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Harnessing the synergy of *Urochloa brizantha* and Amazonian Dark Earth microbiomes for enhanced pasture recovery

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Abstract

Amazonian Dark Earths (ADEs) are fertile soils from the Amazon rainforest that harbor microorganisms with biotechnological potential. This study aimed to investigate the individual and potential synergistic effects of a 2% portion of ADEs and *Urochloa brizantha* cv. Marandu roots (Brazil's most common grass species used for pastures) on soil prokaryotic communities and overall soil attributes in degraded soil. We conducted a comprehensive plant succession experiment in the greenhouse, utilizing vase soil samples for next-generation sequencing of 16 S rDNA, enzymatic activity assays, and soil chemical properties analysis. Univariate and multivariate analyses were performed to understand better the prokaryotic interactions within soil environments influenced by ADEs and *U. brizantha* roots, including differential abundance, diversity, and network analyses. Our findings reveal a complementary relationship between *U. brizantha* and ADEs, each contributing to distinct positive aspects of soil bacterial communities and quality. The combined influence of *U. brizantha* roots and ADEs exhibited synergies that enhanced prokaryotic diversity and enzyme activity. This balance supported plant growth and increased the general availability of beneficial bacteria in the soil, such as *Chujaibacter* and *Curtobacterium* while reducing the presence of potentially pathogenic taxa. This research provided valuable insights into the intricate dynamics of plant-soil feedback, emphasizing the potential for complementary interactions between specific plant species and unique soil environments like ADEs. The findings highlight the potential for pasture ecological rehabilitation and underscore the benefits of integrating plant and soil management strategies to optimize soil characteristics.

Highlights

- *Urochloa brizantha* showed improved growth in degraded soils supplemented with 2% Amazonian Dark Earth (ADE).
- Combined ADE and conditioned soil (CS) treatments elevated arylsulfatase activity, potentially improving sulfur bioavailability.
- ADE increased microbial diversity, particularly taxa involved in plant growth promotion, nitrogen fixation, and cellulolysis.

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- ADE notably increased beneficial bacterial genera such as *Streptomyces*, *Paenibacillus*, and *Solirubrobacter*, essential for soil health and plant growth, with or without *U. brizantha*.

Keywords Amplicon data, Ecological restoration, Microbial ecology, Next-generation sequencing, Soil science

Introduction

Urochloa brizantha cv. Marandu, commonly known as signalgrass, is the most used species for pastures in the Brazilian Amazon rainforest due to its resistance to harsh conditions, such as fire and grazing, and high biomass production [1]. Once studies have highlighted its ability to increase soil organic matter content, it could be a dynamic player in soil health and ecological rehabilitation, fostering a microbial haven that improves nutrient cycling and carbon storage [2, 3]. Furthermore, its dense root system promotes soil aggregation, enhancing water infiltration and erosion resistance [4, 5]. Researchers also demonstrated its effectiveness in improving soil fertility and microbial diversity in previously impoverished soils, providing a most suitable environment for future plants in ecological succession [6].

Amazonian Dark Earths (ADE), by turn, are high-fertility soils formed in part by pre-Columbian Amerindian societies in the Amazon basin, thousands of years ago. These soils and their properties have also been connected with ecological restoration in recent studies due to the presence of a pathogen-suppressive and plant growth-promoting microbial community on them [7–9]. In addition, the nutrient content of ADE is commonly associated with a beneficial environment for plant development, such as trees and subsistence agriculture, leading to a huge potential to be sustainably explored [10].

Although both *U. brizantha* and ADE are promising tools for rehabilitation, many challenges arise. *U. brizantha* exhibits rapid growth and adaptation, but it increases the risk of invasiveness and reduces the establishment of novel species [11, 12], whereas ADE, a non-renewable resource, is protected by genetic and archeological heritage, making it impossible to directly use this soil on a large scale for ecological rehabilitation [13]. For these reasons, it's crucial to understand the interplay between *U. brizantha* and ADE, to open their "black box" and comprehend how to build strategies for the management of *U. brizantha*, as to identify the key factors to mimic the beneficial microbiota provided by ADE in ecological rehabilitation to promote the sustainable use of its microbiome.

Here, we designed an experiment with *U. brizantha* growing up in degraded soil from a pasture in the Amazon rainforest and later growing up with this conditioned soil (CS), with a small amount of 2% of ADE, and with the combination of CS and ADE (CS + ADE). Our hypothesis was that *U. brizantha* would establish negative feedback for itself and ADE would increase growth rate, mass production, and abundance of putative plant growth-promoting bacteria. Our aims were: (i) establish the relationship between growth and bacterial diversity in successive cultivation of *U. brizantha*, and (ii) study how the interplay between ADE and *U. brizantha* affects soil bacterial diversity and composition. Understanding these characteristics is fundamental for planning sustainable strategies to restore the soil and promote long-term health in the Central Amazon pasturelands.

Materials and methods

Soil source

We collected 100kg of control soil from a degraded pasture in a farm located in the municipality of Presidente Figueiredo, state of Amazonas, Brazil, in the Central Amazon (2°2'4" S, 60°1'33"' W). According to the USDA classification system, the soil was classified as Oxisol [14], with clay texture. The climate in the region is tropical hot and wet, with an average temperature of 32 °C and annual precipitation between 2,600 and 3,000 mm [15]. Amazonian Dark Earths (approximately 20kg) were collected from the Embrapa Experimental Station, located in Iranduba, Amazonas, Brazil (03°26'00" S, 60°23'00" W). The climate in the region is also tropical humid (Am), with annual precipitation of 2,100 mm and average temperatures between 25.9 and 27 °C. The soil is classified as Hortic Anthrosol, with a medium texture. Both samplings were made in the first 20 cm of soil, representing the arable layer, at five different points of the area, and were mixed.

Part of the soil was sent to the EMBRAPA's Laboratory of Soil Analysis, in Manaus-AM, for analysis of organic matter, pH, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al^{3+}), sulfur (S), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), sand, clay, and silt. These analyses were carried out following the methods and standards suggested by van Raij and colleagues for Brazilian soils [16]. The other part was kept under refrigeration (4 °C) for two days and then sent to Piracicaba, São Paulo, Brazil, where the experiment was conducted. This study was registered in the Brazilian National System for Management of Genetic Patrimonial and Associated Traditional Knowledge (SISGEN) under the access number AD13FB3.

Experimental design

The greenhouse experiment was conducted in two phases. In phase I (conditioning phase), twenty 3 L pots were filled with control soil (from degraded pasture

collected in the Amazon) and seeds of *Urochloa brizantha* cv. Marandu were sowed in 15 of them. The remaining five pots were kept without plants, as a Negative Control during the experiment. After seed germination, the number of plants in each pot was normalized to all pots having the same number of ramets. These plants were kept in the greenhouse for 60 days and were watered until reaching the field capacity once every 48 h. After that, the aerial part of the plants was then removed and the soil with the remaining roots was used as an inoculum for treatments in phase II.

In phase II, we tested the growth of new seeds of *U. brizantha* in four treatments: 100% of control soil (Control), 80% of Control Soil + 20% of Conditioned Soil with *U. brizantha* (CS), 98% of Control Soil + 2% of fresh Amazonian Dark Earths (ADE), and 78% of Control Soil + 20% of Conditioned Soil + 2% of fresh ADE (CS + ADE). The interest in these treatments lies in evaluating the feedback effects of pasture roots microbiota, ADE microbiota, and the combined effects of these two factors on plant growth. All treatments were also applied to pots without plants as a negative control. The experiment included five replicates per treatment in phase II, with 40 experimental pots, and was kept in the greenhouse for 120 days at 23.8 °C (± 2.9 °C) and 64% (± 11%) of air moisture, watered with deionized water until reaching the field capacity once every 48 h.

Sampling

At the end of the experiment, we measured plant height for each plant using a measuring tape, considering the distance between the soil and the higher plant leaf. The canopy area was measured by measuring two perpendicular pairs of points in the canopies and computing the multiplication of these two distances. All the aerial parts were collected and dried in an oven at 60 °C for 48 h and then weighed to measure the dry matter. Soil samples were collected from the surrounding area close to the roots for DNA extraction and enzyme activity analyses. Samples for DNA extraction were frozen at -20 °C until the extraction time, and samples for enzymes were kept with a breather and refrigerated at 5 °C until the measurements.

Molecular procedures

Microbial DNA was extracted from 0.25 g of soil using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany) using the manufacturers' instructions and the modifications suggested by Venturini and colleagues [17]. The quality was measured using a Nanodrop™ 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), considering as suitable for downstream analysis all samples with DNA concentrations higher than 10 ng uL^{-1} and A260/A280 ratios between 1.70 and 2.00.

Amplification and sequencing were performed by Novogene Corporation Inc. (Sacramento, CA, USA) using standard approaches as defined by the Earth Microbiome Project [18]. The V3-V4 region of the 16 S rDNA was amplified to determine the abundance of prokaryotes (bacteria and archaea) in samples using the updated primers 515 F [19] and 816R [20]. The paired-end sequencing with 2 × 250 bp reads was performed using the Illumina HiSeq 2500 platform. The raw reads used in this work can be found in the Sequence Read Archive (SRA) under the project number PRJNA1157008.

Besides, enzyme activity analyses were performed for the soil of each pot. We tested the activity of acid phosphatases using the colorimetric method proposed by Tabatabai, at pH 5.5 [21]. β-glucosidase activity was evaluated using ρ-nitrophenyl-β-D-glucopyranoside as a substrate [22]. Finally, the activity of arylsulfatase was analyzed by the hydrolysis of potassium p-nitrophenyl sulfate, incubating the soil sample for 1 h at 37 °C [23]. All products were evaluated by colorimetric determination at 410 nm using an ELISA microplate reader LMR FLEX UV-VIS i (Loccus Biotecnologia, Cotia, SP, Brazil).

Data analyses

All bioinformatics and statistical analyses were performed in the R environment (version 4.3.0) using the RStudio software (version 2023.09.1 + 494) [24]. The code for the analyses performed in this study can be found publicly on GitHub at: https://github.com/FreitasAndy/PSFforAmazonianPastures. Figures were produced using the ggplot2 package [25], and some of these figures were edited only for aesthetic purposes (i.e. changing colors and fonts) using the Inkscape 1.3.2 program. Because the data did not adjust to the normal distribution, we proceeded with a downstream analysis suitable for non-parametric data.

We used Kruskal-Wallis followed by the *post hoc* Dunn test [26, 27] to test differences in plant growth (dry matter, root length, canopy area, and plant height), chemical properties (OM, pH, P, K, Ca, Mg, H, Al^{3+}), and enzymatic activity (acid phosphatase, beta-glucosidase, and arylsulfatase).

The raw reads from sequencing were analyzed using the DADA2 pipeline [28], 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 [29]. The resulting ASV table was imported into both a phyloseq object [30] and a microeco's R6 object [31] for downstream analysis.

Observed richness was calculated by considering the number of different taxa identified in each sample (observed diversity), and dominance of taxa was calculated by the inverse Simpson index, considering a

confidence level of 95% by the Kruskal-Wallis test post hoc the Dunn's test with correction by false discovery rate. Beta diversity was calculated by transforming the dataset into a centered log ratio (clr), to reflect the compositional structure of the data. Data ordination was performed using Euclidean, and nonmetric multidimensional scaling was plotted on the two first axes. Significance was calculated by permutational multivariate analysis of variance (PERMANOVA), at the level of significance of 5% and 999 permutations, using the adonis function from the package vegan [32].

Differential abundance analysis of each treatment per plant against the control was accessed by the ALDEx2 algorithm [33], considering significant differences between those with p-value < 0.01 calculated by Welch's test, and also with effect size bigger than 1.

The functional estimation for each sample was carried out using the FAPROTAX tool, which presumes the function of microorganisms from an ASV table based on previously published studies with those taxa [34]. The results were plotted as a heatmap representing key potential functions in soil health and management. Finally, correlation network analyses were performed at the genus level using the SpiecEasi algorithm [35], considering significant correlations higher than 70% with p-values lower than 0.001, thereby capturing only strong and most trustable correlations.

Results

Initial soil came from a very degraded environment

To perform this experiment, we collected soil from a degraded pasture in the Central Amazon that has been covered with *U. brizantha* for over 20 years. Besides the expected conditions of increased compaction and low grass abundance, this soil presented lower organic matter content and nutrient balance (Table 1), especially phosphorus (P), potassium (K), and sodium (Na), in comparison with ADE. In contrast, ADE samples collected from a secondary forest had lower calcium (Ca) and magnesium (Mg) levels. Although the pH did not differ between the soils, the aluminium levels were higher in degraded oxisol (Table 1).

ADE mitigated the negative legacy of *U. brizantha*

The growth aspects in the degraded soil showed that the worst environment for *U. brizantha* to establish and grow was in the conditioned soil by itself. Plants in conspecific soil (CS) presented lower mass, height, and a smaller stem than the Control. However, the inclusion of 2% ADE drove all these factors to the same level as the control with or without the presence of *U. brizantha*, suggesting that ADE inhibits its conspecific negative effects. No difference was observed in root size, once the roots were spread all over the pots in all treatments.

ADE highly influences the soil microbial structure

The sequencing generated from 131,822 to 145,334 high-quality reads per sample, assigned to 55,286 different taxa. The analysis of these data showed that ADE was this study's key driver for diversity (Figs. 1 and 2). All groups were separated by two-dimensional ordination, but the ADE treatment presented the most varied samples, all of them with a high distance from all other groups but heterogeneous among themselves (Fig. 2-A). The ADE + CS treatment was the most diverse in taxa, with all other treatments presenting diversity similar to the control (Fig. 2-B). However, there was no difference in the dominance indexes, even though it was highly variable in each treatment, especially in the CS and ADE groups (Fig. 3-C).

The phyla distribution in all treatments was also similar, with Proteobacteria, Acidobacteriota, Actinobacteria, Chloroflexi, Verrucomicrobia, and Firmicutes being the most common ones, with small variance either among samples or groups (Fig. 3-D), with supposed differences in deeper taxonomic levels.

ADE induces major microbial differences

In the CS treatment, when *U. brizantha* showed less growth than the control, there was a depletion in several genera, such as *Anaerolinea*, *Brevundinomas*,

Table 1 Description of chemical variables for each collected soil

Variable	Amazonian Dark Earth	Pasture Oxisol	*p*-value
pH ($CaCl_2$)	5.2 ± 0.1	5.1 ± 0.2	ns
Organic Matter (%)	46.5 ± 6.5	26.5 ± 8.3	0.02
P ($mg.dm^{-3}$)	160.3 ± 38.4	7.5 ± 1.7	0.02
K ($mmolc.dm^{-3}$)	45.8 ± 20.1	0.4 ± 0.1	0.02
Na ($mmolc.dm^{-3}$)	6.3 ± 2.1	1.1 ± 0.9	0.02
Ca ($mmolc.dm^{-3}$)	6.8 ± 0.6	29.5 ± 1.7	0.02
Mg ($mmolc.dm^{-3}$)	1.2 ± 0.3	15.3 ± 1.0	0.02
H + Al_3 ($mmolc.dm^{-3}$)	4.3 ± 0.6	32.5 ± 10.8	0.02
Base Saturation (%)	82.0 ± 0.8	45.3 ± 3.0	0.02

p-values were calculated for the Kruskal Wallis chi-squared test, considered as significant values with $p < 0.05$. ns = not significantly different

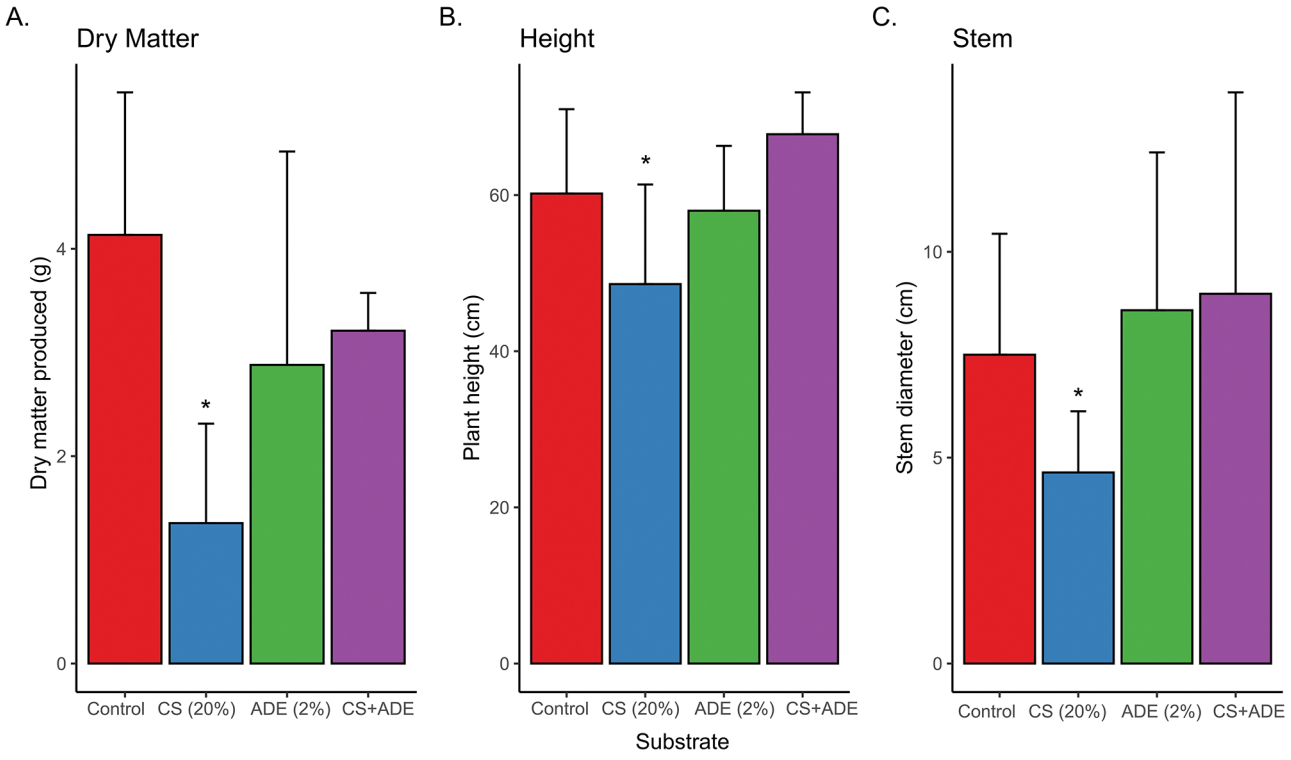

Fig. 1 Growth of *U. brizantha* after 120 days of experiment in four different treatments. (**A**) Production of aerial dry matter. (**B**) Plant height. (**C**) Stem diameter at ground level. *Significantly different from Control calculated by the Kruskal Wallis chi-squared test ($p < 0.05$) post hoc Dunn test

Curtobacterium, *Leptothrix*, *RB41*, and *Sphingopyxis*. On the other hand, *Sphingorhabdus* and *Tychonema CCAP 1459-11B* were increased in CS (Table 2).

The addition of ADE without conditioned soil, which did not alter the plant growth but hardly altered the prokaryotic community, increased the abundance of different genera, such as *Streptomyces*, *Paenibacillus*, *Conella*, and *Lysinibacillus*. Besides, ADE depleted the abundance of *Paenarthobacter* and *Sphingopyxis*.

Finally, the addition of both CS and ADE, although the increase in observed diversity, was the closest treatment to control differential abundance. CS + ADE presented an increase in *Chujaibacter* and a decrease in *Curtobacterium*, *Sphingopyxis* (also decreased in only ADE treatment), and *Tychonema CCAP 1459-11B* (also decreased in only CS treatment).

Putative functions were driven by both CS and ADE

In addition to increasing the abundance of beneficial bacteria, both CS and ADE (alone or together) altered the genetic and functional profile of degraded soil. Treatments with the addition of *U. brizantha* roots (CS and CS + ADE) presented a higher number of phototrophic microorganisms (Fig. 3), as well as other carbon-fixing organisms, such as photosynthetic cyanobacteria. ADE treatment, in turn, increased the potential for nitrogen fixation and cellulolysis. When combined, these treatments presented both characteristics higher than the control, highlighting the cumulative effect of then.

Enzymatic activity is increased by treatments

Regarding enzymes, treatment with just conditioned soil (CS) decreased beta-glucosidase activity with no differences in acid phosphatase and arylsulfatase activities. ADE treatment, on the other hand, increased the activity of beta-glucosidase but did not affect any other enzymes. In the combined effect of both treatments (CS + ADE), the activity of beta-glucosidase was similar to that of the control, suggesting one more time the synergetic effect of each treatment. Surprisingly, the CS + ADE treatment also increased arylsulfatase activity, despite the treatments' null effect apart.

The addition of ADE and CS increased the correlations among microorganisms

Looking at the correlations in soil, we also found increased complexity in soil with the addition of both ADE and CS. CS increased the number of interactions derived from the co-occurrence of taxa (that is, edges) by six-fold compared with the control, whereas ADE alone increased it by 26-fold. However, when placed together, the complexity was less intense than that for each treatment alone, being only 0.46-fold higher than the control. Besides that, the number of vertexes was similar among

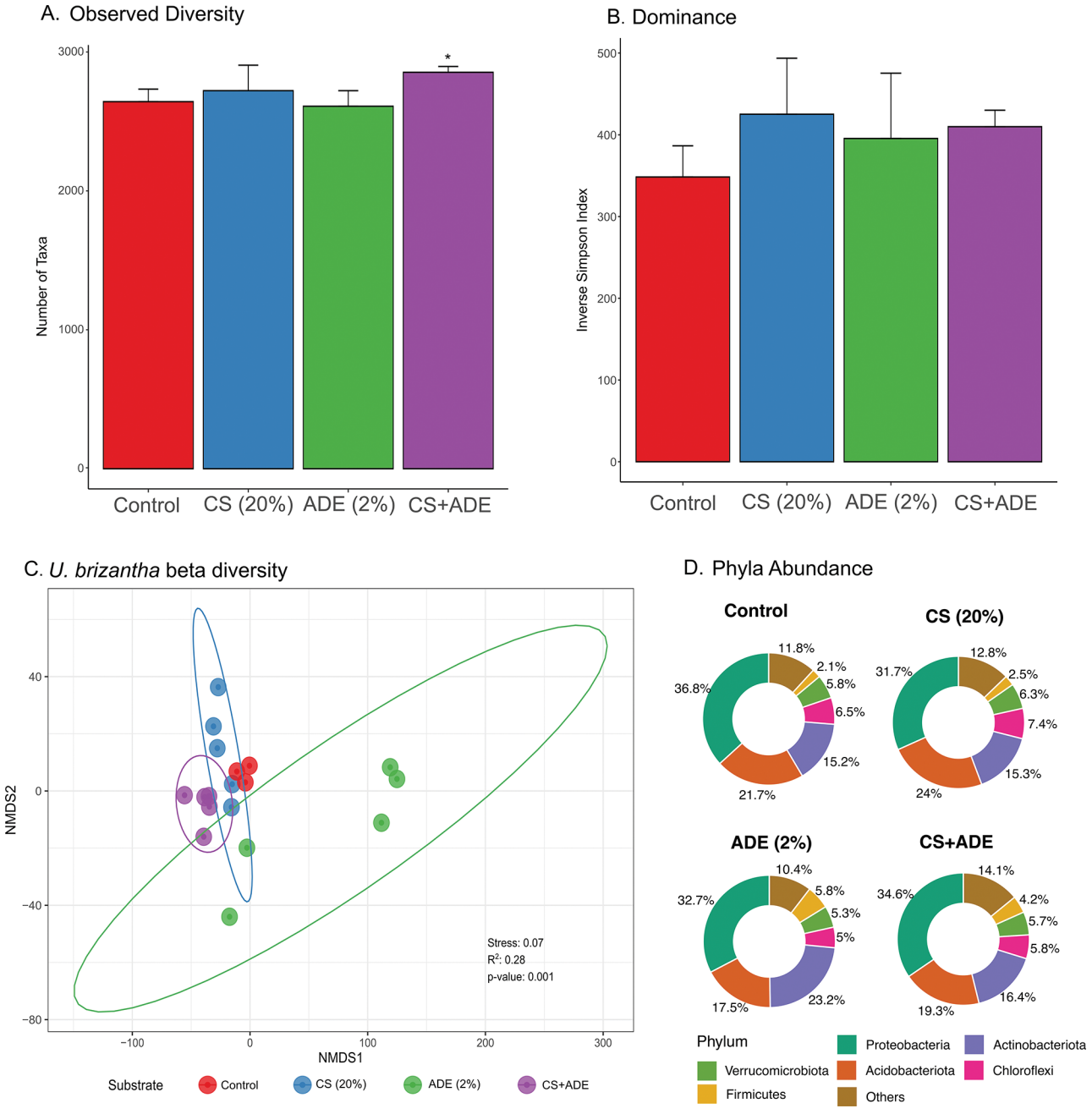

Fig. 2 Microbial structure of soil cultivated with *U. brizantha* in four different treatments. (**A**) Observed diversity calculated by the total number of different taxa found in each treatment. Whiskers represent the standard deviation. *significantly different from control ($p < 0.05$). (**B**) Dominance calculated by the inverse Simpson index. No differences were assigned at 95% of confidence. (**C**) Beta diversity calculated by Euclidean distance and plotted in a non-metric multidimensional scaling. (**D**) Distribution of the top 6 phyla in each treatment and split by sample

all treatments, whereas all other aspects such as average degree, clustering coefficient, density, homogeneity, and centralization were higher in all treatments than in the control. These findings are consistent with the diversity analysis (Fig. 2) showing that taxa did not significantly change among treatments.

Discussion

Microorganisms are crucial for soil health and can drive the functions that improve ecological restoration projects. More than 350,000 km^2 of forest has been deforested in the last 33 years for cattle breeding, and 50% of the Brazilian pastures suffer some level of degradation, with the most important factor being the absence or insufficiency of management in the pastures [36]. Here,

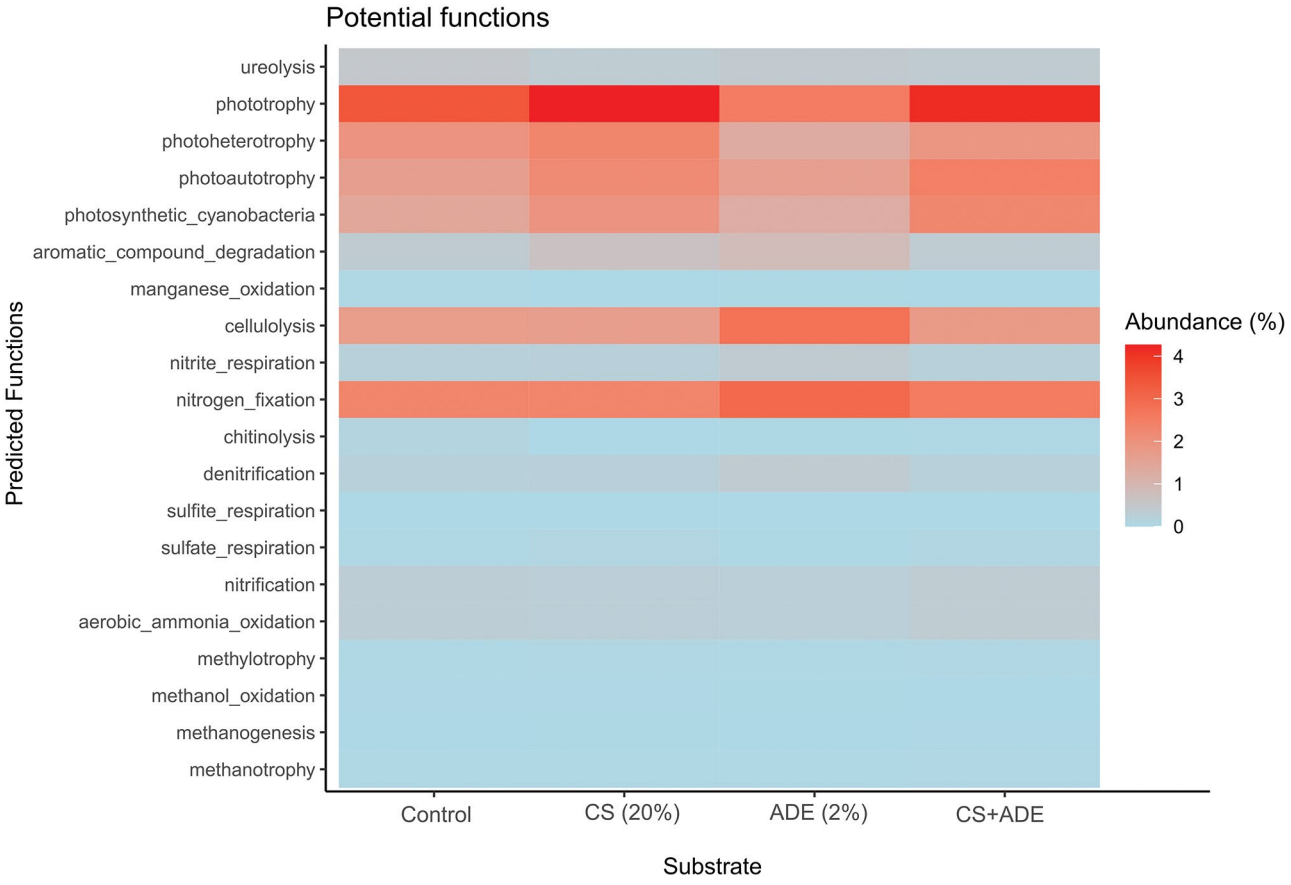

Fig. 3 Heatmap illustrating the predicted functional profiles of microbial communities across different soil treatments. Each row represents a specific microbial function, while each column corresponds to a treatment group. The color intensity of the spots varies from red to blue, with red indicating a higher relative abundance of the associated function, while blue represents a lower abundance. This figure underscores the dynamic shifts in microbial community functions, providing insights into the role of specific treatments in enhancing or suppressing certain microbial activities

we showed lines of evidence that *U. brizantha* could promote the growth of beneficial microorganisms in the soil, which can be potentialized using microorganisms from ADE.

First, we demonstrated that *U. brizantha* promotes a negative legacy in new *U. brizantha* plants (Fig. 1). It is known that conspecific feedback among grasses tends to be negative due to the accumulation of specific pathogens or competition among plants [37], which can favor ecological succession in a well-managed scenario. However, the use of a small portion of ADE (we used only 2% in the experiment) reverted this negative legacy to neutral, which could be interesting in pastures where initial coverage with grass is required at the beginning of the recovery, promoting the later insertion of trees in a more suitable environment if management is properly applied.

A second piece of evidence of the core work from *U. brizantha* and ADE is related to the taxa and functions they brought to the soil. CS treatment increased the abundance of genera known to degrade carbon or act in the nitrogen cycle by reducing ammonia oxidation or nitrite (Fig. 3), improving carbon storage [38, 39]. ADE treatment increased the abundance of several plant growth-promoting genera, such as *Paenibacillus*, *Solirubrobacter*, and *Pedomicrobium* (Table 2), and also produced the most variable community (Fig. 2-C). It increased the abundance of potential nitrogen fixers (Fig. 4), highlighting the importance of ADE microbes in helping the establishment and growth of plants [40–42]. Treatment with ADE also decreased the amount of *Sphingopyxis* and *Paenarthrobacter*, two genera correlated with the degradation of aromatic compounds, such as environmental contaminants [43, 44]. When combined in the treatment CS + ADE, the effect was the increase of genera that degrades complex carbohydrates, such as the ones secreted by *U. brizantha*, into sugars and short-chain organic acids to both plants and other microorganisms, as well as suppressing potential pathogens, showing that the plant can recruit microbes from ADE to improve the root-influenced soil zone [45, 46].

In addition, CS treatment showed decreased activity of beta-glucosidase, the enzyme responsible for breaking down complex carbohydrates such as cellulose, and some variants of hemicelluloses in soils [47]. The activity

Table 2 List of microbial taxa with significant differences between treatments and control group after 120 days of growth of *U. Brizantha*

CS (20%) x Control					
Genera	rab.Control	rab.CS	effect	overlap	we.ep
Anaerolinea	1.32	3.96	3.79	< 0.01	< 0.01
Brevundimonas	2.30	-0.31	-2.03	< 0.01	0.01
Curtobacterium	1.87	-5.23	-3.08	< 0.01	0.01
Leptothrix	2.35	-5.11	-3.01	< 0.01	< 0.01
RB41	4.96	7.21	2.96	< 0.01	0.01
Sphingopyxis	2.31	-5.42	-3.32	< 0.01	< 0.01
Sphingorhabdus	1.44	-5.42	-2.72	< 0.01	0.01
Tychonema CCAP 1459-11B	2.62	-5.18	-3.04	< 0.01	< 0.01
ADE (2%) x Control					
Genera	rab.Control	rab.ADE	effect	overlap	we.ep
Streptomyces	4.24	5.78	4.16	< 0.01	< 0.01
Paenibacillus	2.45	5.44	4.13	< 0.01	< 0.01
Lysinibacillus	2.59	5.04	4.10	< 0.01	< 0.01
Actinoallomurus	2.76	4.23	4.10	< 0.01	< 0.01
Solirubrobacter	2.88	4.61	3.89	< 0.01	< 0.01
Cohnella	0.55	4.05	3.88	< 0.01	< 0.01
Pseudonocardia	2.76	4.40	3.67	< 0.01	< 0.01
Kitasatospora	1.63	3.08	3.53	< 0.01	< 0.01
Luedemannella	4.93	6.77	3.49	< 0.01	< 0.01
Phaselicystis	3.75	5.10	3.36	< 0.01	< 0.01
Actinoplanes	1.57	3.62	3.35	< 0.01	< 0.01
Clostridium sensu stricto 12	2.18	3.60	3.31	< 0.01	< 0.01
Gaiella	5.37	6.77	3.25	< 0.01	0.01
Acidothermus	6.44	8.38	3.16	< 0.01	< 0.01
Conexibacter	5.75	8.28	2.77	< 0.01	0.01
Pedomicrobium	6.27	7.29	2.75	< 0.01	< 0.01
Ruminiclostridium	2.42	4.02	2.66	0.01	< 0.01
Mycobacterium	5.17	7.29	2.58	< 0.01	< 0.01
Micromonospora	2.57	4.31	2.52	< 0.01	0.01
Rugosimonospora	4.01	5.41	2.47	< 0.01	0.01
Clostridium sensu stricto 1	0.10	1.90	2.39	< 0.01	0.01
Dactylosporangium	2.36	4.56	2.34	< 0.01	< 0.01
Plantactinospora	0.03	1.90	2.19	< 0.01	0.01
Actinomadura	1.26	3.02	2.13	< 0.01	0.01
Paenarthrobacter	0.36	-5.52	-2.37	< 0.01	0.01
Sphingopyxis	0.97	-5.61	-2.57	< 0.01	0.01
CS + ADE x Control					
Genera	rab.Control	rab.CS + ADE	effect	overlap	we.ep
Chujaibacter	1.51	3.28	2.91	< 0.01	< 0.01
Curtobacterium	1.81	-5.55	-3.18	< 0.01	< 0.01
Sphingopyxis	2.23	-5.64	-3.24	< 0.01	0.01
Tychonema CCAP 1459-11B	2.55	-5.78	-3.56	< 0.01	< 0.01

rab.: relative abundance, median centered log-ratio value for the group mentioned; effect: effect size of the difference, a median of difference between groups on a log base 2 scale/largest median variation within groups, positive values indicate a higher abundance in the treatment group whereas negative values indicate higher abundance control group; overlap: confusion in assigning an observation Control or treatment; we.ep: the expected value of the Welch test p-value. The table includes all OTUs with effect > 1 and p-value ≤ 0.01

of this enzyme is inversely proportional to the increasing amount of carbon in the soil, which is acquired with the cultivation of *U. brizantha* [48]. ADE treatment increased the activity of this enzyme (Fig. 5), leading to maintenance in the activity of enzymes that increase the carbon fixation and availability to plants for a longer time, which is beneficial to late succession plants and for the environment. Also, the combination of CS + ADE increased the activity of arylsulfatase, the enzyme responsible for transforming organic sulfur into inorganic forms, a process

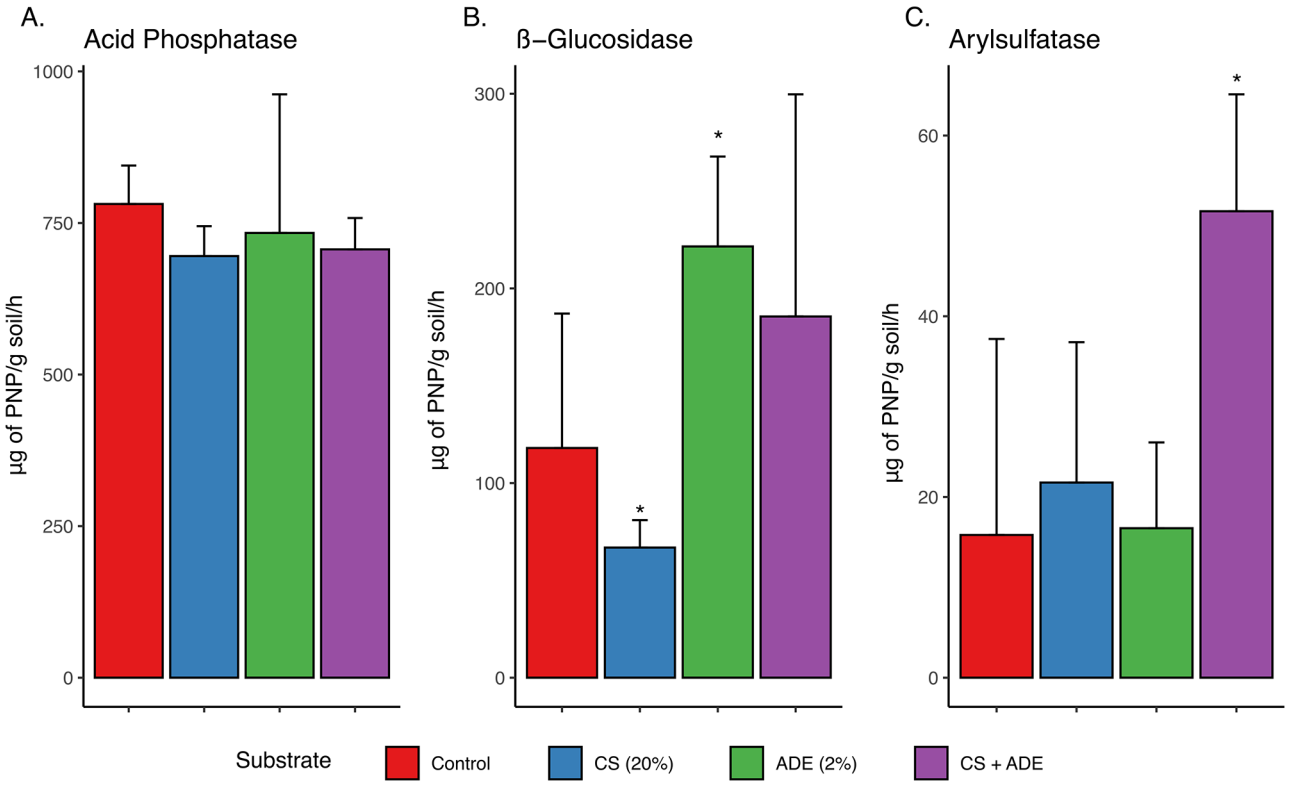

Fig. 4 Bar chart depicting the enzymatic activity levels observed across soil treatments. Each bar represents the mean enzymatic activity for a specific treatment, with error bars indicating the standard deviation. Asterisks (*) denote statistically significant differences between treatments, with p-values < 0.05, as determined by the Kruskal-Wallis chi-squared test followed by Dunn's post hoc test

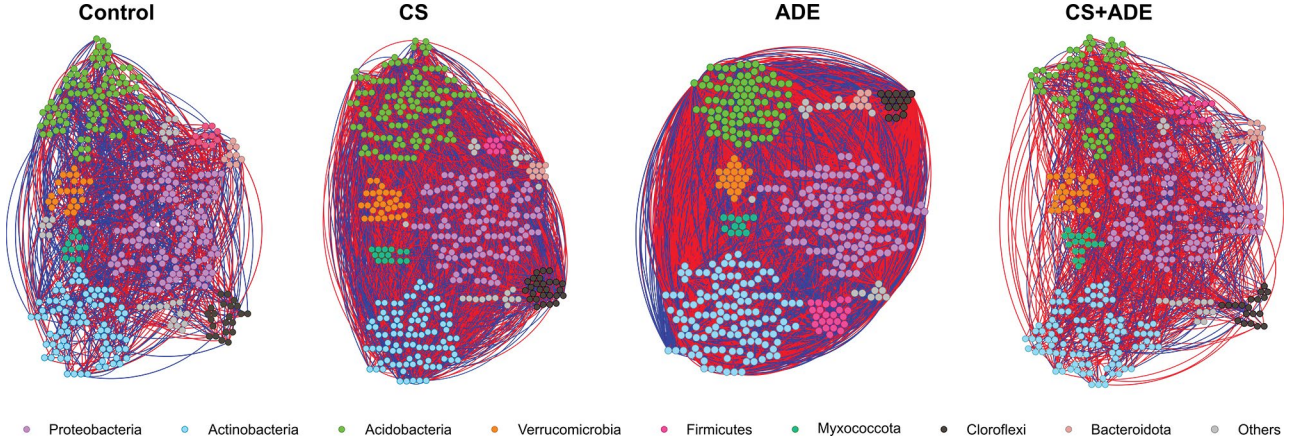

Fig. 5 Co-occurrence network analysis of the active *U. brizantha*-assocociated bacterial community based on the 16 S rRNA gene from the Control (CS), 20% of conditioned soil (CS), 2% of Amazonian Dark Earth (ADE) and the combination of 20% of conditioned soil and 2% of Amazonian Dark Earth (CS + ADE). Significant ($p < 0.01$) positive (magnitude > 0.7) and negative (magnitude, < −0.7) SpiecEasi correlations are respectively denoted by the blue and red edges. Each node represents bacterial taxa at the ASV level, with the color meaning the phylum from that ASV. Detailed topological properties of the networks are provided in Table 3

essential for plant nutrition, as this nutrient is mostly immobilized in soil colloids and is vital for plant growth and yield [49].

Finally, CS and ADE increased the correlations among the prokaryotes, suggesting a more connected community (Fig. 5; Table 3). Fortunately, most of the correlations among prokaryotes were positive in ADE treatment, with the majority of negative relationships relying only on CS + ADE treatment. It suggests control of some taxa for other ones, one more time suggesting a complementary role of *U. brizantha* and ADE in the soil and also explaining the highest diversity found in CS + ADE treatment,

Table 3 Correlations and topological properties of soil microbiome networks

Attribute	Control	CS	ADE	CS + ADE
Nodes[a]	423	446	439	454
Edges[b]	924	6123	26,505	1349
Positive edges[c]	56.5%	42.2%	57.6%	31.0%
Negative edges[d]	43.5%	57.8%	42.4%	69.0%
Average degree[e]	4.37	27.46	120.75	5.94
Average path length[f]	3.86	1,76	1.39	3.02
Network diameter[g]	8.00	3,00	2.00	6.00
Clustering coefficient[h]	0.02	0.08	0.32	0.01
Density[i]	0.01	0.06	0.28	0.01
Heterogeneity[j]	0.76	0.38	0.27	0.52
Centralization[k]	0.09	0.10	0.21	0.04

[a]Microbial taxon (at genus level) with at least one significant ($p < 0.001$) and strong correlation (> 0.7 or < -0.7)

[b]Number of connections/correlations obtained by the SpiecEasi algorithm

[c]Percentage of SpiecEasi-positive correlation (> 0.7 with $P < 0.001$)

[d]Percentage of SpiecEasi-negative correlation (< -0.7 with $P < 0.001$)

[e]The average number of connections per node in the network, the node connectivity

[f]Average network distance between all pairs of nodes or the average length of all edges in the network

[g]The longest distance between nodes in the network, measured in the number of edges

[h]How nodes are embedded in their neighborhood and the degree to which they tend to cluster together

[i]The degree of interconnectedness or the number of connections within the network

[j]The similarity or sameness of attributes among connected taxa

[k]The influence concentration using degree, closeness, and betweenness centrality metrics

once there is probably more competition among them [50].

The combination of this information leads to a combination of good characteristics that can be brought by both *U. brizantha* roots and ADE to the soil. Although grass species are known for increasing carbon content in soils (which is extremely important in degraded environments), the ADE brings plant-growth-promoting bacteria to the environment and increases the interconnection among taxa, leading to higher growth and inhibition of negative legacy from plants. We believe that these data support the choice of *U. brizantha* and ADE for ecological recovery processes as long as management is properly conducted to avoid the overpopulation of grasses and guarantee the survival of other species.

Conclusions

Here we showed that *Urochloa brizantha* and prokaryotes from Amazonian Dark Earths alter the genetic and functional profile of soil. Our results point to a complementary relationship between these two treatments, converging together to an improvement in the soil microbial community and helping to generate a more suitable environment for plant growth in ecological rehabilitation.

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Author contributions
A.S.F. wrote the original draft, performed visualization, methodology, investigation, formal analysis, data curation, and conceptualization. L.F.G.Z., G.S.R., F.M., G.L.M., and S.S.S.Z. contributed to writing – review & editing, data acquisition, and validation. R.E.H. and A.W.M. contributed to conceptualization, provided resources and acquired funding. S.M.T. contributed to writing – review & editing, validation, supervision, resources, project administration, funding acquisition, data curation, and conceptualization. All authors reviewed the manuscript and agree with the submission.

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Data availability
The raw reads used in this work can be found in the Sequence Read Archive (SRA) under the project number PRJNA1157008.The code for the analyses performed in this study can be found publicly on GitHub at: https://github.com/FreitasAndy/PSFforAmazonianPastures.

Declarations

Ethics approval and consent to participate
This study did not involve human participants, human data, or animals, and thus, ethical approval and consent to participate were not required. All experimental protocols and methodologies adhered to relevant institutional and international guidelines for research.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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