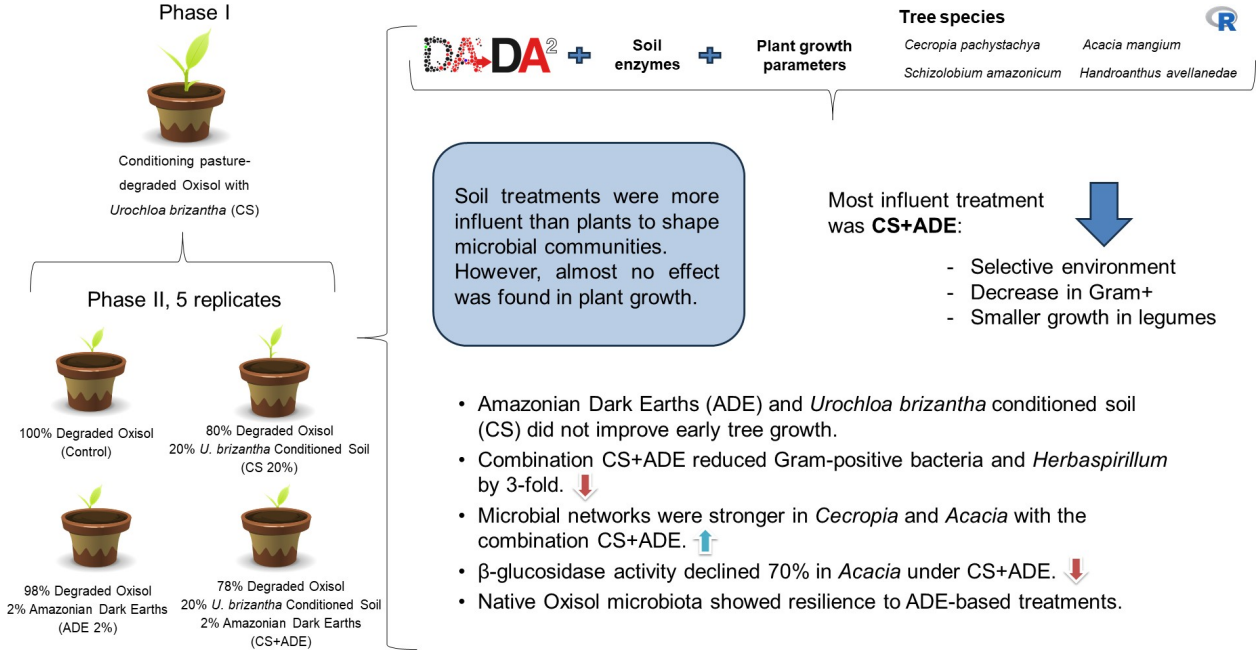
ABSTRACT



• Amazonian Dark Earths (ADE) and *Urochloa brizantha* conditioned soil (CS) did not improve early tree growth.

• Combination CS+ADE reduced Gram-positive bacteria and *Herbaspirillum* by 3-fold.

• Microbial networks were stronger in *Cecropia* and *Acacia* with the combination CS+ADE.

• β-glucosidase activity declined 70% in *Acacia* under CS+ADE.

• Native Oxisol microbiota showed resilience to ADE-based treatments.

Amazonian Dark Earths (ADE) are fertile anthropogenic soils rich in organic matter and microbial diversity, offering potential for restoring degraded tropical soils. We tested the combined effects of ADE (2% w/w) and *Urochloa brizantha* conditioned soil (CS 20%) on soil microbial communities and early growth of four tree species (*Cecropia pachystachya*, *Schizolobium amazonicum*, *Handroanthus avellanedae*, *Acacia mangium*) in a pasture-degraded Oxisol. Plant performance, soil enzyme activity, prokaryotic community structure (16S rRNA sequencing), predicted functions, and co-occurrence networks were evaluated. Neither ADE nor *U. brizantha*, alone or combined, significantly improved tree growth or microbial alpha diversity ($p < 0.05$). However, the combination CS+ADE shifted microbial composition, reducing by 3-fold the abundance of several aerobic Gram-positive taxa (*Actinophytocola*, *Lysinibacillus*, *Rubrobacter*) and nitrogen-fixers (*Herbaspirillum*). Network analyses

showed treatment-specific connectivity changes, especially in Cecropia and Acacia, where CS+ADE increased both positive and negative microbial associations. Functional prediction and enzyme assays revealed a largely stable functional core, except for a 70% decline in β-glucosidase activity in *Acacia* under CS+ADE, indicating altered carbon cycling. Overall, while microbial networks responded strongly, limited ADE input and the stability of native microbiota constrained plant and functional benefits, underscoring the importance of application strategies in restoration.

Keywords biotic interactions, co-occurrence patterns, land degradation, microbial network analysis, plant-soil feedback, tropical forest species.

1 Introduction

Soil degradation stands as a major obstacle to both ecological restoration and sustainable development, particularly in tropical ecosystems. Highly weathered soils, such as the Oxisols prevalent in the Amazon, are characterized by low natural fertility, a condition that severely restricts the growth of native tree species and makes restoration efforts challenging (Anda and Kurnia, 2010; IAC, 2020). Traditional approaches often rely on the addition of chemical amendments and fertilizers, but these methods are frequently unsustainable, can lead to further soil acidification, and often fail to provide lasting improvements in soil quality and plant performance (Gann et al., 2019; Peddle et al., 2025).

Alternatively, more sustainable solutions have emerged, including the use of conditioning pastures to improve soil structure and organic matter, and the application of highly fertile and/or microbial-rich soils (Howard et al., 2017; Cruz et al., 2022). These strategies hold great promise for enhancing soil quality, even though the long-term effects of these approaches on plant growth and the complex interactions within the soil microbial communities remain poorly understood.

The soil microbiome is a central player in nutrient cycling, plant health, and the success of ecological restoration. Changes in land management practices can profoundly alter not only the diversity and composition of these microbial communities, but also their functional roles and co-occurrence patterns. Such shifts can have significant and lasting implications for the health of the entire ecosystem (Bieluczyk et al., 2023; Pedrinho et al., 2024; Tan et al., 2025). Previous studies have demonstrated that Amazonian Dark Earths (ADE), rich soil developed by pre-Columbian societies in the Amazon, can effectively promote plant growth and increase microbial activity, particularly when applied in large proportions (Lombardo et al., 2022; de Freitas et al., 2023, 2025). However, the introduction of a new microbiome (via ADE) into already established, degraded soil microbiotas may not be sufficient to significantly alter the system. Furthermore, there is a lack of integrated evidence evaluating the combined effects of ADE addition and soil conditioning with pastures on microbial communities, soil functions, and the growth of key tropical tree species.

This study addressed these gaps by evaluating the short-term effects of ADE addition (at a 2% proportion) and *Urochloa brizantha*-conditioned soil, both individually and in combination. We hypothesize that the combined approach will synergistically enhance soil conditions and promote plant growth more effectively than either treatment alone. We assessed the impacts on: (i) the initial growth performance of four tree species vital for restoration efforts; (ii) the diversity and composition of the soil bacteriome; and (iii) the predicted functional profiles and bacterial co-occurrence networks. Our findings aimed to provide crucial insights into the synergistic potential of these low-cost, sustainable practices, contributing to more effective and biologically driven strategies for ecological restoration in degraded tropical soils.

2 Materials and methods

2.1 Soil source

The control soil was obtained from a farm located in Presidente Figueiredo, Amazonas, Brazil (2°2′4″ S, 60°1′33″ W), classified as Oxisol according to the USDA system (García-Gaines and Frankenstein, 2015). This soil presented a state of degradation, with high compaction, low base saturation, and a lack of function, resulting in the pasture's inability to grow after 30 years of intensive use. The farm soil was classified as Yellow Oxisol (Latossolo Amarelo), and the region's climate is classified as tropical rainforest (Af) according to the Köppen classification, characterized by a mean annual temperature of approximately 27 ºC and high relative humidity. The Amazonian Dark Earth (ADE) samples were obtained from the Caldeirão Experimental Station in Iranduba, Amazonas (03°26′00" S, 60°23′00" W). The region (including both locations) presents an average annual rainfall ranging from 2750 to 3000 mm, with the period of lowest precipitation occurring between July and September (Alvares et al., 2013).

Both soils were sampled from the top 20 cm (arable layer) at five random locations per site, at least 15 meters from the area's borders, and homogenized. Soil samples were sent to Agrilab Análises Agrícolas (Botucatu, SP, Brazil) for evaluation of organic matter, pH, macronutrients (P, K, Ca, Mg,

S), micronutrients (Cu, Fe, Mn, Zn), Al^{3+}, and texture (sand, clay, silt) following van Raij et al.'s methods for Brazilian soils (van Raij et al., 2001). The remaining soil (approximately 600 kg of oxisol and 10 kg of ADE) was transported to Piracicaba, São Paulo, for the establishment of the experiment. This study was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, acronym in Portuguese) under the access number AD13FB3.

2.2 Experimental design

The greenhouse experiment was conducted in two phases to simulate the conversion of pasture to reforestation. In phase I, 20 three-liter plant pots were filled with a control soil. Seeds of *Urochloa brizantha* cv. Marandu, the most common pasture species in the Amazon, were sown in each pot. Once the seeds germinated, the plant count in every pot was standardized to ensure uniformity. These plants grew for 60 days, receiving water to field capacity every 48 hours. At the end of this phase, the above-ground parts of the plants were removed. The remaining soil, along with the roots, was homogenized into a single mix and saved to be used as inoculum for the corresponding treatments in Phase II.

Phase II of the experiment investigated four distinct soil treatments designed to evaluate the feedback effects of pasture and Amazonian Dark Earth (ADE) microbiota on plant growth. The treatments were as follows: 100% control soil (Control), 80% control soil supplemented with 20% soil from Phase I (CS 20%), 98% control soil supplemented with 2% fresh Amazonian Dark Earth (ADE 2%), and 78% control soil supplemented with 20% conditioned soil and 2% fresh Amazonian Dark Earth (CS+ADE). These treatments were applied to four plant species: two primary successional species (*Cecropia pachystachya* and *Schizolobium amazonicum*) and two secondary successional species (*Handroanthus avellanedae* and *Acacia mangium*). Additionally, we maintained pots without plants to serve as a negative control, excluding the plant effect (Bulk). All plants were germinated by the Bauru Botanical Garden (Bauru, SP, Brazil) and selected for the same size for use in the experiment. The experiment included five replicates per plant species per treatment, resulting in a total of 100 experimental pots. The entire setup was maintained in the greenhouse for 120 days at an average temperature of 23.8 °C (±2.9 °C) and 64% (±11%) air humidity, receiving water to field capacity every 48 hours.

2.3 Experimental sampling

After 120 days of experiment, several plant and soil parameters were quantified. Plant height was determined by measuring the vertical distance from the soil surface to the highest plant node using a measuring tape. Canopy area was calculated by multiplying the lengths of two perpendicular measurements across the plant canopy. All above-ground biomass was harvested, oven-dried at 60 °C for 48 hours, and subsequently weighed to determine dry mass. Roots were carefully extracted from the soil using sterile gloves, and their total length was measured. Finally, soil samples were collected from the rhizosphere for subsequent DNA extraction and enzyme activity analyses. Samples designated for DNA extraction were immediately frozen at −20 °C until processing, while those for enzyme activity were stored with aeration at 4 °C until analysis.

2.4 Molecular procedures

Microbial DNA was extracted from 0.25 g soil aliquots using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany), following manufacturer instructions with modifications by Venturini and colleagues for tropical soils (Venturini et al., 2020). DNA quality was assessed with a Nanodrop™ 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA); samples with DNA concentration exceeding 10 ng μL^{-1} and A260/A280 ratios between 1.70 and 2.00 were deemed suitable for downstream analysis.

Amplification and sequencing were performed by Novogene Corporation Inc. (Sacramento, CA, USA) using standard Earth Microbiome Project protocols (Gilbert et al., 2014). The V3−V4 region of the 16S rRNA gene was amplified with updated 515F and 816R primers to quantify prokaryotic (bacterial and archaeal) abundance (Parada et al., 2016). Paired-end sequencing (2 × 250 bp reads) was conducted on an Illumina Novaseq 6000 platform. Raw reads are publicly available in the Sequence Read Archive (SRA) under project number PRJNA1157008.

Additionally, enzyme activity analyses were conducted on soil from each pot. Acid phosphatase activity was determined using an adapted colorimetric method (Eivazi and Tabatabai, 1977). β-glucosidase activity was assessed using ρ-nitrophenyl-β-D-glucopyranoside as a substrate (Eivazi and Tabatabai, 1988). Finally, arylsulfatase activity was analyzed via the hydrolysis of potassium p-nitrophenyl sulfate (the enzyme's substrate) to p-nitrophenol (PNP), after incubating soil samples for 1 hour at 37 °C (Tabatabai and Bremner, 1970). All reaction products were quantified colorimetrically at 410 nm using an ELISA microplate reader (LMR FLEX UV-VIS i; Loccus Biotecnologia, Cotia, SP, Brazil).

2.5 Data analyses

All bioinformatics and statistical analyses were carried out in the R environment (version 4.4.2) using the RStudio

program (version 2024.09.1) (R Core Team, 2022). The code for the analyses performed here can be publicly found on GitHub available at the website of github.com/FreitasAndy/PSF-CentralAmazon. Figures were produced using the ggplot2 package, and some of these figures were edited only for aesthetic purposes (i.e., changing colors and fonts) using the program Inkscape 1.3.2 (available at the website of inkscape.org).

Given that each group contained fewer than 30 samples, and due to anticipated non-normal distribution of ecological and soil parameters, all data were treated as non-parametric, and subsequent analyses were chosen accordingly. We used the Kruskal-Wallis test (Kruskal and Wallis, 1952), followed by Dunn's post-hoc test (Dinno, 2015), to assess differences across various parameters. These included plant growth metrics (dry mass, root length, canopy area, and plant height), soil texture and chemical properties (organic matter, pH, phosphorus, potassium, calcium, magnesium, potential acidity, aluminum, sulfur, copper, iron, manganese, zinc, sand, clay, and silt), and enzymatic activities (acid phosphatases, beta-glucosidases, and arylsulfatases).

Raw reads from sequencing were analyzed using the DADA2 pipeline (Callahan et al., 2016), considering acceptable sequences with a mean quality score greater than 30. Filtered reads were grouped into amplicon sequence variants (ASVs) and matched to taxonomy using the SILVA database v. 138.1 (Quast et al., 2013). The resulting ASV table was imported into both a phyloseq S4 object (McMurdie and Holmes, 2013) and a microeco's R6 object (Liu et al., 2021) for downstream analysis. Alpha diversity, representing the number of unique taxa within each sample (observed diversity), was compared between groups using the Kruskal-Wallis test, followed by Dunn's post-hoc test for pairwise comparisons, all at a 95% confidence level. For beta diversity analysis, the dataset was transformed using a centered log ratio (CLR) transformation to accurately reflect its compositional structure. Data ordination was then performed using Euclidean distance and visualized via non-metric multidimensional scaling (NMDS) on the first two axes. Statistical significance was determined using Permutational Multivariate Analysis of Variance (PERMANOVA), with a significance level of 5% and 999 permutations, implemented via the adonis function from the vegan package (Oksanen et al., 2015).

Differential abundance analysis was performed for each treatment relative to the control using the ALDEx2 algorithm (Fernandes et al., 2013). Significant differences were identified by a *t*-test (Benjamini-Hochberg adjusted *p*-value < 0.05) and an effect size greater than 1. Functional estimation for each sample was conducted using the FAPROTAX tool (Louca et al., 2016), which predicts microorganism functions from an ASV table based on previously published studies. The results were visualized as a heatmap, highlighting key functions related to soil. Correlation network analyses were performed at the genus level using the SpiecEasi algorithm (Kurtz et al., 2015), considering significant correlations higher than 70% with *p*-values lower than 0.001, thereby capturing only strong and most trustworthy correlations.

3 Results

3.1 Characteristics of the initial degraded Oxisol and ADE

The initial soil presented a microbial community mainly composed of the orders Rhizobiales, Acidobacteriales, Subgroup 2, Burkholderiales, and Chthiobacteriales (Fig. 1, left panel). The chemical composition (Fig. 1, right panel) was nutrient-poor, with only a few elements reaching adequate levels. The pH was acidic (~5.0), aluminum was detected (0.5 $mmol_c$ kg^{-1}), and phosphorus, boron, and potassium levels were low. Calcium and magnesium were within acceptable ranges but may still be affected by high exchangeable acidity. The base saturation indicated moderate fertility, but the high hydrogen content indicated poor nutrient retention. The organic matter content was also low, confirming the degraded state of the soil. On the other hand, the ADE soil was characterized by high fertility and a substantial organic carbon reserve. The pH indicated moderate acidity (5.2 ± 0.1), high organic matter content (46.5 ± 6.5%), and high concentrations of phosphorus (160.3 ± 38.4 mg dm^{-3} and potassium (45.8 ± 20.1 $mmol_c$ dm^{-3}). Calcium and magnesium levels were 6.8 ± 0.6 and 1.2 ± 0.3 $mmol_c$ dm^{-3}, respectively. The potential acidity ($H + Al^{3+}$) was minimal (4.3 ± 0.6 $mmol_c$ dm^{-3}). Consequently, the ADE exhibited a high base saturation (82.0 ± 0.8%), indicating a eutrophic environment with low aluminum toxicity and high nutrient availability (de Freitas et al., 2025).

3.2 ADE increased microbial biomass but had limited effects on plant growth and microbial diversity

The estimated microbial biomass responded clearly to the treatments: the addition of 2% ADE consistently increased microbial biomass in bulk soil and root-associated samples across all species, except for *S. amazonicum* (Fig. 2A), suggesting that ADE can stimulate microbial growth (increase in biomass) in degraded soils, potentially supporting the proliferation of the native microbial community. However, microbial diversity remained mostly stable across treatments, except for a significant reduction in observed ASV richness in *C. pachystachya* grown in Control soil.

Microbial growth was nonetheless not accompanied by

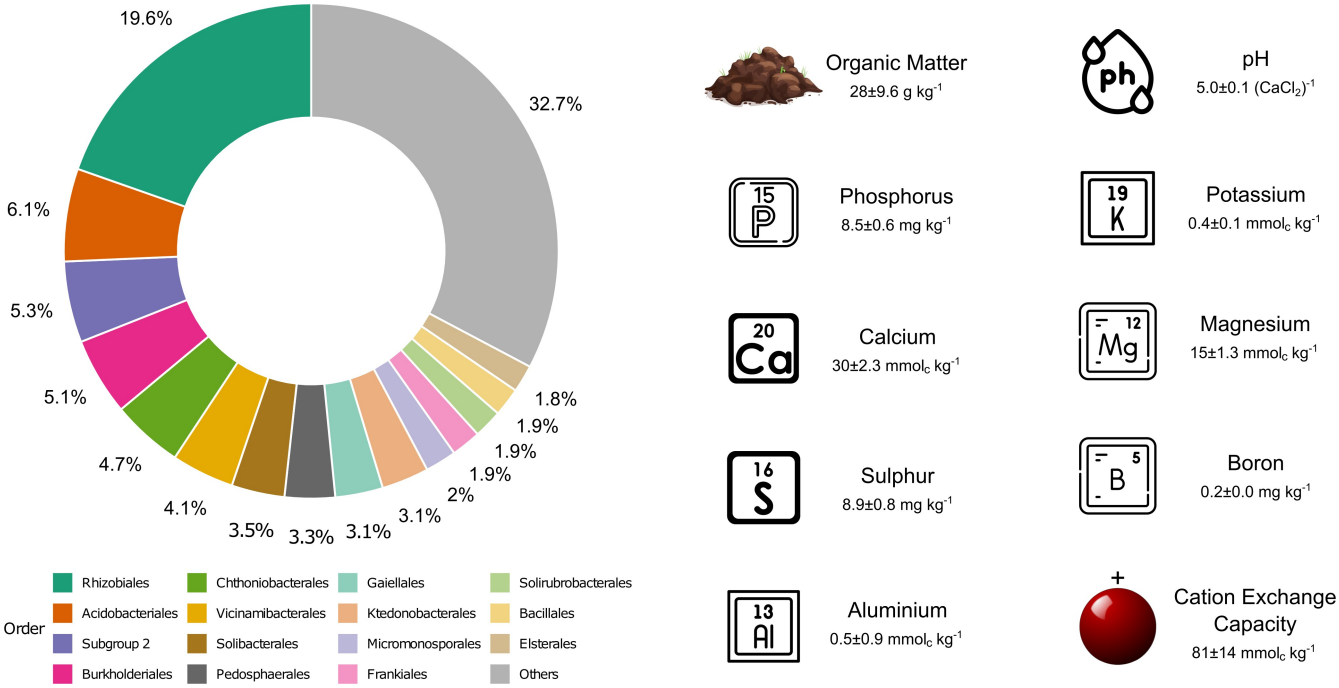


Fig. 1 Microbial and chemical aspects of the initial soil used as Control and base for treatments. The soil was classified as an Oxisol and came from a degraded pasture in the Central Amazon.

increased plant growth. The combination of treatments (CS + ADE) negatively affected *C. pachystachya*, reducing aerial biomass, plant height, and stem circumference, as well as reducing root size in *A. mangium* (Table 1). The only positive growth response was observed in *S. amazonicum*, which showed increased plant height under the CS 2% treatment alone. *H. avellanedae* exhibited no significant differences in growth across treatments.

3.3 Soil treatments, not plant species, primarily shaped microbial community composition

Although little variation was observed in plant development, microbial communities were strongly affected by the different treatments. Beta diversity analysis revealed that the treatments shaped microbial composition more than plant identity. The NMDS ordination showed a clear separation of all treatments from the control, with the CS + ADE treatment positioned farthest from the ADE-alone group, suggesting a synergistic effect of the two amendments on microbial communities (Fig. 3). This spatial pattern was consistent across all tree species, and the Control samples clustered centrally, indicating that each amendment introduced distinct shifts in community structure. Permutational analysis confirmed this separation, with a significant R^2 of 36% ($p < 0.001$), reinforcing the idea that soil management strategies had a more pronounced impact on microbial assemblages than plant species identity.

Differential abundance analysis further supported this observation by identifying 28 microbial genera significantly depleted in the CS + ADE treatment relative to the control, despite no significant effect ($p<0.05$) driven by CS or ADE individually (Table 2). This depletion occurred consistently across the bulk soil and vases with the four evaluated species. Bulk soil CS+ ADE had a decrease in *Actinophytocola* and *Galbitalea*. *Schizolobium amazonicum* CS+ADE had a depletion of *1959-1*, *Anaerolinea*, *Aquisphaera*, *Ellin517*, *Puia*, *RB41*, *UTCFX1*, *Vogesella*. *Cecropia pachystachya* CS+ADE had a reduction in *Acrocarpospora*, *Candidatus Megaira*, *Herbaspirillum*, and *Rubrobacter*. *Acacia mangium* CS+ADE had decreasing *Candidatus Ovatusbacter*, *Flavobacterium*, *Herbaspirillum*, and *Rubrobacter*. Finally, *Handroanthus avellanedae* CS+ADE had a decrease in *Brevundimonas*, *Dokdonella*, *Lysobacter*, *Marmoricola*, *Paenarthrobacter*, *Pseudarthrobacter*, *Pseudoxanthomonas*, *Sphingopyxis*, *Sphingorhabdus*, and *Steroidobacter*.

Genera such as *Anaerolinea*, *Herbaspirillum*, *Rubrobacter*, and *Sphingorhabdus* showed strong effect sizes (up to 3.37). Interestingly, some genera were repeatedly affected across different plant species, including *Herbaspirillum* and *Rubrobacter* (detected in *Cecropia* and *Acacia*), as well as *Sphingorhabdus* and *Pseudarthrobacter* (both present in *Handroanthus* and bulk soil), suggesting that the combined CS + ADE treatment imposed a consistent environmental filter on microbial communities (Table 2). Taken together, these results suggest that microbial shifts were driven primarily by the microbial inputs and physicochemical changes introduced by the treatments, with minimal modulation by the plant host.

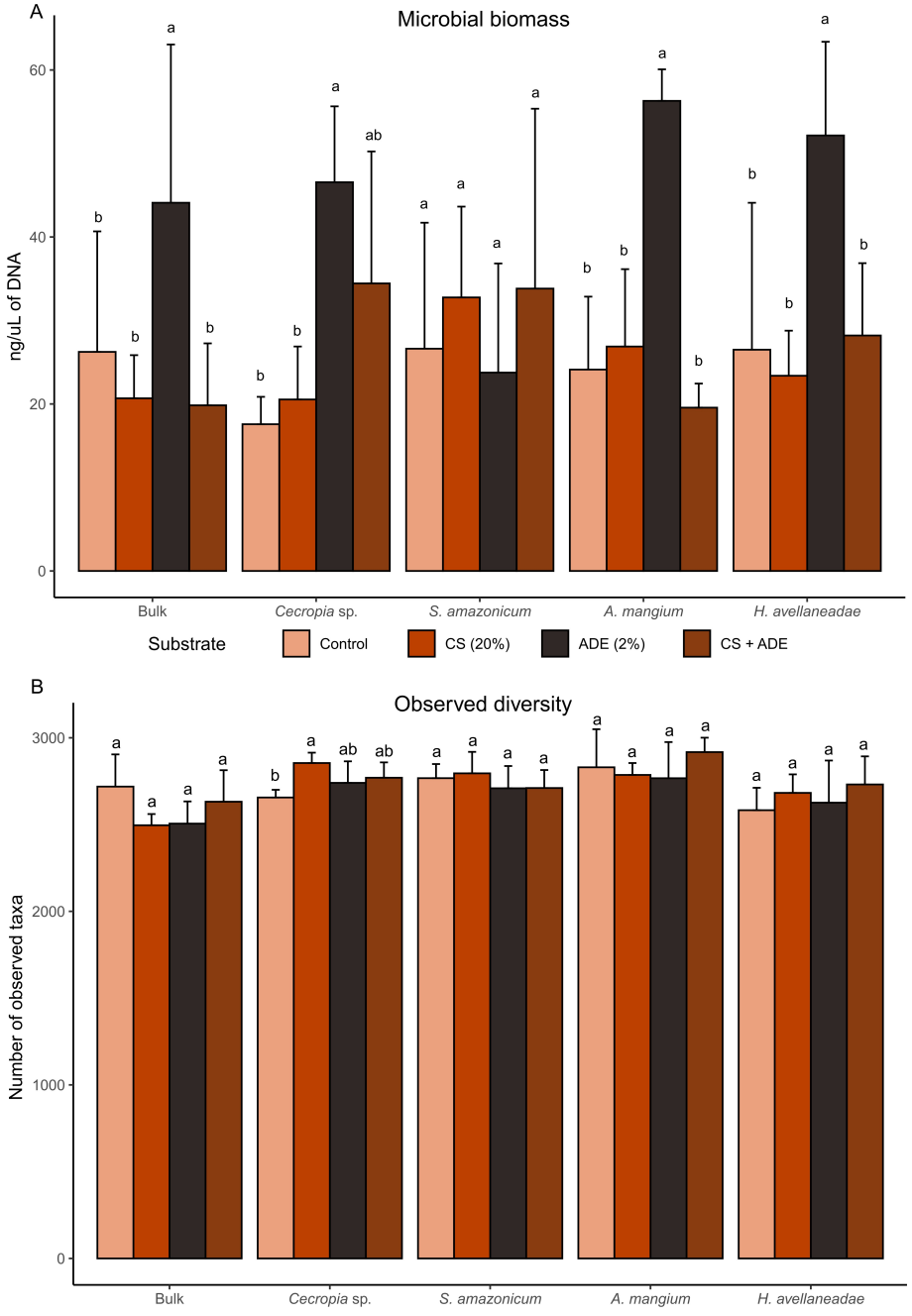


Fig. 2 Microbial aspects of treatments. (A) The estimated microbial biomass calculated based on DNA concentration, where the microbial community is expected to be dominant. (B) Observed diversity measured by the number of different taxa (the different deepest taxonomic levels) found in the samples. Differences were calculated by the Kruskal-Wallis test and Dunn's test post hoc. Data are presented as mean ± standard error. Equal letters mean no difference among treatments ($p > 0.05$), whereas different letters mean differences among treatments (FDR-adjusted p-value < 0.05).

3.4 Microbial networks and functions respond more strongly to soil treatments than to tree identity, revealing treatment-specific patterns of cooperation and competition

The predicted functional profiles of microbial communities also showed shifts associated with soil treatments, with minimal differentiation observed across tree species. Functions related to chemoheterotrophy were the most abundant across all samples, slightly enriched in the bulk soil and across treatments involving ADE and CS (Fig. 4). Control soils exhibited higher relative abundance in photoautotrophy and photoheterotrophy categories in Control *Handroanthus*, whereas the same functions were lower in Control *Acacia*.

Complementary to functional analysis, the activities of β-glucosidase, arylsulfatase, and acid phosphatase did not differ significantly across treatments or tree species,

Table 1 Comparison of growth metrics in tree species subjected to control and feedback treatments.

Tree	Treatment	Plant height (cm)	Root size (cm)	Aerial mass (g)	Canopy area (cm^2)	Stem circunference (cm)
Cecropia pachystachya	Control	36.5 ± 3.70a	14.80 ± 4.95a	7.25 ± 1.26a	691 ± 307.4a	7.85 ± 0.83a
	CS (20%)	29.4 ± 7.89a	15.72 ± 7.60a	4.40 ± 2.41a	541 ± 296.1a	6.30 ± 0.58a
	ADE (2%)	31.2 ± 8.53a	19.52 ± 5.52a	5.98 ± 3.12a	781 ± 321.2a	6.08 ± 1.14a
	CS + ADE	**15.2 ± 11.37b**	10.00 ± 9.19a	**1.77 ± 1.48b**	368 ± 383.8a	**3.50 ± 3.08b**
Schizolobium amazonicum	Control	49.8 ± 10.26b	19.00 ± 6.14a	5.60 ± 2.70a	1012 ± 485.2a	7.56 ± 2.66a
	CS (20%)	**61.2 ± 9.98a**	26.42 ± 12.12a	8.00 ± 1.87a	1688 ± 615.0a	7.92 ± 0.39a
	ADE (2%)	58.8 ± 8.17b	16.90 ± 8.41a	7.20 ± 2.28a	1030 ± 287.9a	7.26 ± 1.40a
	CS + ADE	55.0 ± 3.94b	16.18 ± 6.07a	6.20 ± 1.92a	1267 ± 431.1a	6.78 ± 0.86a
Acacia mangium	Control	21.8 ± 4.44a	17.10 ± 3.65a	2.31 ± 1.64a	545 ± 308.1a	3.50 ± 1.08a
	CS (20%)	25.6 ± 5.32a	15.62 ± 7.28a	2.38 ± 0.93a	602 ± 224.7a	3.10 ± 0.65a
	ADE (2%)	18. ± 6.28a	12.90 ± 6.31a	1.00 ± 0.96a	163 ± 125.9a	2.50 ± 1.17a
	CS + ADE	14.8 ± 8.70a	**6.80 ± 4.87b**	0.73 ± 0.42a	196 ± 134.7a	2.18 ± 1.38a
Handroanthus avellanedae	Control	2.6 ± 7.23a	15.04 ± 1.12a	1.91 ± 1.13a	608 ± 431.6a	3.70 ± 0.86a
	CS (20%)	15.4 ± 9.96a	14.98 ± 8.88a	2.17 ± 2.78a	474 ± 686.9a	2.78 ± 1.51a
	ADE (2%)	19.4 ± 8.32a	11.20 ± 6.76a	2.31 ± 2.15a	642 ± 595.5a	4.16 ± 1.46a
	CS + ADE	15.4 ± 6.31a	16.40 ± 8.48a	1.54 ± 1.16a	522 ± 473.0a	3.46 ± 2.32a

Data are presented in mean ± standard error. Equal letters mean no difference among treatments ($p > 0.05$), whereas different letters mean differences among treatments (FDR-adjusted p-value < 0.05). Significantly different groups are highlighted in bold. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's post hoc test.

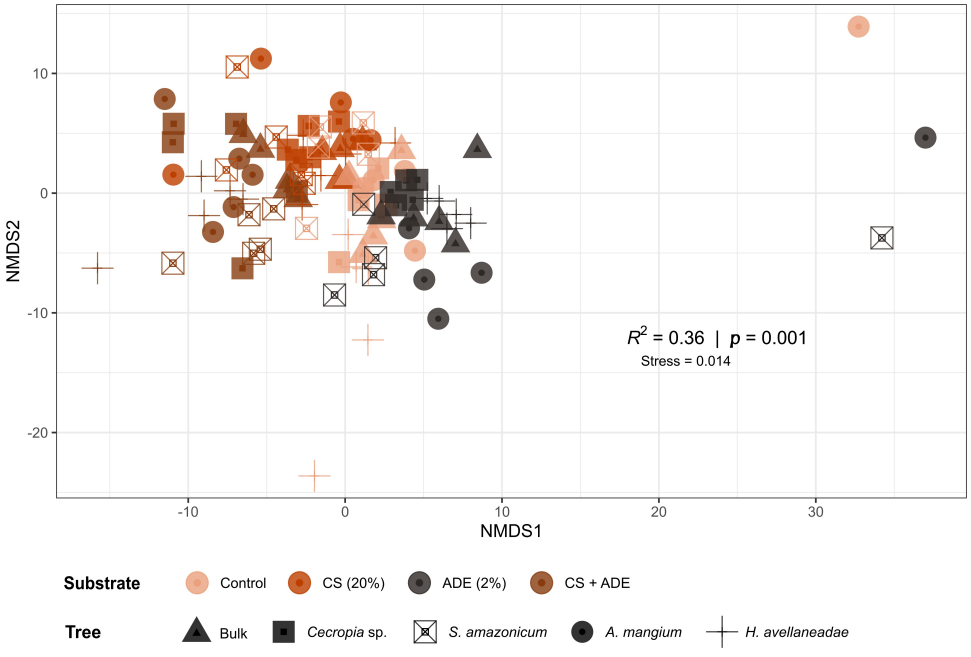


Fig. 3 Bacterial dissimilarity of soil cultivated with four tree species in three different treatments and a Control. Data is based on ASV-level absolute reads transformed by centered log ratios. Each point represents a distinct sample. Different shapes mean different tree species, and different colors mean treatments. Beta diversity was calculated using Euclidean distance and plotted in a non-metric multidimensional scaling (NMDS). R-squared and p-value were calculated by PERMANOVA with 999 permutations.

supporting the observation of a relatively stable functional core. The only exception was observed in *Acacia mangium* under the CS+ADE treatment, which showed a significant decrease in β-glucosidase activity (Table 3), potentially indicating a disruption in microbial cellulose degradation or carbon processing specific to this plant-treatment interaction.

The structure of microbial co-occurrence networks

Table 2 List of microbial taxa with significant (effect >1; adjusted *p*-value <0.05) differences between Treatments and the Control group after 120 days of experiment.

Tree	Genera	Condition	Effect size*	Overlap**	*p*-value***
Bulk	*Actinophytocola*	Decreased in CS+ADE	1.88	0.00	0.05
	Galbitalea	Decreased in CS+ADE	2.26	0.00	0.01
Schizolobium amazonicum	*1959-1*	Decreased in CS+ADE	2.43	0.00	0.00
	Anaerolinea	Decreased in CS+ADE	3.37	0.00	0.00
	Aquisphaera	Decreased in CS+ADE	1.33	0.01	0.05
	Ellin517	Decreased in CS+ADE	1.73	0.01	0.04
	Puia	Decreased in CS+ADE	2.08	0.00	0.05
	RB41	Decreased in CS+ADE	2.39	0.00	0.00
	UTCFX1	Decreased in CS+ADE	1.46	0.01	0.05
	Vogesella	Decreased in CS+ADE	2.26	0.00	0.00
Cecropia pachystachya	*Acrocarpospora*	Decreased in CS+ADE	2.64	0.00	0.01
	Candidatus Megaira	Decreased in CS+ADE	2.47	0.00	0.01
	Herbaspirillum	Decreased in CS+ADE	2.58	0.00	0.02
	Rubrobacter	Decreased in CS+ADE	2.81	0.00	0.00
Acacia mangium	*Candidatus Ovatusbacter*	Decreased in CS+ADE	1.68	0.00	0.00
	Flavobacterium	Decreased in CS+ADE	2.08	0.00	0.00
	Herbaspirillum	Decreased in CS+ADE	1.59	0.00	0.00
	Rubrobacter	Decreased in CS+ADE	1.95	0.01	0.03
Handroanthus avellanedae	*Brevundimonas*	Decreased in CS+ADE	1.78	0.00	0.00
	Dokdonella	Decreased in CS+ADE	2.05	0.01	0.04
	Lysobacter	Decreased in CS+ADE	1.89	0.00	0.00
	Marmoricola	Decreased in CS+ADE	1.39	0.00	0.00
	Paenarthrobacter	Decreased in CS+ADE	1.87	0.00	0.00
	Pseudarthrobacter	Decreased in CS+ADE	2.34	0.00	0.00
	Pseudoxanthomonas	Decreased in CS+ADE	2.11	0.00	0.00
	Sphingopyxis	Decreased in CS+ADE	2.21	0.00	0.00
	Sphingorhabdus	Decreased in CS+ADE	2.49	0.00	0.00
	Steroidobacter	Decreased in CS+ADE	1.79	0.01	0.05

*a median of the difference between Treatment and Control on a log base 2 scale/largest median variation within groups, positive values indicate a higher abundance in the Control group, whereas negative values indicate higher abundance treatment group. **confusion in assigning an observation as Control or treatment, lower is better. ***the expected value of the Wilcoxon test *p*-value, corrected by Benjamini-Hochberg's method. No significant differences in abundance between both CS (20%) and ADE (2%) and Control were found in any plant, nor in the bulk soil.

responded distinctly to treatments across plant species and in bulk soil (Table 4). In the bulk soil, the ADE treatment notably increased the number of positive correlations and raised the average degree, indicating a more interconnected microbial community. For *Cecropia pachystachya*, there was a consistent and successive increase in both nodes and edges following the gradient Control < CS < ADE < CS+ADE. The CS+ADE network also showed a small number of negative correlations, while the CS treatment had the largest network diameter, suggesting a more dispersed community. In *Schizolobium amazonicum*, the ADE treatment decreased the total number of edges and drastically reduced the number of negative correlations, alongside a decrease in average degree and an increase in diameter, features that indicate a more fragmented microbial network. In *Acacia mangium*, ADE reduced the number of negative correlations and increased the average degree, reflecting a more cooperative microbial structure. However, CS+ADE in this species potentially led to an increase in negative correlations, possibly indicating competitive interactions. Finally, in *Handroanthus avellanedae*, the only noticeable change was an increase in average degree under the CS+ADE treatment, suggesting a modest rise in microbial connectivity without major shifts in other topological metrics.

Fig. 4 Heatmap illustrating the predicted functional profiles of microbial communities across various soil treatments and tree species. Each row represents a specific microbial function, while each column corresponds to a treatment group. The color intensity of the spots varies from light grey to dark red, with red indicating a higher relative abundance of the associated function, and light grey representing a lower abundance.

4 Discussion

Active restoration is the chosen method for 75% of the total projects for recovering degraded areas in Brazil. Given this, it's crucial to understand how natural environments (with their nutrients, plants, and microorganisms) can support this process (Brancalion et al., 2016; Alves-Pinto et al., 2017). Here, we showed that the application of Amazonian Dark Earth (ADE) and conventional soil (CS), alone or in combination, had limited effects on plant performance and overall microbial diversity and composition in early stages of restoration. Contrary to expectations, plant growth metrics showed no significant improvement across treatments, indicating that the introduced soil amendments did not translate into short-term plant productivity gains, at least within the timeframe of this study. Similarly, microbial alpha diversity remained largely stable across soils and plant species, and community composition showed minimal shifts, suggesting that the soil microbiome in these systems is either resilient to amendment inputs or that the effects require more time to manifest. This functional and compositional stability implies that core microbiome functions may persist across treatments, possibly buffering the soil ecosystem from abrupt changes, probably with the initial soil harboring a stable microbiota that only changes if a strong disturbance occurs in the system (Kulmatiski and Kardol, 2008; van der Putten et al., 2016).

Interestingly, although our broader analysis showed limited effects of soil amendments on plant performance and microbial diversity, the CS + ADE treatment (the one with more mass input) stood out as the most disruptive, particularly for *C. pachystachya* and *A. mangium*. Contrary to previous results reported by our group (de Freitas et al., 2025), the combination of *U. brizantha* conditioning with ADE amendment reduced shoot growth in *Cecropia* and root development in *Acacia*. These negative effects may be linked to shifts in the rhizosphere microbial community, particularly a marked depletion of several aerobic Gram-positive bacteria, including *Actinophytocola*, *Acrocarpospora*,

Table 3 Soil enzymatic activity levels observed across soil treatments and tree species growth in a degraded pasture oxisol (*n*=5, per group).

Tree Species	Substrate	Beta glucosidase	Acid phosphatase	Arylsulfatase
Bulk Soil	Control	139.7 ± 19.9a	467.1 ± 65.8a	12.8 ± 4.6a
	CS (20%)	126.1 ± 30.6a	518.0 ± 42.4a	17.9 ± 5.1a
	ADE (2%)	148.8 ± 14.7a	624.4 ± 56.5a	15.7 ± 4.0a
	ADE + CS	196.5 ± 36.2a	633.9 ± 24.2a	8.5 ± 4.9a
Cecropia pachystachya	Control	283.0 ± 52.8a	573.3 ± 60.0a	16.5 ± 8.4a
	CS (20%)	165.9 ± 19.1a	587.1 ± 56.6a	5.0 ± 3.5a
	ADE (2%)	187.4 ± 17.5a	626.2 ± 92.5a	6.0 ± 2.6a
	ADE + CS	225.0 ± 80.7a	690.5 ± 35.0a	24.7 ± 7.0a
Schizolobium amazonicum	Control	185.5 ± 15.1a	510.7 ± 56.6a	16.4 ± 3.9a
	CS (20%)	350.0 ± 57.4a	714.0 ± 72.6a	9.5 ± 4.7a
	ADE (2%)	222.8 ± 39.8a	500.7 ± 31.3a	10.8 ± 2.7a
	ADE + CS	118.6 ± 57.3a	780.7 ± 84.6a	18.6 ± 4.2a
Acacia mangium	Control	229.5 ± 23.7a	653.1 ± 65.6a	13.4 ± 6.6a
	CS (20%)	222.3 ± 22.9a	754.5 ± 74.3a	4.5 ± 1.5a
	ADE (2%)	156.0 ± 13.9ab	608.9 ± 46.4a	14.7 ± 5.1a
	ADE + CS	**69.0 ± 3.4b**	642.3 ± 33.4a	5.9 ± 2.4a
Handroanthus avellaneadae	Control	244.9 ± 40.9a	531.3 ± 32.7a	11.6 ± 4.5a
	CS (20%)	193.6 ± 37.7a	705.9 ± 50.1a	9.1 ± 2.5a
	ADE (2%)	181.3 ± 2.6a	634.2 ± 43.7a	6.9 ± 3.3a
	ADE + CS	271.8 ± 39.3a	714.5 ± 29.8a	10.0 ± 3.8a

Data are presented as mean ± standard error. Equal letters mean no difference among treatments ($p > 0.05$), whereas different letters mean differences among treatments (FDR-adjusted $p < 0.05$). Significantly different groups are highlighted in bold. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's post hoc test.

Galbitalea, *Lysinibacillus*, *Marmoricola*, *Paenarthrobacter*, and *Rubrobacter* (Table 2). Mechanistically, this suppression likely stems from the disruption of critical plant-microbe symbioses and altered nutrient cycling. The depleted taxa are functionally essential: *Herbaspirillum* is a known nitrogen fixer, while *Actinobacteria* like *Acrocarpospora* and *Rubrobacter* serve as primary degraders of complex carbohydrates (Brenner et al., 2005; Vos et al., 2011). Their reduction suggests that the CS+ADE environment may have induced a nutrient lock, where the recalcitrant carbon typical of ADE could not be efficiently mineralized, depriving the plants of available nutrients. Although this interpretation is supported by the literature, it cannot be conclusively confirmed in the absence of chemical data collected at the end of the experiment. Furthermore, *Urochloa* species are known to release allelopathic compounds (e.g., saponins) and biological nitrification inhibitors (BNI). While ADE is typically a microbial booster, its specific adsorption properties might have concentrated these inhibitory root exudates rather than buffering them, thereby suppressing the recruitment of beneficial symbionts required by *Acacia* and *Cecropia* (Macedo et al., 2019).

The most dynamic responses were found in microbial co-occurrence network structures, where treatment effects were more pronounced. In particular, ADE increased microbial connectivity and positive interactions in bulk soil and some rhizospheres, reflecting a more cooperative microbial structure, even in the absence of major taxonomic or functional turnover. ADE effect was previously mentioned in several papers as an enhancer of microbial development in different conditions and concentrations (Lima et al., 2015; Lucheta et al., 2017; de Freitas *et al.*, 2023, 2025; Pellegrinetti et al., 2023). Negative interactions decreased under ADE in legume species (*Schizolobium*, *Acacia*), indicating ADE can reduce competition among microorganisms. As we could not find any differential abundance related to pathogenic bacteria, we believe these reductions in negative correlations can improve the environment for the establishment of species in the long term.

Regarding the use of ADE, it is important to note that the applied quantity was relatively low (2% ADE, equivalent to 60 g per pot), substantially below nutrient input levels typically used in ecological restoration practices (Silva et al., 2015). This subfield-relevant dosage (⩾10%–20%) may have constrained both nutrient availability and the establishment of ADE-associated microbial communities, thereby limiting

Table 4 Correlations and topological properties of soil bacterial networks.

Tree	Treatment	Nodes	Edges (+/−)	Average degree	Average path length	Network diameter	Clustering coefficient	Density	Heterogeneity	Centralization	Modularity
Bulk	Control	110	244 (19% + ; 81%−)	4.44	3.02	7.00	0.03	0.04	0.48	0.05	0.42
	CS (20%)	103	295 (30% + ; 70%−)	5.73	2.53	5.00	0.07	0.06	0.48	0.06	0.38
	ADE (2%)	100	421 (43% + ; 57%−)	8.42	2.09	4.00	0.09	0.09	0.42	0.10	0.29
	CS + ADE	107	214 (30% + ; 70%−)	4.00	3.16	8.00	0.03	0.04	0.55	0.06	0.46
Cecropia pachystachya	Control	35	22 (27% + ; 73%−)	1.26	1.29	3.00	0.00	0.04	0.35	0.02	0.91
	CS (20%)	99	149 (28% + ; 72%−)	3.01	3.75	9.00	0.03	0.03	0.54	0.07	0.56
	ADE (2%)	106	210 (35% + ; 65% −)	3.96	2.99	7.00	0.03	0.04	0.53	0.07	0.47
	CS + ADE	120	672 (43% + ; 57% −)	11.20	1.95	4.00	0.11	0.09	0.36	0.09	0.26
Schizolobium amazonicum	Control	110	225 (43% + ; 57% −)	4.09	2.97	6.00	0.05	0.04	0.51	0.05	0.48
	CS (20%)	121	287 (35% + ; 65% −)	4.74	2.79	6.00	0.04	0.04	0.50	0.06	0.44
	ADE (2%)	109	155 (86% + ; 14% −)	2.84	4.16	9.00	0.01	0.03	0.51	0.04	0.60
	CS + ADE	126	315 (35% + ; 65% −)	5.00	2.79	6.00	0.04	0.04	0.49	0.06	0.41
Acacia mangium	Control	118	218 (43% + ; 57% −)	3.69	3.36	8.00	0.05	0.03	0.57	0.05	0.53
	CS (20%)	113	275 (57% + ; 43% −)	4.87	2.68	7.00	0.05	0.04	0.63	0.12	0.42
	ADE (2%)	119	366 (85% + ; 15% −)	6.15	2.46	5.00	0.07	0.05	0.51	0.09	0.35
	CS + ADE	105	129 (28% + ; 72% −)	2.46	4.41	12.00	0.03	0.02	0.60	0.04	0.69
Handroanthus avellanedae	Control	120	193 (35% + ; 65% −)	3.22	3.74	9.00	0.02	0.03	0.55	0.05	0.57
	CS (20%)	109	207 (38% + ; 62% −)	3.80	3.19	7.00	0.03	0.04	0.51	0.06	0.50
	ADE (2%)	95	130 (32% + ; 68% −)	2.74	3.86	9.00	0.03	0.03	0.61	0.07	0.60
	CS + ADE	118	310 (30% + ; 70% −)	5.25	2.65	5.00	0.05	0.04	0.55	0.07	0.40

Nodes: microbial taxon (at genus level) with at least one significant ($p < 0.001$) and strong correlation (> 0.7 or < − 0.7); Edges: number of connections/correlations obtained by the SpiecEasi algorithm; Average degree: the average number of connections per node in the network, the node connectivity; Average path length: average network distance between all pairs of nodes or the average length of all edges in the network; Network diameter: the longest distance between nodes in the network, measured in the number of edges; Clustering coefficient: how nodes are embedded in their neighborhood and the degree to which they tend to cluster together; Density: the degree of interconnectedness or the number of connections within the network; Heterogeneity: the similarity or sameness of attributes among connected taxa; Centralization: the influence concentration using degree, closeness, and betweenness centrality metrics.

the plant growth responses observed in this study. Accordingly, the absence of a clear plant growth improvement with ADE alone is likely attributable to subthreshold application rather than inherent ineffectiveness. Previous studies have demonstrated that higher ADE proportions (e.g., ~20%) can more than double tree growth, particularly when ADE is integrated with native soil at the onset of the experiment (de Freitas et al., 2023). In contrast, ADE was applied here primarily as a microbial inoculant rather than as a bulk soil amendment or fertilizer. This reduced dosage, together with the presence of an established resident soil microbiota, likely limited microbial colonization and functional expression, thereby attenuating potential effects on plant performance (Souza et al., 2025). Importantly, ADEs are protected archaeological soils, which motivated a conservative application strategy focused on microbial transfer rather than soil replacement. Notably, the strongest plant growth responses were observed when ADE was combined with the complementary treatment, further suggesting that higher inoculum levels enhance functional outcomes without necessarily requiring the use of larger quantities of bulk ADE material.

Overall, these findings suggest that while soil amendments may not rapidly change plant performance or microbial community structure, they can subtly reshape microbial interaction patterns and functional potentials. We cannot affirm these shifts will be persistent, but such changes could lay the groundwork for longer-term ecological shifts, while short-term restoration outcomes may not be immediately evident in plant or microbial diversity metrics.

5 Conclusions

Our results demonstrated that the addition of *Urochloa brizantha* and Amazonian Dark Earth (ADE) to degraded Oxisols significantly altered microbial network structures and functional potential, particularly under the combined CS+ADE treatment, which acted as a microbial suppressor. However, these microbial and functional shifts did not translate into consistent improvements in tree growth or root development across the studied species, at least in the short term. In some cases, such as *Cecropia pachystachya* and *Acacia mangium*, the CS+ADE treatment was associated with growth suppression. These findings suggest that while ADE and *Urochloa*-based soil conditioning can improve microbial communities, such modifications alone are insufficient to restore plant productivity in degraded Amazonian Oxisols.

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Conflicts of interest

The authors declare no conflict of interest.

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