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Boosting tree growth in the Amazon rainforest using Amazonian Dark Earths

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Abstract

The restoration of degraded tropical ecosystems, particularly in the Amazon, requires innovative and sustainable solutions. This study investigates the potential of Amazonian Dark Earth (ADE), a highly fertile and resilient soil, as a microbial bio-inoculant to improve the growth of two key tree species, *Schizolobium amazonicum* and *Handroanthus avellanedae*. By applying a small volume of ADE, we observed a significant improvement in the growth of both tree species, characterized by enhanced plant height and stem diameter at breast height. These positive results are linked to ADE's ability to fundamentally restructure the soil's microbial communities. Our findings reveal that ADE acts as a powerful suppressive soil, selectively depleting a wide range of opportunistic and pathogenic bacterial and fungal genera, while simultaneously promoting the establishment of a new, beneficial microbial community. We observed a notable decrease in pathogens, such as the fungus *Lasiodiplodia* and the bacteria *Pseudoxanthomonas*, alongside a significant increase in well-known biocontrol agents and plant-growth promoters, including the fungi *Metarhizium* and *Tomentella* and the bacteria *Rhizobium* and *Enterobacter*. The high nutrient content of the ADE may create a negative feedback loop that reduces the need for certain microbial functions, such as nitrogen fixation, but this targeted microbial "re-wiring" is the key mechanism driving improved plant health. Our work demonstrates that ADEs true value lies in its living microbial community, offering a sustainable and effective strategy for accelerating the restoration of degraded tropical landscapes.

Keywords Biochar, Pioneer species, Mycoparasites, Rhizosphere, Suppressive soil

Introduction

The conversion of tropical forests to agriculture and cattle breeding areas has led to widespread deforestation and the degradation of vast pasturelands. In Brazil, this has resulted in millions of hectares of unproductive, compacted soil with low organic matter [1]. This degradation not only hinders land use but also creates a vicious cycle of environmental loss and inefficiency, as it increases the pressure for new deforestation. Furthermore, the conversion of forest to agricultural land releases massive amounts of stored carbon, contributing significantly to global warming. Degraded soils have a lower capacity for carbon sequestration, exacerbating climate change and

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threatening both food security and ecosystem stability [2].

The accelerated degradation of tropical ecosystems, particularly in the Amazon rainforest, in the last decades has intensified the search for sustainable and efficient ecological restoration strategies [3]. The manipulation of soil communities through soil inoculation has been shown to be a powerful tool for the restoration of degraded terrestrial ecosystems in temperate ecosystems [4]. However, many processes are not well understood in tropical ecosystems, especially in the context of Amazonian soil degradation. The interactions of the soil microbial communities can be a key process to understand how effective the process of soil inoculation is. In this context, Amazonian Dark Earth (ADE) emerges as a promising model of highly fertile soil. Formed from the activity of pre-Columbian peoples thousands of years ago, ADE's unique composition (rich in organic matter and essential nutrients) fosters a high and resilient microbial diversity, setting it apart from the adjacent low-fertility soils [5]. The potential of ADE as a soil inoculum showed a potential for higher biomass and plant growth under greenhouse experiments [6]. However, the potential of ADE as a soil inoculum has never been tested under field conditions for forest restoration.

The interactions between microbes can represent microbial resilience, offering a unique opportunity to investigate ADE as a potential for biotechnological application on natural and long-term conditions. These microbes, including plant-growth-promoting bacteria and mycorrhizal fungi, can enhance nutrient availability, modulate plant immunity, and suppress pathogens [7, 8]. Their application represents a sustainable alternative to conventional chemical inputs. This practice aims to restore microbial diversity and accelerate ecological processes, offering a low-cost, effective solution for re-establishing native species [9]. Here, we aimed to investigate the role of ADE as a soil inoculum for the restoration of degraded agricultural land. Our main objective was to evaluate how a small-volume application of ADE affects the growth of two key Amazonian tree species, *Schizolobium amazonicum* (primary succession specie) and *Handroanthus avellanedae* (secondary succession specie). We hypothesize that ADE's rich and resilient microbiome will selectively restructure the microbial communities in the plant's rhizosphere, suppressing opportunistic and pathogenic microbes while promoting the increase in abundance of plant-growth-promoting and biocontrol agents.

Materials and methods

Site description and experimental design

The experiment was performed in an experimental site belonging to the Brazilian Agricultural Research Corporation (EMBRAPA), located in Itacotiara, AM, Brazil (2°53'25"S, 59°58'06" W). The experimental area was a 1.2 ha plot of a former cassava cultivation field surrounded by native forest. The soil was classified as Oxisol [10] and the weather is classified as Rainy Tropical (Amw) according to the Köppen classification, characterized by an annual average temperature of 28 °C, high humidity (75–85%) for most of the year, and a short dry season. Annual rainfall ranges from 1,750 to 2,500 mm [11]. The plant species composition of the surrounding forest area is mainly composed of *Protium hebetatum*, *Eschweilera coriacea*, *Licania oblongifolia*, and *Pouteria minima*. The experiment was established at the beginning of autumn, in April 2023.

The effect of ADE as a soil inoculum was tested in two native Amazonian tree species with important characteristics for forest restoration. We selected *Schizolobium amazonicum,* a fast-growing leguminous pioneer species, due to its ability to colonize degraded soils rapidly [12]. We also selected *Handroanthus avellanedae*, a non-leguminous secondary succession species, due to its potential for timber in commercial reforestation [13]. We used commercial seeds (sold by Arbocenter Comércio de Sementes Ltda.) from both species and germinated them in seedling pots (290 cm^3) filled with 290 g of Amazonian Dark Earth (ADE). Seedling production was carried out in the EMBRAPA Western Amazon nursery, located in Itacotiara, Brazil. Seedling species confirmation was carried out by Dr. Aleksander Westpahl Muniz (listed coauthor). Seedling pots with coconut fiber (290 cm^3 with a density of ~0.72 g cm^{-3}) was used as a conventional treatment control. The ADE used in the study was collected from a secondary forest area at the EMBRAPA Experimental Station in Manaus, Brazil (2°53'25"S, 59°58'06" W). To ensure representative sampling, we selected five random sampling points located more than 10 meters from the area's borders. Each point was subdivided into three triangular subpoints, spaced 15 meters apart. At each subpoint, soil was collected from the 0–20 cm depth and mixed. The ADE fertility was characterized by conventional methods (see information below).

Seedlings of uniform size were selected 15 days after germination and transferred to the field. The experiment followed a randomized block design with three blocks of 6 × 36 m. Each block contained seedlings from one of the plant species, grown either with ADE or with a control. We used six plants per treatment as replicates, totaling 72 plants. To minimize environmental interference, individual plants were spaced 2 meters apart, and blocks were separated by 3 meters. The experiment was conducted without the use of fertilizers to mimic natural restoration conditions. Weed control was performed manually throughout the experiment. After six months, measurements and samples were collected. For each

treatment, we collected five soil samples (0–20 cm) for DNA sequencing, totaling 20 samples. We also collected five soil cores (0–20 cm) per treatment for physicochemical analyses before the beginning of the experiment, taken in a cross-section around the block. Plant growth measurements were assessed by plant height and stem diameter at breast height (DBH) for *S. amazonicum,* and stem diameter at ground level was taken for *H. avellanedae* since they were too small for DBH.

Soil physicochemical analysis

The initial physicochemical soil attributes were measured following the recommended protocols [14]. Briefly, pH was measured in a $CaCl_2$ solution (0.01 mol L 1); the soil organic matter (OM) was evaluated by oxidation in potassium dichromate [15]; P, K^+, Ca^{2+}, and Mg^{2+} were extracted using ion exchange resins; K^+ was quantified using a colorimetric method, while Ca^{2+} and Mg^{2+} were measured by atomic absorption spectrophotometry (PerkinElmer 3100, USA). SO_4^{2-} was extracted with a $Ca_3(PO_4)^2$ solution (0.01 mol L^{-1}) and quantified by turbidimetry. Al^{3+} was extracted with a KCl solution (1.0 mol L^{-1}) and quantified by titration with NaOH solution (0.025 mol L^{-1}). Micronutrients, including Fe, Cu, Mn, and Zn, were extracted using diethylenetriaminepentaacetic acid (DTPA) and quantified via atomic absorption spectrophotometry. Cation exchange capacity (CEC) was estimated based on the sum of Ca^{2+}, Mg^{2+}, K^+, Al^{3+}, and H^+. The sum of exchangeable bases (SB) was calculated based on the sum of Ca^{2+}, Mg^{2+}, and K^+.

DNA extraction, sequencing, and bioinformatics

Genomic DNA was extracted from 0.25 g of every sample using the DNeasy PowerLyzer PowerSoil© kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations with additional adaptations proposed for tropical soil samples [16]. The quality of the extraction was measured using a spectrophotometer NanoDrop 2000© (Thermo Fisher, Waltham, MA, USA). All samples were approved in quality control (A280/A260 between 1.70 and 2.00, concentration > 10 ng μL^{-1}). The V3–V4 region of the 16S rDNA was amplified by PCR to determine the abundance of prokaryotes (bacteria and archaea) in samples using the updated primers 515F [17] and 816 R [18]. The region ITS was amplified by PCR to determine the abundance of fungi using the primers ITS1f and ITS2 [19]. The paired-end sequencing with 2 × 250 bp reads was performed using the Illumina NovaSeq 6000 platform, and followed the recommendations from the Earth Microbiome Project [20].

Raw sequencing reads were processed using the DADA2 pipeline [21]. We kept sequences with a mean quality score greater than 30 and grouped the filtered reads into amplicon sequence variants (ASVs). We assigned taxonomy to the ASVs by matching them against the SILVA database (v. 138.1) [22]. The resulting ASV table was then converted into a phyloseq S4 object and a microeco R6 object for further analysis [23, 24]. The raw reads used in this work can be found in the Sequence Read Archive (SRA) under the project number PRJNA1346081.

Statistical analysis

All data wrangling and statistical analyses were carried out in the R language (v. 4.4.2) using RStudio (v. 2024.09.1) [25]. The code for the analyses performed in this study can be found publicly on GitHub at: https://github.com/FreitasAndy/ADE-in-the-field. Figures were produced using the ggplot2 package [26], and some of these figures were edited only for aesthetic purposes (i.e., changing colors and fonts) using the Inkscape 1.3.2 program. Data normality was tested using the Shapiro-Wilk test [27]. When parametric, we used analysis of variance (ANOVA) followed by the Tukey post hoc test to evaluate differences in plant growth parameters [28]. When data were considered non-parametric, we used the Kruskal-Wallis test followed by Dunn's post-hoc test with a false discovery rate (FDR) to evaluate differences [29]. Significance level was determined as 95% ($p < 0.05$).

We measured alpha diversity to assess the richness and dominance of the microbial communities. Richness was calculated as the number of unique taxa identified in each sample. Dominance was measured using the inverse Simpson index [30]. For beta diversity, we transformed the dataset using a centered log ratio (clr) to account for the compositional nature of the data. We then used non-metric multidimensional scaling (NMDS) based on Euclidean distance to visualize the community structure on the first two axes. We tested for significant differences between groups using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations and a significance level of 5% via the adonis function in the vegan package [31].

We used the ALDEx2 algorithm to identify microbial taxa with significant differences in abundance between the ADE treatment and the control for each plant species [32]. We considered a genus to be differentially abundant if it had a Welch's test p-value of less than 0.01 and an effect size greater than 1. Finally, we performed correlation network analysis at the genus level using the SpiecEasi algorithm to identify co-occurring bacterial, archaeal, and fungal groups. [33]. We focused on strong, highly reliable correlations, considering only those with a significance threshold of $p < 0.001$ and a correlation coefficient greater than 0.7.

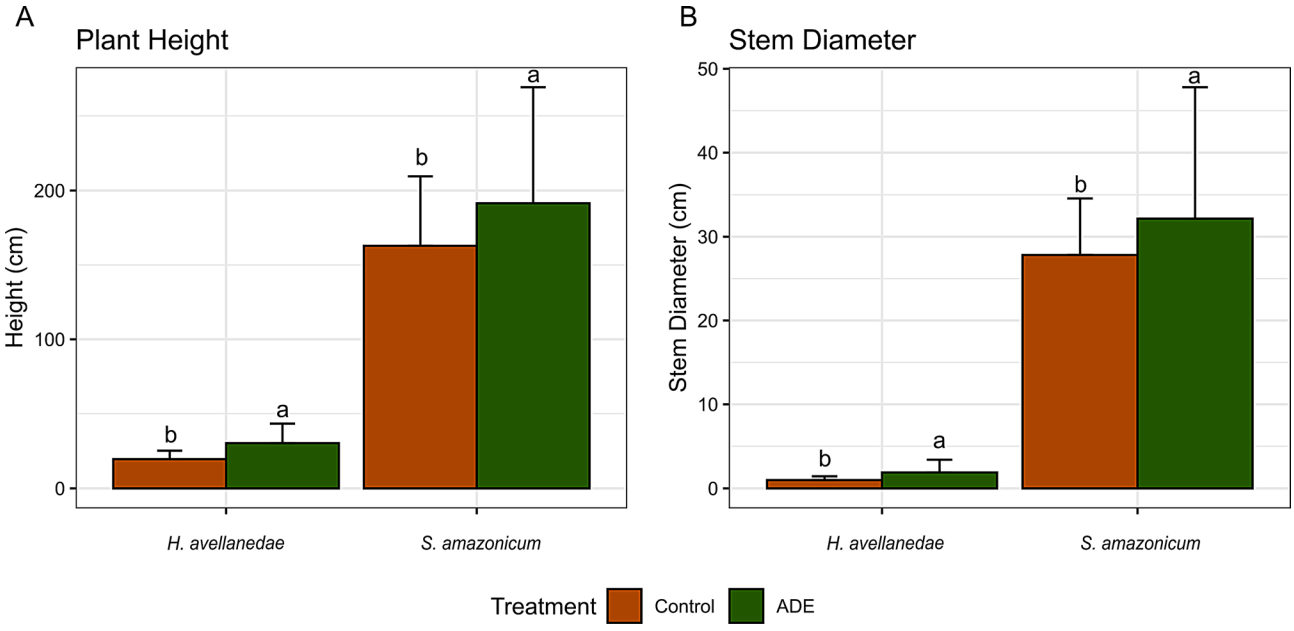

Fig. 1 Growth of *Handroanthus avellaneade* and *Schizolobium amazonicum* after 180 days of experiment in control and ADE treatments. (**A**) Plant height. (**B**) Stem diameter at ground level. Data are shown in mean + positive standard deviation. Different letters mean differences between ADE and Control calculated by ANOVA and Tukey's test post hoc

Table 1 Description of the main soil chemical attributes in both the experimental area where the experiment was conducted and the Amazonian Dark Earth site where ADE was collected

Measure	Experimental Area	ADE	Significance
pH (CaCl)	4.5 ± 0.2	5.2 ± 0.1	***
C (g kg^{-1})	21.0 ± 4.6	25.6 ± 5.6	***
OM (%)	36.0 ± 8.0	44.0 ± 9.6	***
P (mg dm^{-3})	3.8 ± 1.9	156.7 ± 33.0	***
K (mg dm^{-3})	62.2 ± 26.2	40.5 ± 17.9	*
Na (mg dm^{-3})	4.3 ± 1.3	5.6 ± 1.6	*
Ca ($cmol_c$ dm^{-3})	1.0 ± 0.5	6.7 ± 1.8	***
Mg ($cmol_c$ dm^{-3})	0.6 ± 0.3	1.1 ± 0.3	***
Al ($cmol_c$ dm^{-3})	0.4 ± 0.2	0.1 ± 0.1	***
H+Al ($cmol_c$ dm^{-3})	3.5 ± 0.9	4.3 ± 0.4	**
SB ($cmol_c$ dm^{-3})	1.8 ± 0.7	7.9 ± 2.2	***
CEC ($cmol_c$ dm^{-3})	5.4 ± 1.3	12.2 ± 2.1	***

Data are presented in mean ± standard deviation. *p-value < 0.05. **p-value < 0.01. ***p-value < 0.001. C: carbon; OM: organic matter; P: phosphorus; K: potassium; Na: sodium; Ca: calcium; Mg: magnesium; Al: aluminum; H+Al: potential acidity of the soil; SB: sum of bases; CEC: cation exchange capacity

Results and discussion

Incoculating Amazonian Dark Earths for seedling production increases the plant growth

All plants were alive after 180 days of the experiment. Seedlings produced with ADE developed better than those from the Control. *S. amazonicum* ones grew on average 20% more in ADE after 180 days of experiment ($p < 0.05$) and had the stem approximately 15% bigger ($p < 0.05$). The ADE effect in *H. avellanedae* was even higher. The species grew to approximately 55% ($p < 0.05$) and had a stem that was approximately 88% bigger ($p < 0.05$) (Fig. 1). As *S. amazonicum* is a fast-growing species, ideal for ecological restoration projects, once it receives good sunlight and is not demanding of many nutrients [34], we already expected the species to exhibit greater growth due to ADE attributes such as higher organic matter (OM) content, higher soil pH and overall higher fertility compared to the soil from the experimental area (Table 1). In fact, after six months of experimentation, we already had most of the trees with a height of more than 1.5 meters, with those produced using ADE being of a larger size. The increase in growth of *H. avellanedae* also highlights the potential of ADE as a growth booster for non-primary succession species in the Amazon, which commonly presents more challenges in establishing, especially in degraded areas [35]. We have already shown that larger amounts of ADE (20%) could boost the establishment of trees for ecological restoration, independently of successional stage [6], and here demonstrated in the field that the need for ADE could be reduced in the field, with similar results for both a primary and secondary succession species.

ADE inoculum promoted higher microbial diversity

Alpha diversity was similar among bacterial communities (Figs. 2A and 2C), but fungal diversity and dominance of taxa (Figs. 2B and 2D) were strongly increased in *H. avellanedae*, despite no effect found in *S. amazonicum*. We expected an increased diversity in both prokaryotes and fungal diversity in the two species, but we believe the

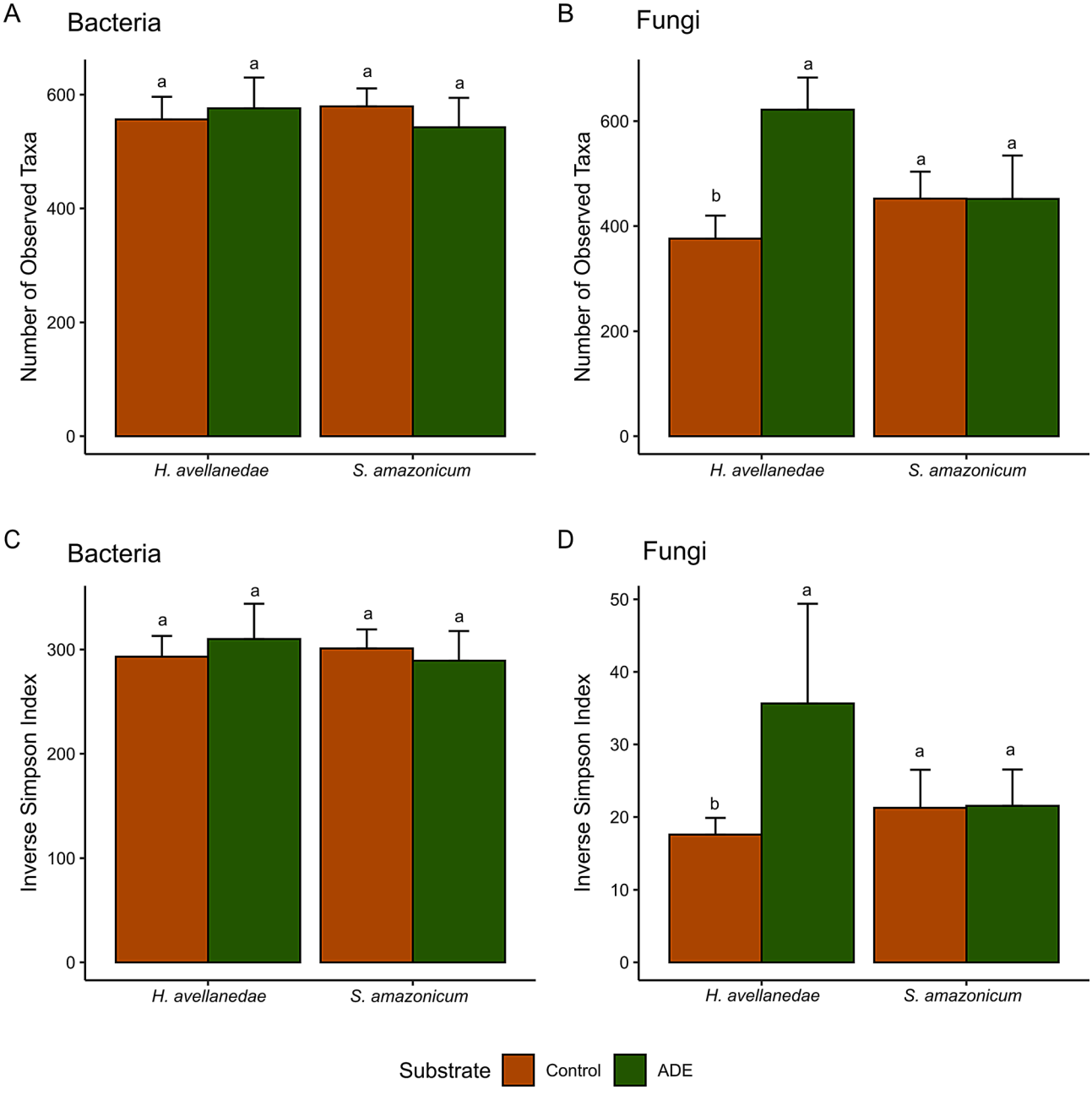

Fig. 2 Diversity indexes of *Handroanthus avellaneade* and *Schizolobium amazonicum* after 180 days of experiment in control and ADE treatments. (**A**) Bacterial observed diversity, considering the number of different taxa found in each treatment. (**B**) Observed fundal diversity. (**C**) Bacterial inverse Simpson index, used as an indicator of dominance of species. (**D**) Fungal inverse Simpson index. Data are shown in mean + positive standard deviation. Different letters mean differences between ADE and Control calculated by Kruskal-Wallis and Dunn's test post hoc

absence of effects in *S. amazonicum* is mainly due to the Amazonian soils' resilience to changes without a strong event of disturbance [36]. On the other hand, soil eukaryotes used to respond quicker than bacteria and archaea when changes in soil happen [37], and the diversity of fungi is higher in ADEs than in agricultural soils [38], leading to a tendency for ADEs to enhance the establishment and development of fungal communities.

Looking at the abundance of microorganisms despite the count of taxa, the standards are clearer. Similarly, to the alpha diversity, the beta diversity analysis showed the *H. avellanedae* ADE communities as the most dissimilar compared to the other groups, considering both prokaryotes and fungi (Figs. 3A and 3B). Although the dissimilarity among the other groups was smaller, each one was separated from the other, highlighting once

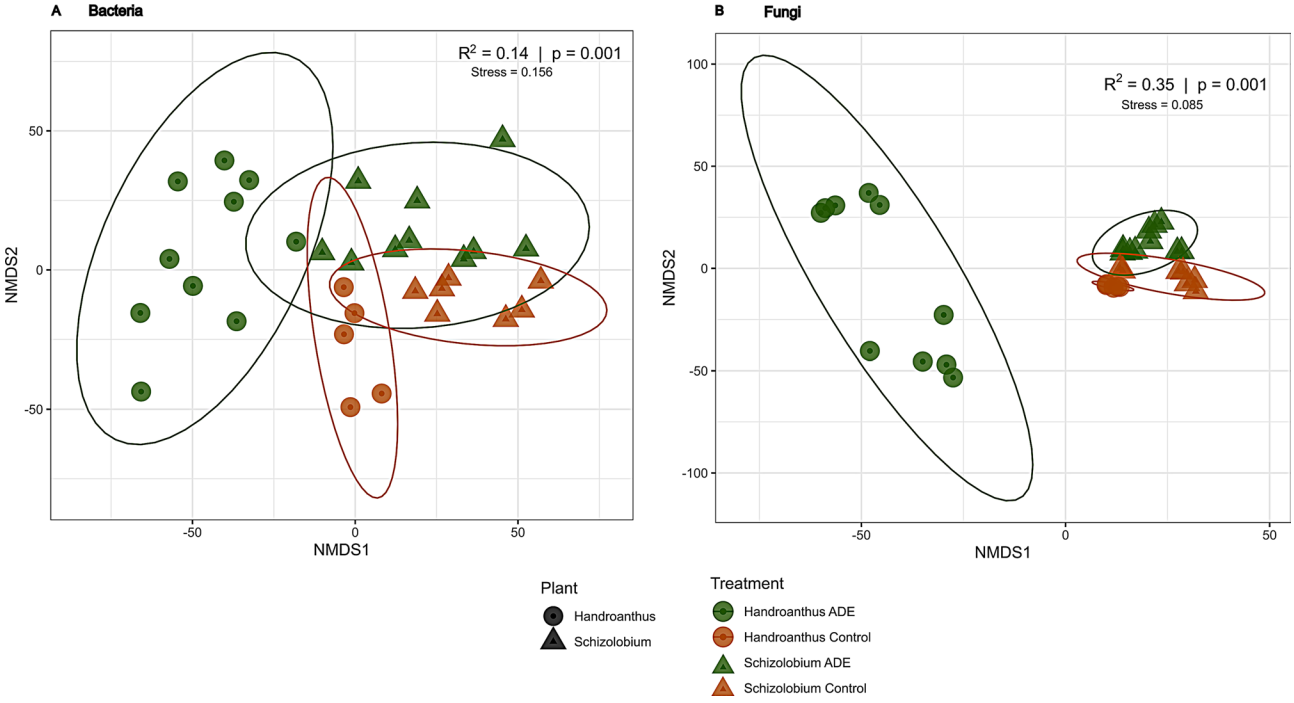

Fig. 3 Beta diversity calculated by Euclidean distance and plotted in a non-metric multidimensional scaling. (**A**) Prokaryotes. (**B**) Fungi. R-squared and p-values were calculated by PERMANOVA with 999 permutations

more that the differences in growth are probably driven by microorganisms.

One of the main criticisms of soil transference and usage as enhanced for plants in the argument is that the new soil can affect plant growth only because of its nutrients. It can be true in huge amounts of high-fertility soils, but it is not the case here. Each tube, when the seedlings were produced, had a small amount of soil (290 cm^3), and the plants were normalized by size when planted in the soil. Additionally, the amount of nutrients in the ADEs we used was way lower than the amount commonly found in commercial fertilizers. Finally, when in the soil, the nutrients are dissolved in the original Oxisol from the experimental field. Knowing all of this, we tested the microbial distribution among treatments.

ADE treatments shaped the fungal community (but not the prokaryotic one) in S. amazonicum

The microbial distribution of phyla was slightly different between treatments (Fig. 4). The core prokaryotic microbiome was mainly composed of Proteobacteria, Actinobacteriota, Acidobacteriota, Chloroflexi, and Firmicutes (Fig. 4A). Control samples had, on average, a higher percentage of Acidobacteriota than ADE treatments, probably due to the low pH (~4.5) from the original soil [39]. Despite the dominance of Ascomycota, *S. amazonicum* plants cultivated with ADE recruited more Mortierellomycota than the control ones, alongside a reduction in the relative abundance of Basidiomycota. Those patterns are key indicators of rhizosphere re-establishment and biostimulation driven by both *S. amazonicum* and ADE. Mortierellomycota species are often associated with healthy, nutrient-rich soils and are known to be plant-growth promoters, especially in the early stages of plant establishment. They thrive in the new, more favorable conditions created by the ADE [40]. Furthermore, the ADE must have provided a more immediately available, simpler organic matter source that favored the faster-growing Mortierellomycota over the slower, lignin-specializing Basidiomycota. The ADE effectively bypassed the need for the long-term, slow decomposition process that Basidiomycota are known for [41].

Producing seedlings of *S. amazonicum* with ADE significantly restructured the soil fungal community around the plant. ADE acted as a selective filter that both suppressed and promoted specific microbial taxa. On one hand, ADE treatment resulted in a significant decrease in the relative abundance of *Penicillium*, *Myrothecium*, and Basidiomycota-related yeasts like *Papiliotrema* and *Saitozyma*, as well as the ectomycorrhizal *Serendipita* (Table 2). This decline suggests the suppression of fungal groups often associated with disturbed, nutrient-poor, or stressed environments [42, 43]. The reduction in potentially pathogenic (*Myrothecium*) indicates a successful transition toward a healthier, more balanced soil ecosystem, as ADE is known for being a suppressive soil [44]. On the other hand, ADE provided conducive conditions for other fungal groups. Notably, there was a substantial

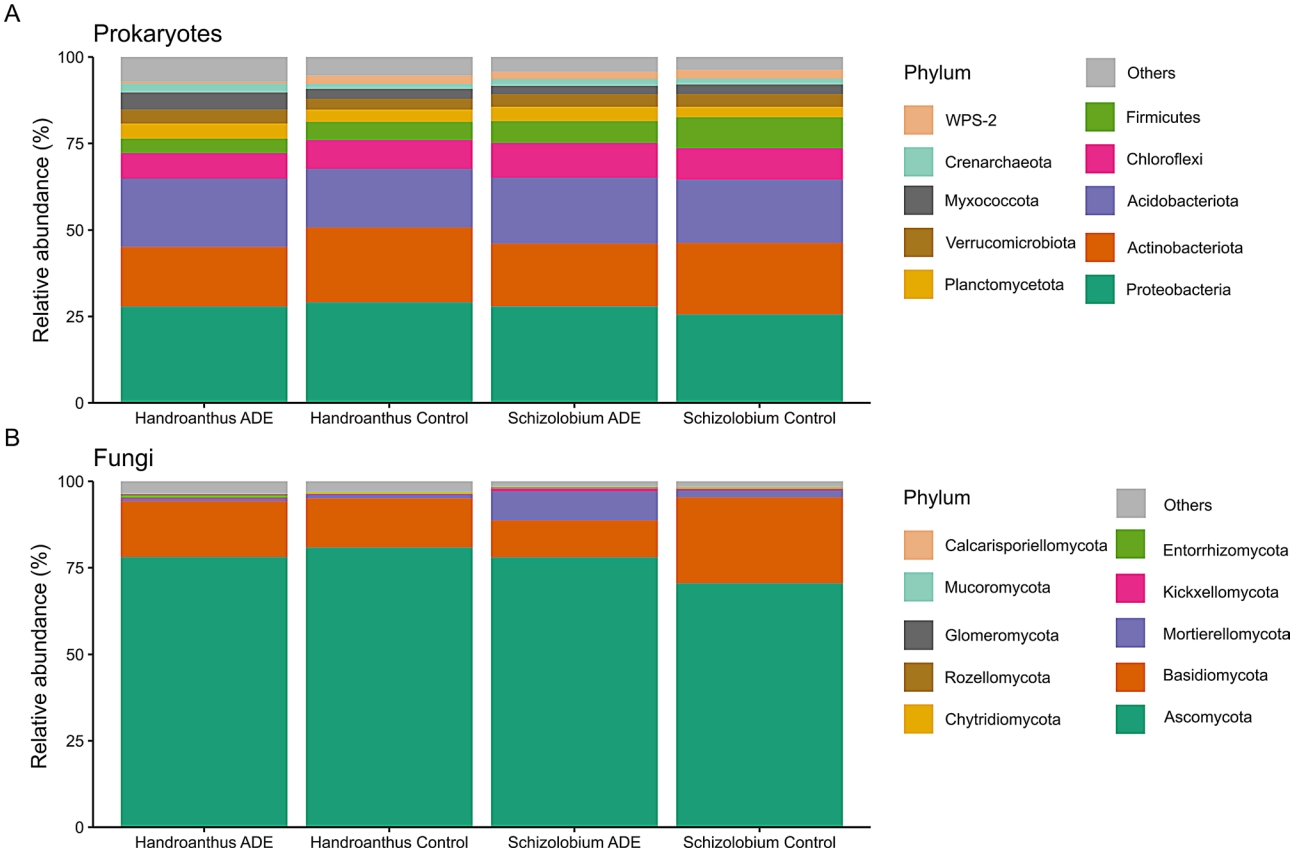

Fig. 4 Top 10 phyla abundance in *Handroanthus avellanedae* and *Schizolobium amazonicum* in ADE treatment and control. (**A**) Prokaryotes abundance, including bacteria and archaea. (**B**) Fungal abundance

increase in taxa such as *Setophoma*, *Pyxidiophora*, *Ascobolus*, *Cercophora*, *Apiotrichum*, and *Vanrija* (Table 2). These fungi represent key functional groups: *Setophoma* and some yeasts (*Apiotrichum*, *Vanrija*) are likely involved in the decomposition of specific organic matter, while *Pyxidiophora* acts as a mycoparasite, potentially helping to regulate the new fungal community [45]. Furthermore, the promotion of coprophilous taxa like *Ascobolus* and *Cercophora* highlights the unique, nutrient-rich, and dung-like nature of ADE, which fosters a distinct ecological niche [46].

ADE treatments steered microbial abundance in H. avellanedae

The ADE treatment also performed as a powerful microbial inoculum and a suppressive soil, with potential to suppress some pathogenic genera, in the *H. avellanedae* rhizosphere. ADE caused a notable depletion of several bacterial genera, including *Achromobacter*, *Rhizobium*, *Aureimonas*, *Chitinophaga*, *Chryseobacterium*, *Ellin516*, *Enterobacter*, *Haoranjiania*, *Labrys*, *Larkinella*, *Leifsonia*, *Pandoraea*, *Pseudoxanthomonas*, *Roseateles*, *Siphonobacter*, *Sphingobacterium*, and *Stenotrophomonas*. This shift, unlike the previous findings with *S. amazonicum*, highlights the combined effect of ADE's unique physicochemical properties and the specific plant-host interactions in shaping the microbial community, even in small amounts. This is a critical observation, as the high nutrient content of ADE may create a negative feedback loop, suppressing beneficial functional groups, such as nitrogen-fixers (e.g., *Rhizobium* and *Enterobacter*), that are energetically unnecessary in a nutrient-rich environment. Although we did not measure the total nitrogen content, a previous study showed that the values of nitrogen were around 40% higher in the same ADE used in our study compared to the original soil [47]. Consequently, while ADE promotes short-term plant growth by providing a reserve of available nutrients, it could potentially inhibit the long-term, self-sustaining biological processes of nutrient cycling. This observation challenges the current state of the art, where ADE's power in ecological restoration relies on both nutrients and microbiota [6, 48]. However, it opens a field of study regarding the amplitude of each aspect (nutrients and microbes) in the plant growth promotion driven by ADE.

Regarding fungi, ADE treatment also had a profound and selective effect on the fungal community in *H. avellanedae* rhizosphere, increasing the soil's suppressive

Table 2 List of microbial genera with significant differences between the ADE treatment and the control group after 180 days of experiment

Tree Species	Superior Taxonomy	Genus	Abundance ADE	Abundance Control	Effect	Overlap	p-value
Schizolobium amazonicum	Bacteria	-	-	-	-	-	-
	Fungi	*Setophoma*	5.20	3.76	−1.72	0.02	0.02
		Penicillium	5.64	7.30	1.52	0.05	0.04
		Pyxidiophora	3.75	−5.23	−2.89	0.00	0.02
		Ascobolus	2.04	−5.05	−2.62	0.00	0.02
		Myrothecium	5.05	6.69	1.75	0.01	0.02
		Cercophora	4.53	3.06	−1.42	0.08	0.05
		Serendipita	−3.08	3.19	1.41	0.01	0.04
		Papiliotrema	5.42	10.17	2.69	0.00	0.00
		Saitozyma	5.27	7.72	2.19	0.00	0.00
		Apiotrichum	5.47	3.85	−1.44	0.05	0.04
		Vanrija	2.98	−4.43	−2.37	0.00	0.03
Handroanthus avellanedae	Bacteria	*Achromobacter*	−2.11	6.43	2.97	0.00	0.00
		Rhizobium	−1.73	6.04	2.12	0.02	0.00
		Aureimonas	−1.98	6.18	2.97	0.00	0.00
		Chitinophaga	−1.66	6.57	2.44	0.00	0.00
		Chryseobacterium	−1.13	7.17	2.23	0.00	0.00
		Ellin516	−2.03	5.37	2.78	0.00	0.00
		Enterobacter	−1.74	5.00	2.08	0.03	0.00
		Haoranjiania	−2.16	6.09	2.98	0.00	0.00
		Labrys	−0.46	6.21	1.15	0.08	0.03
		Larkinella	−1.56	3.87	2.05	0.00	0.01
		Leifsonia	−2.02	6.38	3.37	0.00	0.00
		Pandoraea	−2.13	5.97	3.16	0.00	0.00
		Pseudoxanthomonas	−1.67	5.89	2.59	0.00	0.00
		Roseateles	−2.18	5.65	3.16	0.00	0.00
		Siphonobacter	−2.07	5.52	2.80	0.00	0.00
		Sphingobacterium	−2.03	5.02	2.26	0.00	0.00
		Stenotrophomonas	−2.20	6.21	2.78	0.00	0.00
	Fungi	*Lasiodiplodia*	3.50	5.96	1.70	0.01	0.01
		Aaosphaeria	5.34	7.62	1.94	0.01	0.00
		Paraconiothyrium	−5.71	2.58	2.76	0.00	0.00
		Neosetophoma	−6.25	0.24	2.40	0.00	0.00
		Setophoma	4.63	1.71	−2.14	0.01	0.01
		Curvularia	6.10	3.55	−2.28	0.02	0.01
		Neoroussoella	−5.16	3.73	3.24	0.00	0.00
		Setoarthopyrenia	−6.25	0.97	2.87	0.00	0.00
		Shiraia	3.98	−6.11	−4.00	0.00	0.03
		Exophiala	−5.01	1.71	1.51	0.01	0.02
		Chaetomella	5.90	3.47	−1.84	0.02	0.01
		Lipomyces	4.81	2.04	−2.55	0.00	0.01
		Cyberlindnera	−0.80	3.48	1.69	0.00	0.02
		Neopestalotiopsis	5.61	0.66	−3.23	0.00	0.00
		Diaporthe	−3.65	4.42	2.26	0.00	0.00
		Paragibellulopsis	−0.71	6.55	2.68	0.00	0.01

Table 2 (continued)

Tree Species	Superior Taxonomy	Genus	Abundance ADE	Abundance Control	Effect	Overlap	p-value
		Nectriella	4.71	−0.74	−5.57	0.00	0.00
		Metarhizium	6.27	4.49	−1.78	0.03	0.03
		Bisifusarium	3.04	0.64	−1.76	0.01	0.02
		Xenomyrothecium	4.00	−6.22	−3.31	0.00	0.03
		Pseudodactylaria	−5.83	0.29	2.00	0.01	0.01
		Humicola	5.66	3.63	−1.42	0.05	0.04
		Serendipita	−0.27	3.45	1.60	0.00	0.04
		Tomentella	5.21	1.87	−2.20	0.00	0.01
		Atractiella	2.70	−6.09	−2.32	0.00	0.04
		Chrysozyma	2.41	−6.45	−1.85	0.04	0.04
		Hannaella	−3.93	2.38	1.78	0.00	0.01
		Papiliotrema	4.87	7.00	2.09	0.01	0.00
		Sonoraphlyctis	−5.29	2.04	2.39	0.00	0.00
		Entorrhiza	4.30	2.50	−1.76	0.03	0.03

Relative abundance was calculated by the 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 Control group whereas negative values indicate higher abundance in the ADE group; overlap: confusion in assigning an observation Control or ADE; p-value: the expected value of the Welch test p-value corrected by Benjamini-Hockberg method. The table includes all genera with effect > 1 and p-value ≤ 0.05

potential against pathogens while simultaneously restructuring the symbiotic relationships between the plant and its fungal partners (Table 2). The ADE-amended soil exhibited a notable decrease in opportunistic and pathogenic fungal genera, including *Exophiala*, *Serendipita*, *Cyberlindnera*, *Lasiodiplodia*, *Hannaella*, *Aaosphaeria*, *Pseudodactylaria*, *Papiliotrema*, *Diaporthe*, *Sonoraphlyctis*, *Neosetophoma*, *Paragibellulopsis*, *Paraconiothyrium*, *Setoarthopyrenia*, and *Neoroussoella*. This depletion, particularly of known pathogens like *Lasiodiplodia*, is consistent with our findings in the bacterial community, where ADE treatment led to a significant decrease in numerous genera, including potential pathogens, highlighting ADE's broad role as a suppressive soil [49, 50].

Additionally, the selective suppression was accompanied by a shift towards a new set of beneficial fungi. ADE inoculum promoted a variety of plant-growth promoters and biocontrol agents, with a notable increase in genera such as *Nectriella*, *Shiraia*, *Xenomyrothecium*, *Neopestalotiopsis*, *Lipomyces*, *Atractiella*, *Curvularia*, *Tomentella*, *Setophoma*, *Chrysozyma*, *Metarhizium*, *Bisifusarium*, *Entorrhiza*, and *Humicola*. The increase in biocontrol agents like *Metarhizium* and *Nectriella* suggests that the ADE is actively fostering a community that can protect the plant from pests and diseases [51]. However, the concurrent decrease in some mycorrhizal fungi like *Serendipita*, while other mycorrhizae such as *Tomentella* increased, indicates a complex trade-off which can be interpreted as negative feedback where the ADE's high nutrient content reduces the plant's need to invest in some symbiotic relationships, as it can acquire nutrients more directly from the inoculum soil. The ADE's function is not just to add nutrients but to re-engineer the soil microbiome, replacing opportunistic and pathogenic taxa with a new, more specialized community that aligns with the plant's needs in a nutrient-rich environment.

ADE restructures H. avelllanedae but not S. amazonicum microbial networks

Finally, the co-occurrence analysis revealed that the ADE treatment also reshaped plant-microbe interactions in the rhizosphere. *H. avelllanedae* showed a stronger impact on the network structure compared to *S. amazonicum* (Fig. 5). The ADE inoculum on *H. avellanedae* increased positive interactions, network density (898 edges, average degree 18.9), resulting in a non-modular (0.18) network with a balanced ratio of positive and negative correlations compared to the control. This massive increase in microbial connectivity in *H. avellannedae* is consistent with previous reports of ADE increasing microbial integration in other species [52], suggesting its effect is pronounced in non-leguminous systems. Conversely, the ADE inoculum resulted in fewer differences in the *S. amazonicum* network properties. The network remained consistently dense (0.16~0.20), highly connected, and non-modular (0.16~0.20) under both conditions, suggesting that the established, complex belowground interactions are typical of this primary succession legume species and already drive a highly connected community that is resistant to structural alteration by the ADE inoculum [34].

Conclusions

Considering all the findings, we showed that the application of a small volume of ADE had a strong effect on both *Schizolobium amazonicum* and *Handroanthus avellanedae* growth. The most significant finding was that ADE is not just a reservoir of soil nutrients, but can also be a highly effective bio-amendment that fundamentally

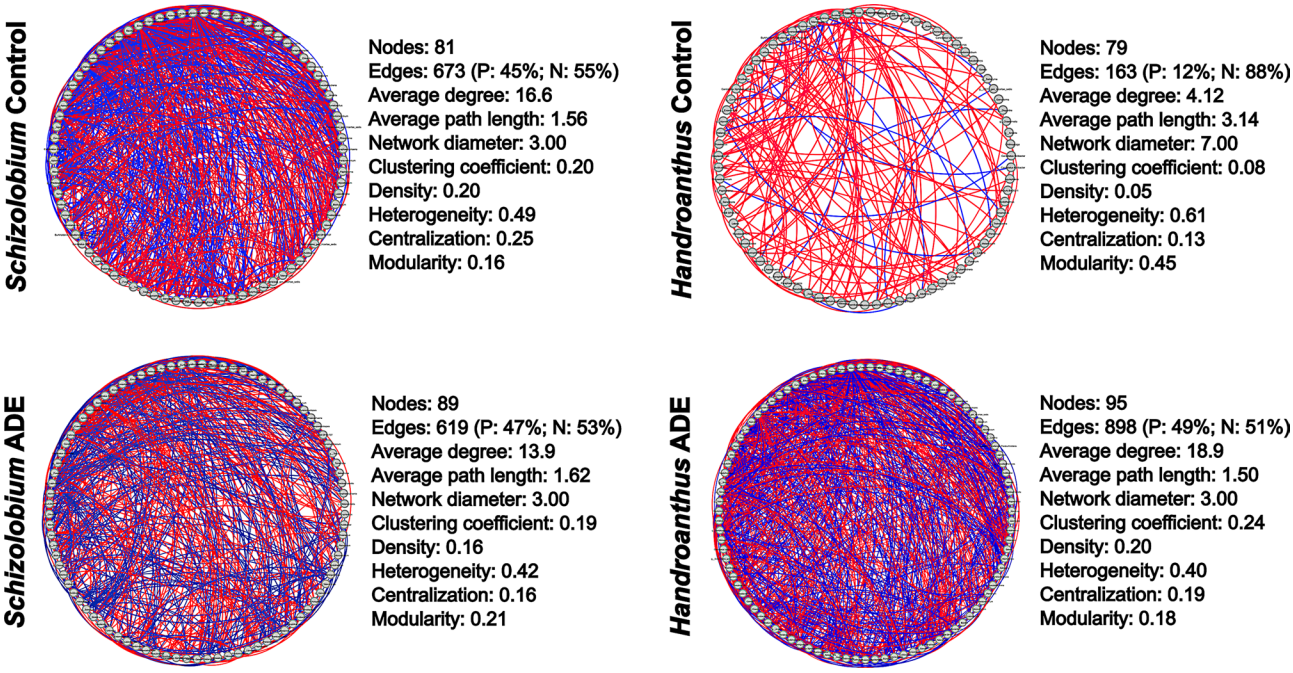

Fig. 5 Co-occurrence network analysis of microbial communities in *Schizolobium amazonicum* and *Handroanthus avellanedae* soils under control and ADE treatments. Nodes represent individual microbial taxa, and edges represent significant correlations ($R^2 > 0.70$, $p < 0.001$). Red lines indicate negative correlations, and blue lines indicate positive correlations. The accompanying tables quantify network topology parameters, including the total number of nodes, edges (with the percentage of positive (P) and negative (N) correlations), Average degree, Average path length, Network diameter, Clustering coefficient, Density, Heterogeneity, Centralization, and Modularity

restructures the soil microbiome. It functioned as a suppressive soil, actively selecting against a wide range of opportunistic and pathogenic bacteria and fungi, while promoting a new, beneficial microbial community. This selective pressure resulted in the depletion of stress-tolerant taxa and pathogens, while simultaneously fostering a diverse array of plant-growth promoters and biocontrol agents. While ADE's high nutrient content may create negative feedback that reduces the need for certain microbial functions, such as nitrogen fixation, this targeted microbial reshape is a key mechanism through which ADE improves soil health and supports robust plant establishment. In conclusion, ADE's value lies in its living microbial community, which acts as a powerful inoculum to restore degraded soils and engineer a healthier rhizosphere for sustainable reforestation.

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Author contributions
ASF conceived the study, performed experiments and data analyses, and wrote the manuscript. GLM performed the experiments, contextualized, and wrote the manuscripts. JAD carried out formal analysis and reviewed the manuscript. REH acquired funding and coordinated the project. AWM conceived the study and reviewed the manuscript. SMT acquired funding, formally supervised, and wrote the manuscript.

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Data availability
All sequences generated and analyzed during the current study are available in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1346081. Additional processed data and scripts used in the analyses are available at https://github.com/FreitasAndy/ADE-in-the-field.

Declarations

Ethics approval and consent to participate
Soil and plant samples were collected in accordance with Brazilian regulations on access to genetic resources (SISGEN registration no. AD13FB3). No human or animal subjects were involved in this study, and therefore, no institutional ethics approval was required.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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