









Article

Non-Specific Interactions of Rhizospheric Microbial Communities Support the Establishment of *Mimosa acutistipula* var. *ferrea* in an Amazon Rehabilitating Mineland

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Abstract: *Mimosa acutistipula* var. *ferrea* (Fabaceae) is endemic to ferruginous tropical rocky outcrops in the eastern Amazon, also known as *canga*. *Canga* are often associated with mining activities and are the target of protection and rehabilitation projects. *M. acutistipula* stands out in this biodiversity hotspot with high growth rates, even in rehabilitating minelands (RMs). However, little is known about the diversity of soil microorganisms interacting with *M. acutistipula* in *canga* and RMs. This study analyzed the rhizosphere-associated bacterial and fungal microbial communities associated with *M. acutistipula* growing in an RM and a native shrub *canga*. The fungal phylum Ascomycota was the dominant taxa identified in the rhizosphere of the *canga* (RA: 98.1) and RM (RA: 93.1). The bacterial phyla Proteobacteria (RA: 54.3) and Acidobacteria (RA: 56.2) were the dominant taxa identified in the rhizosphere in the *canga* and RM, respectively. Beneficial genera such as *Bradyrhizobium*, *Rhodoplanes*, and *Paraconiothyrium* were identified in the rhizosphere of *M. acutistipula* in both areas. However, the analyses showed that the fungal and bacterial diversity differed between the rhizosphere of the *canga* and RM, and that the microbial taxa adapted to the *canga* (i.e., *Rasamsonia*, *Scytalidium*, *Roseiarcus*, and *Rhodomicrobium*) were lacking in the RM. This influences the microbe-mediated soil processes, affecting long-term rehabilitation success. The results showed that *M. acutistipula* established non-specific interactions with soil microorganisms, including beneficial taxa such as nitrogen-fixing bacteria, mycorrhizal fungi, and other beneficial endophytes, well known for their importance in plant adaptation and survival. High levels of microbe association and a plant's ability to recruit a wide range of soil microorganisms help to explain *M. acutistipula*'s success in rehabilitating minelands.

Keywords: Amazon; *canga* ecosystem; iron mining; mineland rehabilitation; soil; symbiosis

1. Introduction

Brazil is one of the largest iron producers and has the second-largest reservoirs in the world [1]. Serra dos Carajás in the state of Pará covers Brazil's main iron ore reservoirs [2]. Here, iron ore extraction occurs in rocky outcrops [3], where a typical and highly diverse vegetation conforms to an ecosystem known as *canga* [4]. Iron mining directly affects *canga*, altering biological diversity and ecological services in this ecosystem [5]. Therefore, the screening of native plants from *canga* with good yield in post-mining areas is mandatory to reduce biodiversity loss and enhance mineland rehabilitation.

Iron mining in Brazil essentially occurs in open-cast mines. These sites are characterized by the removal of the soil surface layer, soil physical, chemical and biological alterations, soil organic matter depletion, nutrient loss, erosion, and soil acidification [6,7]. In this context, the rehabilitation of minelands is necessary to generate a positive diversity offset [8,9]. The revegetation of post-mining areas using native species is the most recommended alternative to reducing biodiversity loss and progressively recovering the plant coverage in biodiversity hotspots such as the Amazon [10,11]. Here, the interaction of native plants with soil-borne microorganisms is essential to contribute to mineland rehabilitation. Specifically, beneficial rhizosphere-inhabiting microorganisms contribute to abiotic/biotic stress tolerance by direct (i.e., production of plant growth-promoting metabolites, phytohormones, and nutrient solubilization) and/or indirect mechanisms (i.e., protection against phytopathogens, immobilization of metals, and changes in gene expression) [12,13]. Therefore, to identify the microbial diversity associated with plants growing in rehabilitated minelands (RMs), evaluating the underground effects of rehabilitation projects in mining areas in *canga* is necessary.

The soils from *canga* are composed of ferruginous fragments in which plant establishment and growth are affected by environmental factors such as the low availability of essential nutrients, low water holding capability, and high soil surface temperatures [14,15]. *Mimosa acutistipula* var. *ferrea* Barneby (Fabaceae) inhabits environments characterized by a herbaceous shrub physiognomy and can grow after being sown in waste piles in RMs [16]. Symbiosis with soil-borne microorganisms has been described as essential for plants from the Fabaceae family to fit to challenging environments such as RMs [14,17]. Therefore, it is expected that symbiotic microorganisms, as well as other beneficial rhizosphere-associated microorganisms, influence the establishment of native plants in RMs.

In the RMs of the eastern Amazon, few studies have analyzed the interaction of native plants with soil microorganisms. Hence, determining the diversity of soil-borne microorganisms associated with the rhizosphere of plants growing in RMs is necessary for identifying changes in the microbial diversity that underline the loss of essential microbe-mediated process that can influence long-term rehabilitation [18]. The aims of this study were: (i) To describe and compare the rhizosphere-associated fungal and bacterial communities of *M. acutistipula* growing in its native environment (*canga*) and RMs, and (ii) to identify beneficial soil-borne microorganisms that contribute to plant establishment in RMs.

2. Materials and Methods

2.1. Sampling

Soil samples were collected in a native shrub *canga* (hereafter, "*canga*"; 6°00'41.0" S 50°17'45.0" W) and in waste piles of an iron mineland in the *canga*, with a revegetation program started in 2014 (hereafter, "RM"; 6°02'32.0" S 50°07'04.0" W), in Serra dos Carajás, Pará, Brazil. *Canga* soils are characterized by the high adsorption of phosphorous by iron and aluminum oxides and the low availability of soluble forms in the soil solution [19,20]. The revegetation in the RM started in 2014 with commercial and native plants, where fertilization with NPK 04-14-08, mulch, organic compost, and AG60 fixer was used (Table S1). Compound samples from rhizospheric and bulk soils were collected from four *M. acutistipula* plants growing in the *canga* and RM. The rhizospheric soil was collected by gently shaking the roots, whereas the bulk soil was collected 5 m away from each sampled plant, at a depth of 10 cm and with no presence of other plants. The samples were immediately stored in 50 mL Falcon tubes in ice, transported to the laboratory, and kept at −80 °C until further processing.

2.2. DNA Extraction and Sequencing

Total DNA was extracted from 250 mg of soil using a PowerSoil DNA Isolation Kit (QIAGEN, Hilden, Germany), according to the manufacturer's recommendations. The

DNA concentration was determined with a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and the quality was verified in a 1% electrophoresis agarose gel.

The amplicon libraries for the bacteria were prepared according to the Illumina 16S Metagenomic Sequencing Library Preparation Protocol (Illumina, San Diego, CA, USA). For the fungi, the amplicon libraries were prepared with the respective modifications (fungal primers and PCR conditions). For the bacteria, the V3–V4 region of the 16S rRNA gene was amplified using the primer set S-D-Bact-0341-b-S-17-N (5'-TCGTCGGCAGCGTCA GATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3') and S-D-Bact-0785-a-A-21-N (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'), whereas for the fungi, the ITS region of the 18S rRNA gene was amplified using the primer set ITS7i (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGARTCATCGAAT CTTTG-3') and ITS4i (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCC TCCGCTTATTGATATGC-3'). The PCR mix contained 12.5 µL of 2x Kapa Hifi HotStart Ready Mix (Sigma-Aldrich, St Louis, MI, USA), 5 µL of each primer (1 µM), and 2.5 µL of DNA. The PCR cycle for bacteria consisted of an initial denaturing of 3 min at 95 °C, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The PCR cycle for the fungi consisted of an initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 1 min, extension at 72 °C for 30 s, and a final extension at 72 °C for 7 min. The concentrations of the PCR fragments were measured in a Qubit fluorometer using a Qubit™ ds DNA HS Assay (Thermo Fisher Scientific, Waltham, MA, USA). The size and quality of the PCR fragments were estimated on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) using a DNA 1000 chip. The PCR reactions were purified with an AMPure XP purification kit (Beckman Coulter, Brea, CA, USA), and the libraries were further processed with a Nextera XT kit (Illumina). The libraries were standardized to a concentration of 4 nM and processed following Illumina 16S Metagenomic Sequencing Library Preparation (Illumina). The 16S rRNA and 18S rRNA gene libraries were sequenced in a MiSeq-Illumina platform using a MiSeq V3 reagent kit (600 cycles; Illumina) in the human and medical genetics laboratory at Universidade Federal do Pará (Belém, PA, Brazil).

2.3. Sequence Analyses

The ITS and 16S sequences from the rhizospheric and bulk soils were analyzed using the PIMBA pipeline (Pipeline for MetaBarcoding Analysis) [21]. The sequences were trimmed and filtered by quality using PRINSEQ v0.20.4, and forward and reverse sequences were merged using PEAR v0.9.19 [22]. Reads were replicated, singletons removed, and the sequences were truncated to 200 for fungi and 240 for bacteria. Chimeras were filtered, and the sequences were grouped into operative taxonomic units (OTUs) using VSEARCH v2.8.2 at a similarity of $\geq 97\%$. Taxonomic assignment was performed using the UNITE database and the Ribosomal Database Project for fungi and bacteria [23,24]. The sequences obtained in this study were submitted to the NCBI Sequence Read Archive (<http://trace.ncbi.nlm.nih.gov/Traces/sra/>, accessed on 13 February 2021) under accession number PRJNA701717.

2.4. Functional Analysis of Rhizosphere-Associated Microbial Communities

The variations in the inferred functions of bacterial and fungal communities associated with *M. acutistipula* were estimated according to Liang et al. [25], with minor modifications. Briefly, the predicted ecological roles of the fungal OTUs were assigned using FUNGuild v1.1 [26], whereas the predicted functions of the bacterial OTUs were assigned using FAPROTAX v1.2.4 [27], both in Python v3.8.2. The data were plotted in R software v3.6.3 (R Core Team 2018; <https://www.R-project.org>) using the viridis, dplyr, and scales packages.

2.5. Statistical Analyses

Specific data analyses and graphs were performed in R software. Graphs for each sample were constructed considering the alpha and beta diversity using the ggplot2 and vegan packages. Principal coordinate analysis (PCoA) was constructed using “weighted UniFrac distances” in the phyloseq package [28]. Statistical significance of the community data was estimated using permutational multivariate analysis of variance (PERMANOVA) via the function “Adonis” (vegan package). Alpha diversity was estimated as the Shannon and Simpson diversity indices using the vegan package. The shared and unique fungal and bacterial OTUs among different samples were calculated and visualized with Venn diagrams using the MicEco package.

Similarly, heatmaps were constructed with the relative abundance (RA) of beneficial OTUs using the heatmaply and phyloseq packages. In addition, a redundancy RDA analysis was constructed to reveal the relationships between beneficial microbiota and soil properties using the vegan, mvpart, and labdsv packages. Clustering analysis of the data was performed using the “hclust” function in the pvclust package. The linear discriminant analysis (LDA) effect size (LEfSe) was performed with the Kruskal–Wallis test [29] and the effect size was estimated with a logarithmic score of >3.0 .

3. Results

The ITS2 sequencing produced a total of 3,158,043 raw reads across 16 input libraries. After quality filtering, 2,324,549 amplicon sequences were considered. The number of fungal OTUs in the samples ranged from 197 to 737 and was, on average, 496 (Table S2). The soils from RMs showed more fungal sequences (1,202,317; comprising 557 ± 189 OTUs) than *canga* (1,122,232; comprising 434 ± 115 OTUs) (Table S2). Additionally, the rhizospheric soil samples showed more sequences (1,318,674; comprising 556 ± 116 OTUs) than the bulk soil samples (1,005,875; comprising 435 ± 188 OTUs) (Table S2). The Shannon diversity index showed higher values in the samples from RMs, whereas the Simpson diversity index was similar in all samples (Figure 1a,b). Venn diagrams of the ITS sequences showed 88 core OTUs in the four samples, and soils from *canga*-B, RM-B, RM-R, and *canga*-R had 133, 203, 128, and 221 unique OTUs, respectively (Figure S1).

The 16S sequencing produced a total of 3,116,392 raw reads across 16 input libraries. After quality filtering 363,908 sequences were considered. The number of bacterial OTUs ranged from 234 to 513 and was, on average, 380 (Table S3). The soils from RMs showed a higher number of sequences (197,775; comprising 456 ± 70 OTUs) than *canga* (166,133; comprising 305 ± 75 OTUs), whereas the bacterial sequences from bulk soils (218,350; comprising 387 ± 107) were higher than that from rhizospheric soils (145,558; comprising 373 ± 107 OTUs) (Table S3). The Shannon and Simpson diversity indices were similar in all the samples' values in soils from RMs (Figure 1c,d). Venn diagrams of the bacterial sequences showed 159 core OTUs, being 15, 84, 43, and 55 unique OTUs in *canga*-B, RM-B, RM-R, and *canga*-R, respectively (Figure S1).

In *canga*, the analysis of the fungal sequences showed Ascomycota as the dominant taxa (Figure 2), whereas Acidobacteria and Proteobacteria were the dominant bacteria (Figure 3). Glomeromycota was also detected in bulk soils, with an RA higher in bulk soil samples (Table S4). At the genus level, different fungal taxa were more abundant in the rhizospheric (i.e., *Mycosphaerella*, *Scytalidium*, and *Talaromyces*) (Figure S2) or bulk soils (i.e., *Periconia*, *Curvularia*, and *Alternaria*) (Figure S2 and Table S4), but most sequences were not assigned to known taxa (Figure S2). Regarding the bacterial genera, *Bradyrhizobium* and *Rhodoplanes* were the most abundant in the rhizospheric and bulk soil samples (Figure S3 and Table S4).

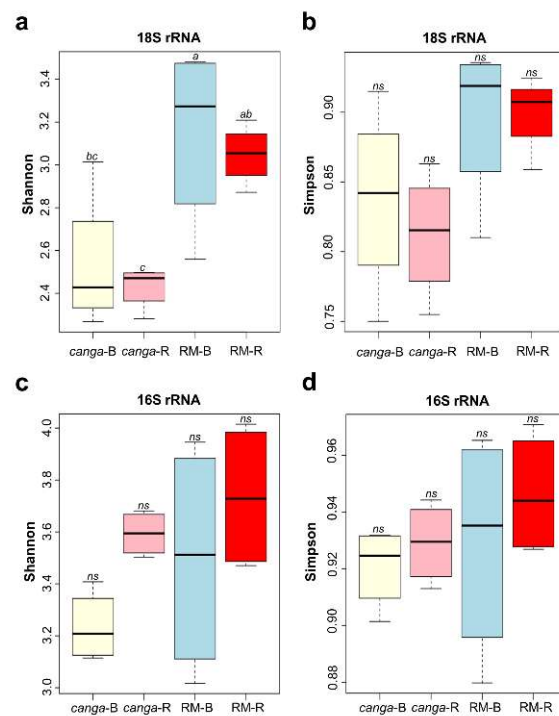


Figure 1. Shannon and Simpson diversity indices of fungal 18S rRNA (a,b) and bacterial 16S rRNA (c,d) sequences in rhizospheric (R) and bulk (B) soil samples associated with *Mimosa acutistipula* growing in a *canga* (*canga*) or rehabilitating mineland (RM) in Serra dos Carajás, eastern Amazon. Different lowercase letters indicate statistical differences ($p > 0.05$).

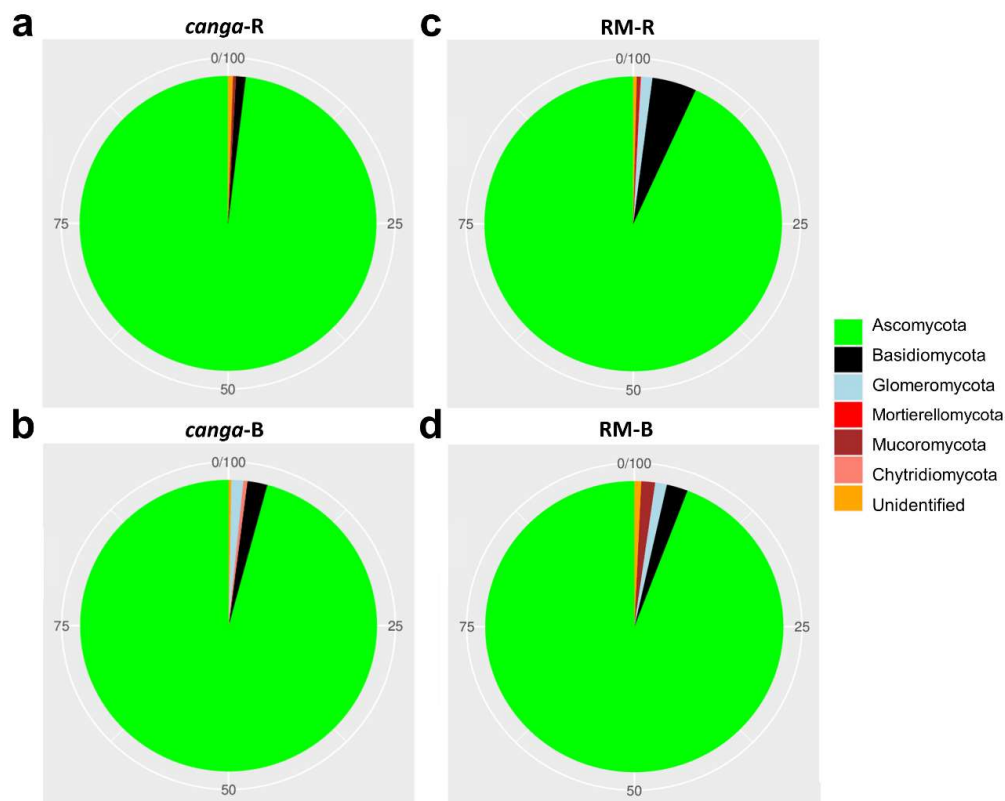


Figure 2. Relative abundance at the phylum level of major fungal 18S rRNA sequences associated with: (a) Rhizosphere (R) of *Mimosa acutistipula* growing in a *canga*; (b) bulk (B) soil of *M. acutistipula* growing in a *canga*; (c) rhizosphere of *M. acutistipula* growing in a rehabilitating mineland (RM); (d) bulk soil of *M. acutistipula* growing in a rehabilitating mineland in Serra dos Carajás, eastern Amazon ($n = 4$).

In the RM, analysis of the fungal sequences identified Ascomycota as the dominant taxa (Figure 2), whereas Proteobacteria and Acidobacteria were the dominant bacterial taxa (Figure 3). At the genus level, most sequences were not assigned to known taxa (Figure S2), followed by *Fusarium* and *Coniosporium* in the rhizospheric and bulk soils (Figure S2 and Table S4). Regarding the bacterial sequences, different genera were dominant in the rhizospheric (i.e., *Sphingomicrobium* and *Pseudomonas*) and bulk soils (i.e., *Serratia* and *Hyphomicrobium*) (Figure S3 and Table S4).

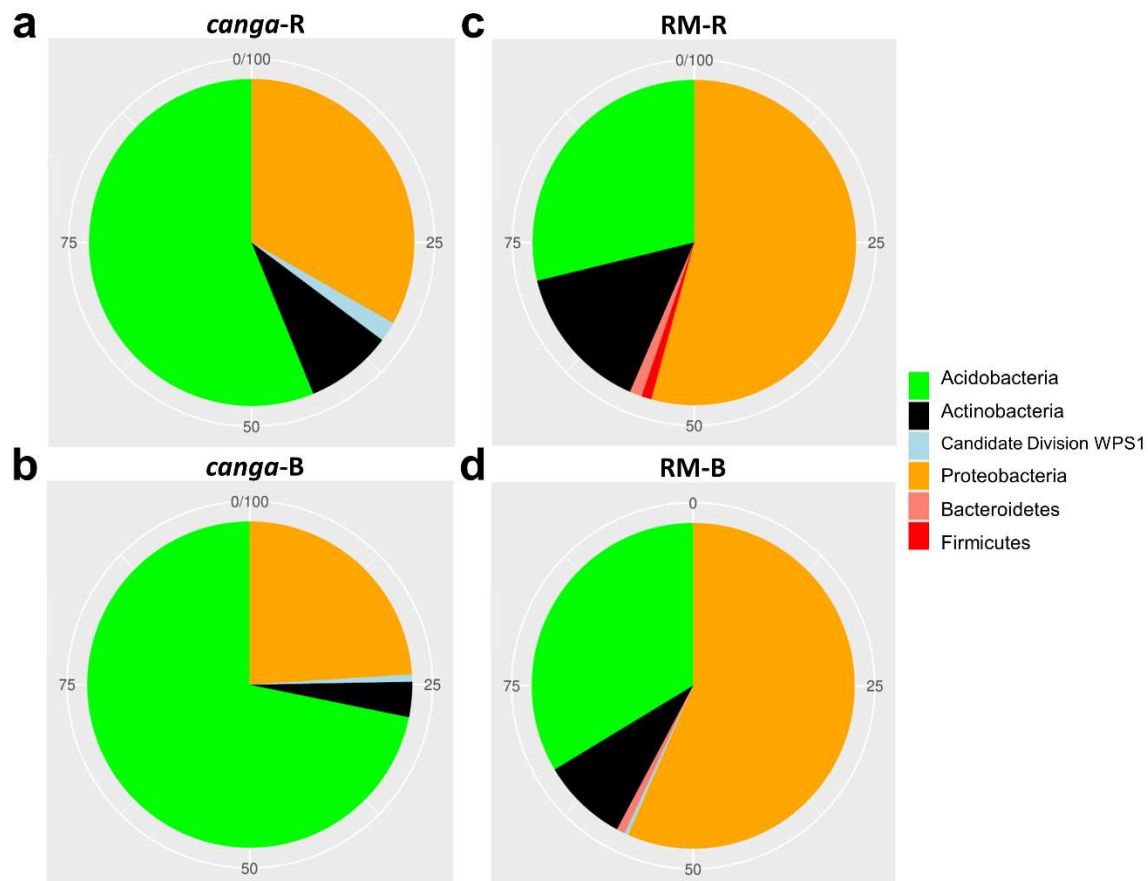


Figure 3. Relative abundance at the phylum level of major bacterial 16S rRNA sequences associated with: (a) Rhizosphere (R) of *Mimosa acutistipula* growing in a *canga*; (b) bulk (B) soil of *M. acutistipula* growing in a *canga*; (c) rhizosphere of *M. acutistipula* growing in a rehabilitating mineland (RM); (d) bulk soil of *M. acutistipula* growing in rehabilitating mineland in Serra dos Carajás, eastern Amazon ($n = 4$).

PCoA and cluster analyses of the microbial community structure among the different samples showed that bacterial (PERMANOVA: $p = 0.003$) and fungal (PERMANOVA: $p = 0.001$) communities were distinct between both sampling environments, but similar when comparing the bulk and rhizospheric soils at each sampling environment (Figure 4 and Figure S4).

Differences in the microbial communities' composition were estimated by calculating the LEfSe scores at the family and genus levels and were focused in the rhizosphere. A total of 24 distinct fungal taxa were identified in the rhizosphere of *M. acutistipula* established in a *canga* or RM. Most of these taxa were related to Ascomycota and Basidiomycota (Figure 5). A total of 26 preferential bacterial taxa were identified in *M. acutistipula* growing in a *canga* or RM, most of which were related to Proteobacteria and Actinobacteria (Figure 5).

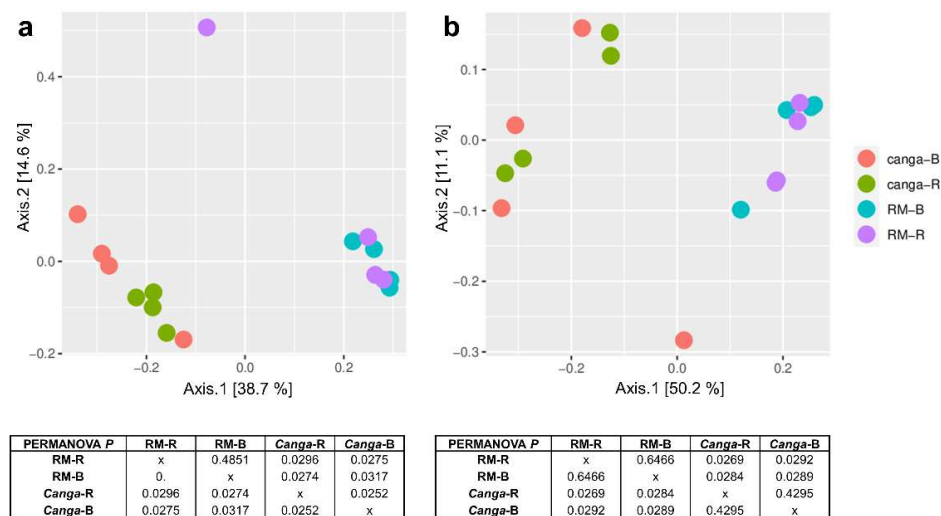


Figure 4. Principal coordinate analysis for cumulative sum scaling (CSS)-normalized counts of the fungal 18S rRNA (a) and bacterial 16S rRNA (b) sequences obtained from the rhizospheric (R) and bulk (B) soils samples of *Mimosa acutistipula* growing in a *canga* (*canga*) and rehabilitating mineland (RM) in Serra dos Carajás, eastern Amazon.

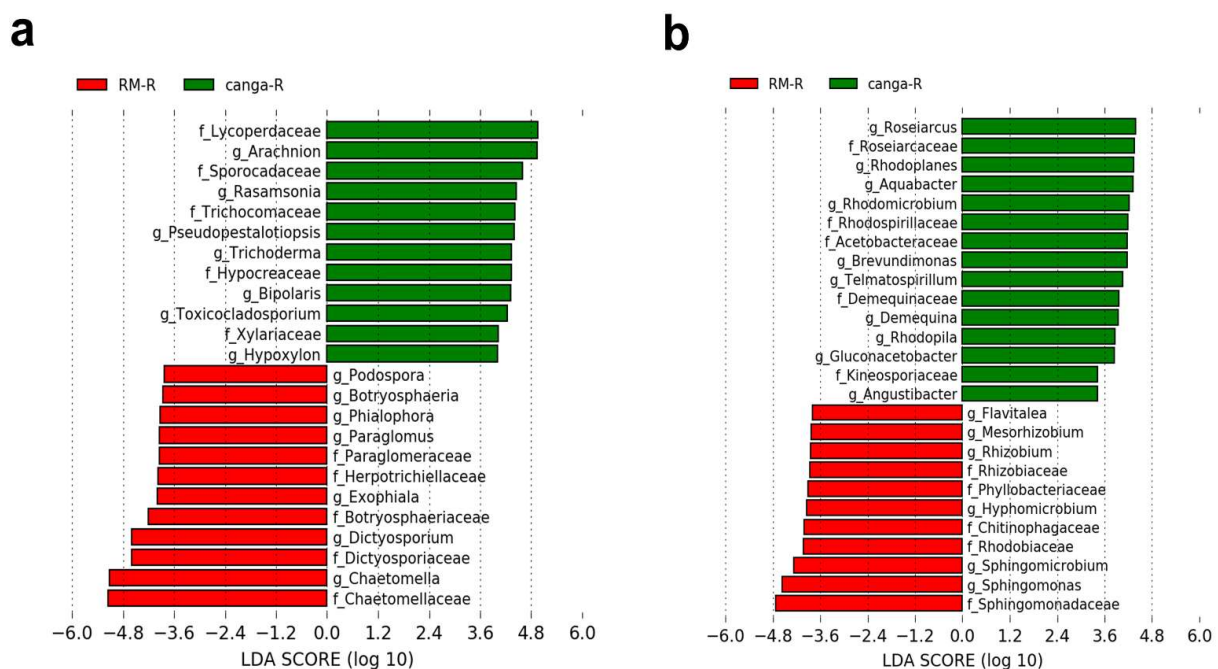


Figure 5. Linear discriminant analysis (LDA) of effect size (LEfSe) scores, identifying preferential taxa in the rhizosphere of *Mimosa acutistipula* in a *canga* (*canga*) or rehabilitating mineland (RM) in Serra dos Carajás, eastern Amazon: (a) Preferential fungal 18S rRNA; (b) preferential bacterial 16S rRNA sequences.

A total of eight trophic modes were predicted in the fungal OTUs, being the categories saprotroph (45.4%) and pathotroph_saprotroph (31.2%) most abundant in the soils from the RM, whereas pathotroph_symbiotroph (24.6%) was more abundant in the soils from the *canga* (Figure 6a). The category saprotroph_symbiotroph (2.6%) was exclusively identified in the soils from the *canga*, whereas symbiotroph was identified in the soils from both the *canga* (1.8%) and RM (1.5%) (Figure 6a). Similarly, 34 predicted functions were identified in the bacterial OTUs. Among them, chemoheterotrophy (7.79% and 10.2%), aerobic_chemoheterotrophy (7.65% and 10.1%), and nitrogen fixation (4.81% and 3.82%) were the most abundant predicted functions in the *canga* and RM, respectively. In the bulk soils, nitrate_ammonification (0.92% and 0.85%), nitrite_ammonification

(0.82% and 0.88%), and chitinolysis (0.84% and 0.87%) were present in the *canga* and RM, respectively (Figure 6b). The categories dark_oxidation_of_sulfur_compounds (2.78%) and aro-matic_compound_degradation (0.57%) were associated with the soils from the RM, whereas anoxygenic_photoautotrophy_S_oxidizing (1.71%) and dark_iron_oxidation (0.76%) were exclusive to the soils from the *canga* (Figure 6b).

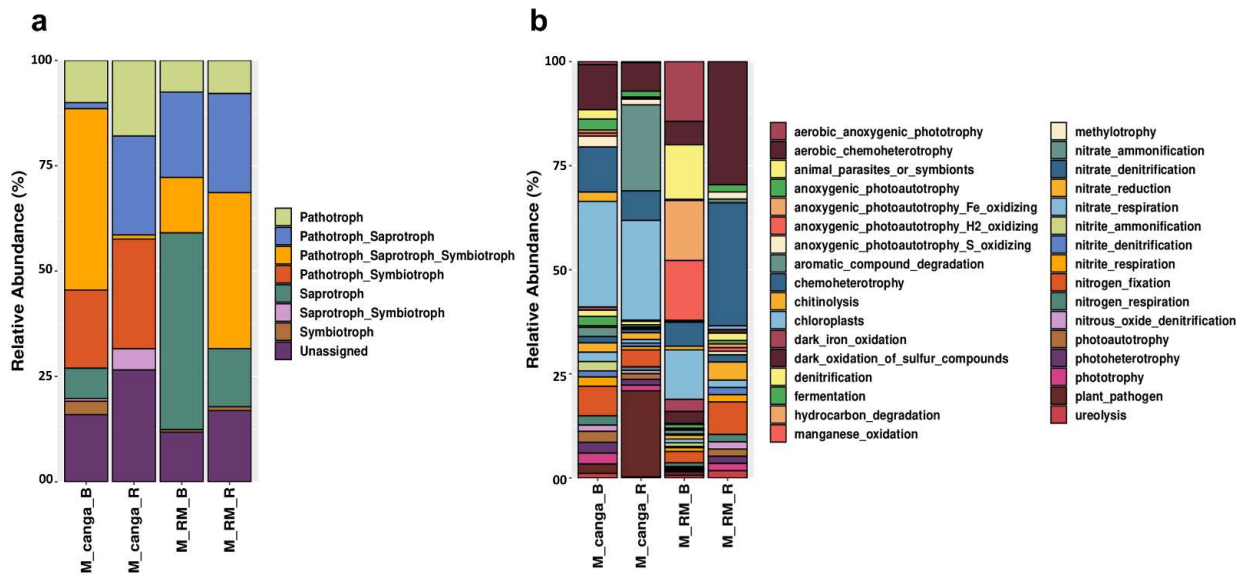


Figure 6. Functional analysis of the communities of 18S rRNA (a) and bacterial 16S rRNA (b) obtained from *Mimosa acutistipula* soils from plants growing in a *canga* ecosystem (*canga*) or a rehabilitating mineland (RM) in Serra dos Carajás, eastern Amazon.

Heatmaps showing the RA of potential beneficial taxa showed that they were distinct among the *canga* and RM for both the fungal and bacterial sequences (except for *Bradyrhizobium*) (Figure 7). The RDA analysis showed that soil characteristics influence the abundance of beneficial genera without a clear tendency among variables (Figure S5).

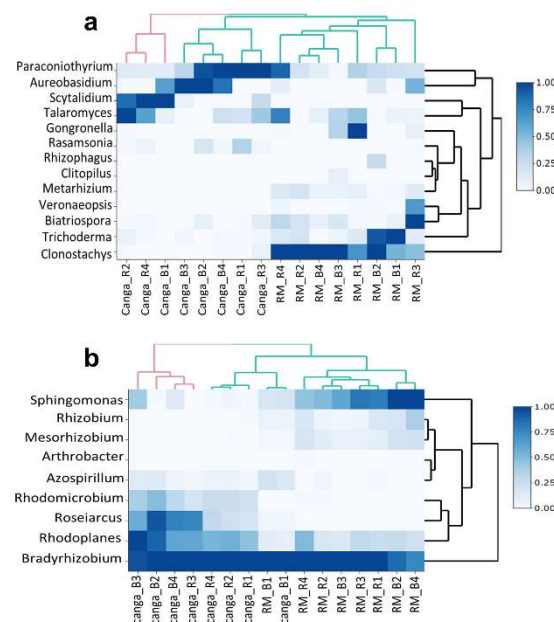


Figure 7. Heatmap showing the relative abundance between beneficial fungal 18S rRNA (a) and bacterial 16S rRNA (b) sequences in rhizospheric (R) and bulk (B) soil samples of *Mimosa acutistipula* growing in a *canga* (*canga*) or a rehabilitating mineland (RM) in Serra dos Carajás, eastern Amazon. Numbers after B and R refer to the soil replicate ($n = 4$).

4. Discussion

Rehabilitation of degraded areas affected by mining has become an urgent issue, especially in biodiversity hotspots such as the Amazon [30]. Few studies have analyzed the effects of mineland rehabilitation in the interaction between soil microbial populations and plants [31,32]. Therefore, this study described and characterized the rhizosphere-associated fungal and bacterial communities of *M. acutistipula* growing in a native *canga* ecosystem and RM, providing novel evidence about the microbial communities interacting with the plant during mineland rehabilitation.

Ascomycota and Proteobacteria were the most abundant fungal and bacterial phyla detected in both sampling ecosystems, namely, a *canga* and RM (Figures 2 and 3), which agrees with recent studies analyzing the microbial communities associated with plants growing in stressful ecosystems such as post-mining areas [33,34]. Several taxa belonging to such phyla were identified (i.e., *Scytalidium*, *Paraconiothyrium*, *Talaromyces*, *Rhizobium*, *Rhodomicrobium*, and *Mesorhizobium*) interacting with *M. acutistipula* in both soil substrates (Figures S2 and S3). However, specific beneficial taxa can also interact with *M. acutistipula* in a *canga* (i.e., *Rhodomicrobium* and *Roseiarcus*) or RM (i.e., *Sphingomonas* and *Mesorhizobium*) (Figure 7 and Table S4). Such microbial taxa are known for their role in essential microbe-mediated processes such as nutrient mineralization, biological control, and stress tolerance [35–37]—mechanisms that are key to sustaining growth in RMs.

The obtained diversity indices in the fungi and bacteria were similar between ecosystems (Figure 1). Nonetheless, the composition of the fungal and bacterial communities was different between the *canga* and RM (Figure 4). Such changes in microbial diversity in disturbed ecosystems underline a shift in the diversity of dominant microbial communities, as well as the loss of microbial taxa involved in specific processes such as nutrient cycling, biocontrol, and promotion of stress tolerance [38,39]. Despite the changes in the fungal and bacterial taxa (Figures 4 and 5), beneficial microorganisms were detected in the rhizosphere of *M. acutistipula* growing in the *canga* and RM (Figure 7). This difference in the composition of microbial communities is influenced by the low presence of plants colonizing RMs, which decreases the selectivity of microbial taxa and competition for resources [40], thus promoting the changes in microbial communities detected in RMs (Figures 2 and 7). As a *canga* is a severe ecosystem with high insolation rates, shallow and oxidic soils, intense dry and wet seasons, and low water holding capacity [41], the microbial diversity associated with native plants is also submitted to these stressful environmental conditions. Here, the presence of specific beneficial microorganisms in the rhizosphere of *M. acutistipula* can be related to beneficial functions to fit with these challenging environmental conditions, which is in line with recent studies analyzing the role of rhizospheric soil communities in the establishment of plants growing in metalliferous soils [42,43].

Microbial interactions in the plant rhizosphere are essential for growth in severe environments such as a *canga*. In the rhizosphere of *M. acutistipula* growing in both sampling points, bacterial taxa commonly associated with tolerance to metal stress were also identified, including members of the phyla Proteobacteria and Actinobacteria (i.e., *Sphingomonas* and *Actinotalea*) (Table S3) [44,45]. One of the main roles of these soil microorganisms is to contribute to the alleviation of metal stress in plants by the production of natural chelators, which decreases the bioavailability of metals [46], such as those detected in both soil substrates (Table S1). Additionally, the presence of nodule-forming bacteria in the rhizosphere of plants adapted to harmful environments is considered fundamental for symbiotic nitrogen fixation and plant growth in metal-polluted areas [47]. Thus, the preferential taxa involved in N mineralization in the rhizosphere of *M. acutistipula* (i.e., *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Rhodomicrobium*, and *Roseiarcus*) indicate a strong affinity with these microorganisms (Figure 7), which can contribute to the establishment of plants in RMs. These results are in line with those of Oliveira et al. [48], who detected several nodule-forming bacteria in soil samples from RMs after iron mining in the Quadrilátero Ferrífero region, southeast Brazil. Therefore, such bacterial taxa are vital for the adaptation of this species in *canga* ecosystems, as well as in RMs. Further studies must be performed

in order to identify the specificity of the beneficial taxa associated with vital structures such as nodules or healthy roots.

The study results showed several fungal taxa with beneficial roles for plants growing under severe environments, including dark septate endophytes (DSEs), AMFs, and free-living fungi. Fungal taxa belonging to DSEs (i.e., *Myceliophthora*, *Thielavia*, *Coniosporium*, and *Exophiala*) were detected with more abundance in soils associated with the RM (Figure 5 and Table S4). Fungi belonging to DSEs have been reported as common rhizosphere-associated fungi that promote abiotic stress tolerance in plants [42,49] in order to grow in stressful environments such as RMs. Similarly, the fungal phylum Glomeromycota was detected in the rhizosphere of plants growing in the RM (Table S4). This fungal phylum identified in the *canga* and RM includes AMFs, which can expand the root system, causing a greater part of the soil to be exploited when searching for resources [50]. As a consequence, plants improve their P uptake and are more resistant to abiotic stress [51]. In RMs, the presence of these microorganisms is fundamental due to the low level of organic matter in the soil after mining (Table S1). This is particularly important in oxidic soils such as those in a *canga*, where the high adsorption rates of P by iron and aluminum oxides limit their bioavailability (Table S1) [20]. Moreover, beneficial fungi belonging to the phyla Ascomycota and Basidiomycota were identified in the rhizosphere of the *canga* and/or RM (i.e., *Paraconiothyrium*, *Scytalidium*, and *Talaromyces*) (Figure 7), which include genera with known roles in the promotion of stress tolerance, enhancement of nutrient uptake, growth promotion, and biocontrol capabilities [52,53]. Therefore, rhizosphere-inhabiting fungi associated with *M. acutistipula* play crucial roles in the establishment at initial stages of mineland rehabilitation, but their benefits for *M. acutistipula* must be confirmed in further analyses.

As demonstrated in this study, *M. acutistipula* can associate without specificity with microbial taxa inhabiting the soil substrate (Figures 2 and 3). Plants with this behavior play critical roles in the rehabilitation of mining areas, participating in essential ecosystem services such as nutrient cycling, carbon storage, and symbiosis that can contribute to ecosystem rehabilitation [54]. However, other plant species need specific interaction with soil microbes for growth, being this dependence a bottleneck in the life cycle, affecting the long-term rehabilitation of an altered ecosystem [55,56]. Such specific interactions with soil microorganisms further limit plant establishment in the rehabilitated ecosystem, promoting invasive/exotic species colonization or affecting plant/microbe-related ecosystem services [57,58]. Therefore, a comprehensive management of soil substrates and their associated microbial communities can be further addressed in order to establish other native *canga* species in RMs.

M. acutistipula has been classified as a promising native plant for mineland rehabilitation in the eastern Amazon by their high drought stress tolerance and capability of hyperaccumulation of metals [59]. In fact, this study showed that *M. acutistipula* interacts with beneficial fungi and bacteria, independent of the sampling environment (Figure 7). However, despite beneficial soil microbes interacting with the *M. acutistipula* rhizosphere in RMs, the differences in rhizosphere-associated microorganisms with *canga* ecosystems hide key underground microbial-related processes that can alter mineland rehabilitation (Figure 6). The results agree with recent studies analyzing the performance of *M. acutistipula* in degraded minelands [54,59] and provide information about specific microbial taxa interacting with the *M. acutistipula* rhizosphere. An additional strategy to enhance mineland rehabilitation is the search for plants evolved in a severe *canga* ecosystem that have established non-specific interactions with soil microbes and plant them in future RMs in order to protect the ecosystem services associated with native *canga* ecosystems.

5. Conclusions

This study showed that non-specific interactions with rhizosphere microorganisms characterize the growth of *M. acutistipula* in a native *canga* ecosystem and RM in the eastern Amazon. Here, interaction with beneficial taxa independent of the soil substrate is one of

the mechanisms explaining why *M. acutistipula* is suitable for use in rehabilitation programs. Therefore, a plant's facility to recruit beneficial microorganisms from the soil substrate, especially those able to establish mycorrhizal or nitrogen fixation symbiosis, is key to selecting plants for further revegetation programs. Moreover, the presence of key microbial groups in soil substrates is crucial for the rehabilitation program.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/pr9112079/s1>, Figure S1: Venn diagram of soil fungal (A) and bacterial OTUs (B) in rhizospheric (R) or bulk (B) soil substrate samples of *Mimosa acutistipula* growing in a *canga* ecosystem (*canga*) or a rehabilitating mineland (RM) in Serra dos Carajás, eastern Amazon; Figure S2: Relative abundance at the genus level of major fungal 18S rRNA sequences obtained from *Mimosa acutistipula* growing in a *canga* ecosystem (*canga*) or a rehabilitating mineland (RM) in Serra dos Carajás, eastern Amazon; Figure S3: Relative abundance at the genus level of bacterial 16S rRNA sequences obtained from *Mimosa acutistipula* growing in a *canga* ecosystem (*canga*) or a rehabilitating mineland (RM) in Serra dos Carajás, eastern Amazon; Figure S4: Clustering of fungal 18S rRNA (a) and bacterial 16S rRNA (b) sequences in rhizospheric (R) or bulk (B) soil samples of *Mimosa acutistipula* (M) growing in a *canga* ecosystem (*canga*) or a rehabilitating mineland (RM) in Serra dos Carajás, eastern Amazon; Figure S5: Redundancy analysis (RDA) showing the influence of soil characteristics on the preferential beneficial taxa associated with *Mimosa acutistipula* growing in a *canga* (*canga*) (a) or a rehabilitating mineland (RM) (b) in Serra dos Carajás, eastern Amazon; Table S1: Physical and chemical characteristics of soil substrates associated with *Mimosa acutistipula* growing in a *canga* or rehabilitating mineland (RM); Table S2: Fungal 18S rRNA sequences obtained in rhizospheric and bulk soil samples from *Mimosa acutistipula* growing in a *canga* (*canga*) or a rehabilitating mineland (RM); Table S3: Bacterial 16S rRNA sequences obtained in rhizospheric and bulk soil samples from *Mimosa acutistipula* growing in a *canga* (*canga*) or rehabilitating mineland (RM); Table S4: Most abundant fungal and bacterial taxa identified in soils associated with *Mimosa acutistipula* growing in a *canga* or rehabilitating mineland (RM) in Serra dos Carajás, eastern Amazon.

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References

1. Salgado, A.A.R.; do Carmo, F.F. ‘Quadrilátero Ferrífero’: A Beautiful and Neglected Landscape between the Gold and Iron Ore Reservoirs. In *Landscapes and Landforms of Brazil*, 1st ed.; Carvalho, B., Rodrigues, A., Cordeiro, L., Eds.; Springer: Dordrecht, The Netherlands, 2015; Volume 1, pp. 319–330.
2. Santos, R.; Milanez, B. The Global Production Network for iron ore: Materiality, corporate strategies, and social contestation in Brazil. *Extr. Ind. Soc.* **2015**, *2*, 756–765. [[CrossRef](#)]

3. Gastauer, M.; Vera, M.P.O.; De Souza, K.P.; Pires, E.S.; Alves, R.; Caldeira, C.F.; Ramos, S.J.; Oliveira, G. A metagenomic survey of soil microbial communities along a rehabilitation chronosequence after iron ore mining. *Sci. Data* **2019**, *6*, 190008. [[CrossRef](#)] [[PubMed](#)]
4. Jacobi, C.M.; Carmo, F.F.; Vincent, R.C.; Stehmann, J. Plant communities on ironstone outcrops: A diverse and endangered Brazilian ecosystem. *Biodivers. Conserv.* **2007**, *16*, 2185–2200. [[CrossRef](#)]
5. Costa, W.F.; Ribeiro, M.; Saraiva, A.M.; Imperatriz-Fonseca, V.L.; Giannini, T. Bat diversity in Carajás National Forest (Eastern Amazon) and potential impacts on ecosystem services under climate change. *Biol. Conserv.* **2018**, *218*, 200–210. [[CrossRef](#)]
6. Artico, M.; Firpo, B.A.; Artico, L.L.; Tubino, R.M.C. Integrated use of sewage sludge and basalt mine waste as soil substitute for environmental restoration. *REM—Int. Eng. J.* **2020**, *73*, 225–232. [[CrossRef](#)]
7. Maiti, S.K.; Ahirwal, J. Ecological Restoration of Coal Mine Degraded Lands: Topsoil Management, Pedogenesis, Carbon Sequestration, and Mine Pit Limnology. In *Phytomanagement of Polluted Sites: Market Opportunities in Sustainable Phytoremediation*, 1st ed.; Pandey, V., Baudhdh, K., Eds.; Elsevier: Dhanbad, India, 2019; pp. 83–111.
8. André, J.C.; Alvarez, F.L.; Rivero, J.F.L. Caracterização dos impactos ambientais e sociais na exploração de rochas e minerais industriais no desenvolvimento local no município de Sumbe (Angola)/Characterization of environmental and social impacts. *Cadernos CIMEAC* **2019**, *9*, 210–237. [[CrossRef](#)]
9. Dong, L.; Tong, X.; Li, X.; Zhou, J.; Wang, S.; Liu, B. Some developments and new insights of environmental problems and deep mining strategy for cleaner production in mines. *J. Clean. Prod.* **2018**, *210*, 1562–1578. [[CrossRef](#)]
10. Rashid, M.I.; Mujawar, L.H.; Shahzad, T.; Almeelbi, T.; Ismail, I.M.; Oves, M. Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. *Microbiol. Res.* **2016**, *183*, 26–41. [[CrossRef](#)]
11. Buta, M.; Blaga, G.; Paulette, L.; Păcurar, I.; Roșca, S.; Borsai, O.; Grecu, F.; Sînziana, P.E.; Negrușier, C. Soil Reclamation of Abandoned Mine Lands by Revegetation in Northwestern Part of Transylvania: A 40-Year Retrospective Study. *Sustainability* **2019**, *11*, 3393. [[CrossRef](#)]
12. Berthelot, C.; Leyval, C.; Foulon, J.; Chalot, M.; Blaudez, D. Plant growth promotion, metabolite production and metal tolerance of dark septate endophytes isolated from metal-polluted poplar phytomanagement sites. *FEMS Microbiol. Ecol.* **2016**, *92*, fiw144. [[CrossRef](#)]
13. Farrar, K.; Bryant, D.; Cope-Selby, N. Understanding and engineering beneficial plant–microbe interactions: Plant growth promotion in energy crops. *Plant Biotechnol. J.* **2014**, *12*, 1193–1206. [[CrossRef](#)]
14. Gastauer, M.; Sarmiento, P.S.D.M.; Santos, V.C.A.; Caldeira, C.F.; Ramos, S.J.; Teodoro, G.S.; Siqueira, J.O. Vegetative functional traits guide plant species selection for initial mineland rehabilitation. *Ecol. Eng.* **2020**, *148*, 105763. [[CrossRef](#)]
15. Vincent, R.d.C.; Meguro, M. Influence of soil properties on the abundance of plant species in ferruginous rocky soils vegetation, southeastern Brazil. *Rev. Bras. Bot.* **2008**, *31*, 377–388. [[CrossRef](#)]
16. Carvalho, C.; Forester, B.R.; Mitre, S.K.; Alves, R.; Imperatriz-Fonseca, V.L.; Ramos, S.J.; Resende-Moreira, L.C.; Siqueira, J.O.; Trevelin, L.C.; Caldeira, C.F. Combining genotype, phenotype, and environmental data to delineate site-adjusted provenance strategies for ecological restoration. *Mol. Ecol. Resour.* **2021**, *21*, 44–58. [[CrossRef](#)] [[PubMed](#)]
17. Prasad, R.; Chhabra, S.; Gill, S.S.; Singh, P.K.; Tuteja, N. The microbial symbionts: Potential for crop improvement in changing environments. In *Advancement in Crop Improvement Techniques*; Woodhead Publishing: Sawston, UK, 2020; pp. 233–240. [[CrossRef](#)]
18. Jurburg, S.D.; Salles, J.F. Functional Redundancy and Ecosystem Function—The Soil Microbiota as a Case Study. In *Biodiversity in Ecosystems—Linking Structure and Function*, 1st ed.; Blanco, J., Lo, Y., Roy, S., Eds.; IntechOpen: London, UK, 2015; pp. 29–49.
19. Fink, J.R.; Inda, A.V.; Bayer, C.; Torrent, J.; Barrón, V. Mineralogy and phosphorus adsorption in soils of south and central-west Brazil under conventional and no-tillage systems. *Acta Sci. Agron.* **2014**, *36*, 379–387. [[CrossRef](#)]
20. Lehmann, J.; Cravo, M.d.S.; Vasconcelos de Macêdo, J.L. Phosphorus management for perennial crops in central Amazonian upland soils. *Plant Soil* **2001**, *237*, 309–319. [[CrossRef](#)]
21. Oliveira, R.R.; Silva, R.L.; Nunes, G.L.; Oliveira, G. PIMBA: A Pipeline for MetaBarcoding Analysis. *bioRxiv* **2021**. [[CrossRef](#)]
22. Zhang, J.; Kobert, K.; Flouri, T.; Stamatakis, A. PEAR: A fast and accurate Illumina Paired-End reAd merger. *Bioinformatics* **2013**, *30*, 614–620. [[CrossRef](#)]
23. Abarenkov, K.; Nilsson, H.; Larsson, K.; Alexander, I.J.; Eberhardt, U.; Erland, S.; Høiland, K.; Kjoller, R.; Larsson, E.; Pennanen, T.; et al. The UNITE database for molecular identification of fungi—Recent updates and future perspectives. *New Phytol.* **2010**, *186*, 281–285. [[CrossRef](#)] [[PubMed](#)]
24. Cole, J.R.; Wang, Q.; Fish, J.A.; Chai, B.; McGarrell, D.M.; Sun, Y.; Brown, C.T.; Porras-Alfaro, A.; Kuske, C.R.; Tiedje, J.M. Ribosomal Database Project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* **2013**, *42*, D633–D642. [[CrossRef](#)] [[PubMed](#)]
25. Liang, T.; Yang, G.; Ma, Y.; Yao, Q.; Ma, Y.; Ma, H.; Hu, Y.; Yang, Y.; Wang, S.; Pan, Y.; et al. Seasonal dynamics of microbial diversity in the rhizosphere of *Ulmus pumila* L. var. *sabulosa* in a steppe desert area of Northern China. *PeerJ* **2019**, *7*, e7526. [[CrossRef](#)]
26. Nguyen, N.H.; Song, Z.; Bates, S.T.; Branco, S.; Tedersoo, L.; Menke, J.; Schilling, J.S.; Kennedy, P.G. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **2016**, *20*, 241–248. [[CrossRef](#)]
27. Louca, S.; Parfrey, L.W.; Doebeli, M. Decoupling function and taxonomy in the global ocean microbiome. *Science* **2016**, *353*, 1272–1277. [[CrossRef](#)]

28. McMurdie, P.J.; Holmes, S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **2013**, *8*, e61217. [[CrossRef](#)]
29. Segata, N.; Izard, J.; Waldron, L.; Gevers, D.; Miropolsky, L.; Garrett, W.S.; Huttenhower, C. Metagenomic biomarker discovery and explanation. *Genome Biol.* **2011**, *12*, R60. [[CrossRef](#)] [[PubMed](#)]
30. Ribeiro, R.A.; Giannini, T.C.; Gastauer, M.; Awade, M.; Siqueira, J.O. Topsoil application during the rehabilitation of a manganese tailing dam increases plant taxonomic, phylogenetic and functional diversity. *J. Environ. Manag.* **2018**, *227*, 386–394. [[CrossRef](#)] [[PubMed](#)]
31. Rodríguez-Rodríguez, R.M.; Kemmelmeier, K.; Pedroso, D.D.F.; Pinto, F.A.; dos Santos, J.V.; Gastauer, M.; Caldeira, C.F.; Ramos, S.J.; Siqueira, J.O.; Carneiro, M.A.C. Native arbuscular mycorrhizal fungi respond to rehabilitation in iron ore mining areas from the Eastern Brazilian Amazon. *Pedobiologia* **2021**, *89*, 150768. [[CrossRef](#)]
32. Rodríguez-Rodríguez, R.M.; Guimarães, A.A.; de Castro, J.L.; Siqueira, J.O.; Carneiro, M.A.C.; Moreira, F.M.D.S. Rhizobia and endophytic bacteria isolated from rainforest fragments within an iron ore mining site of the Eastern Brazilian Amazon. *Braz. J. Microbiol.* **2021**, 1–14. [[CrossRef](#)] [[PubMed](#)]
33. Vieira, C.K.; Borges, L.G.D.A.; Marconatto, L.; Giongo, A.; Stürmer, S.L. Microbiome of a revegetated iron-mining site and pristine ecosystems from the Brazilian Cerrado. *Appl. Soil Ecol.* **2018**, *131*, 55–65. [[CrossRef](#)]
34. Deng, J.; Yin, Y.; Zhu, W.; Zhou, Y. Response of soil environment factors and microbial communities to phytoremediation with *Robinia pseudoacacia* in an open-cut magnesite mine. *Land Degrad. Dev.* **2020**, *31*, 2340–2355. [[CrossRef](#)]
35. Goh, Y.K.; Marzuki, N.F.; Pa, T.N.F.T.; Goh, T.-K.; Kee, Z.S.; Goh, Y.K.; Yusof, M.T.; Vujanovic, V.; Goh, K.J. Biocontrol and Plant-Growth-Promoting Traits of *Talaromyces apiculatus* and *Clonostachys rosea* Consortium against Ganoderma Basal Stem Rot Disease of Oil Palm. *Microorganisms* **2020**, *8*, 1138. [[CrossRef](#)]
36. Shu, W.; Pablo, G.P.; Jun, Y.; Danfeng, H. Abundance and diversity of nitrogen-fixing bacteria in rhizosphere and bulk paddy soil under different duration of organic management. *World J. Microbiol. Biotechnol.* **2011**, *28*, 493–503. [[CrossRef](#)]
37. Lladó, S.; López-Mondéjar, R.; Baldrian, P. Forest Soil Bacteria: Diversity, Involvement in Ecosystem Processes, and Response to Global Change. *Microbiol. Mol. Biol. Rev.* **2017**, *81*, e00063-16. [[CrossRef](#)] [[PubMed](#)]
38. Masse, J.; Prescott, C.E.; Renaut, S.; Terrat, Y.; Grayston, S.J. Plant Community and Nitrogen Deposition as Drivers of Alpha and Beta Diversities of Prokaryotes in Reconstructed Oil Sand Soils and Natural Boreal Forest Soils. *Appl. Environ. Microbiol.* **2017**, *83*, e03319-16. [[CrossRef](#)]
39. Chen, Y.-J.; Leung, P.M.; Wood, J.L.; Bay, S.K.; Hugenholtz, P.; Kessler, A.J.; Shelley, G.; Waite, D.W.; Franks, A.E.; Cook, P.L.M.; et al. Metabolic flexibility allows bacterial habitat generalists to become dominant in a frequently disturbed ecosystem. *ISME J.* **2021**, *15*, 2986–3004. [[CrossRef](#)]
40. Kuz'yakov, Y.; Xu, X. Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytol.* **2013**, *198*, 656–669. [[CrossRef](#)] [[PubMed](#)]
41. Skiryicz, A.; Castilho, A.; Chaparro, C.; Carvalho, N.; Tzotzos, G.; Siqueira, J.O. Canga biodiversity, A matter of mining. *Front. Plant Sci.* **2014**, *5*, 1–10. [[CrossRef](#)]
42. Xu, R.; Li, T.; Cui, H.; Wang, J.; Yu, X.; Ding, Y.; Wang, C.; Yang, Z.; Zhao, Z. Diversity and characterization of Cd-tolerant dark septate endophytes (DSEs) associated with the roots of *Nepal alder* (*Alnus nepalensis*) in a metal mine tailing of southwest China. *Appl. Soil Ecol.* **2015**, *93*, 11–18. [[CrossRef](#)]
43. Hou, J.; Liu, W.; Wu, L.; Ge, Y.; Hu, P.; Li, Z.; Christie, P. Rhodococcus sp. NSX2 modulates the phytoremediation efficiency of a trace metal-contaminated soil by reshaping the rhizosphere microbiome. *Appl. Soil Ecol.* **2018**, *133*, 62–69. [[CrossRef](#)]
44. Bilal, S.; Khan, A.L.; Shahzad, R.; Kim, Y.-H.; Imran, M.; Khan, M.J.; Al-Harrasi, A.; Kim, T.H.; Lee, I.-J. Mechanisms of Cr(VI) resistance by endophytic *Sphingomonas* sp. LK11 and its Cr(VI) phytotoxic mitigating effects in soybean (*Glycine max* L.). *Ecotoxicol. Environ. Saf.* **2018**, *164*, 648–658. [[CrossRef](#)]
45. Li, Y.; Chen, F.; Dong, K.; Wang, G. *Actinotalea ferrariae* sp. nov., isolated from an iron mine, and emended description of the genus *Actinotalea*. *Int. J. Syst. Evol. Microbiol.* **2013**, *63*, 3398–3403. [[CrossRef](#)]
46. Seneviratne, M.; Seneviratne, G.; Madawala, H.; Vithanage, M. Role of Rhizospheric Microbes in Heavy Metal Uptake by Plants. In *Agro-Environmental Sustainability*, 1st ed.; Singh, J., Seneviratne, G., Eds.; Springer: Singapore, 2017; pp. 147–163.
47. Checcucci, A.; Bazzicalupo, M.; Mengoni, A. Exploiting Nitrogen-Fixing Rhizobial Symbionts Genetic Resources for Improving Phytoremediation of Contaminated Soils. In *Enhancing Cleanup of Environmental Pollutants*, 1st ed.; Anjum, N., Singh, S., Tuteja, N., Eds.; Springer: Fiorentino, Italy, 2017; pp. 275–288.
48. Oliveira, A.; Azarias Guimarães, A.; da Costa, A.M.; Louzada Rodrigues, T.; de Soares Carvalho, T.; Reis Sales, F.; de Souza Moreira, F.M. Plant growth-promoting rhizobacterial communities from an area under the influence of iron mining and from the adjacent phytophysionomies which have high genetic diversity. *Land Degrad. Dev.* **2020**, *31*, 2237–2254. [[CrossRef](#)]
49. Ortiz, J.; Soto, J.; Fuentes, A.; Herrera, H.; Meneses, C.; Arriagada, C. The Endophytic Fungus *Chaetomium cupreum* Regulates Expression of Genes Involved in the Tolerance to Metals and Plant Growth Promotion in *Eucalyptus globulus* Roots. *Microorganisms* **2019**, *7*, 490. [[CrossRef](#)] [[PubMed](#)]
50. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*, 3rd ed.; Academic Press: New York, NY, USA, 2010.
51. Tekaya, M.; Mechri, B.; Mbarki, N.; Cheheb, H.; Hammami, M.; Attia, F. Arbuscular mycorrhizal fungus *Rhizophagus irregularis* influences key physiological parameters of olive trees (*Olea europaea* L.) and mineral nutrient profile. *Photosynthetica* **2017**, *55*, 308–316. [[CrossRef](#)]

52. Li, X.; He, X.; Hou, L.; Ren, Y.; Wang, S.; Su, F. Dark septate endophytes isolated from a xerophyte plant promote the growth of *Ammopiptanthus mongolicus* under drought condition. *Sci. Rep.* **2018**, *8*, 1–11. [[CrossRef](#)] [[PubMed](#)]
53. Naraghi, L.; Heydari, A.; Rezaee, S.; Razavi, M. Biocontrol Agent *Talaromyces flavus* Stimulates the Growth of Cotton and Potato. *J. Plant Growth Regul.* **2012**, *31*, 471–477. [[CrossRef](#)]
54. Ramos, S.J.; Gastauer, M.; Mitre, S.K.; Caldeira, C.F.; Silva, J.R.; Neto, A.E.F.; Oliveira, G.; Filho, P.W.M.S.; Siqueira, J.O. Plant growth and nutrient use efficiency of two native Fabaceae species for mineland revegetation in the eastern Amazon. *J. For. Res.* **2019**, *31*, 2287–2293. [[CrossRef](#)]
55. Burghardt, L.T. Evolving together, evolving apart: Measuring the fitness of rhizobial bacteria in and out of symbiosis with leguminous plants. *New Phytol.* **2019**, *228*. [[CrossRef](#)] [[PubMed](#)]
56. Meng, Y.-Y.; Fan, X.-L.; Zhou, L.-R.; Shao, S.-C.; Liu, Q.; Selosse, M.-A.; Gao, J.-Y. Symbiotic fungi undergo a taxonomic and functional bottleneck during orchid seeds germination: A case study on *Dendrobium moniliforme*. *Symbiosis* **2019**, *79*, 205–212. [[CrossRef](#)]
57. Zhou, Z.; Wang, C.; Jiang, L.; Luo, Y. Trends in soil microbial communities during secondary succession. *Soil Biol. Biochem.* **2017**, *115*, 92–99. [[CrossRef](#)]
58. Barfknecht, D.F.; Li, G.; Martinez, K.A.; Gibson, D.J. Interactive disturbances drive community composition, heterogeneity, and the niches of invasive exotic plant species during secondary succession. *Plant Ecol. Divers.* **2021**, 1–13. [[CrossRef](#)]
59. Silva, J.R.; Gastauer, M.; Ramos, S.J.; Mitre, S.K.; Neto, A.E.F.; Siqueira, J.O.; Caldeira, C.F. Initial growth of Fabaceae species: Combined effects of topsoil and fertilizer application for mineland revegetation. *Flora* **2018**, *246–247*, 109–117. [[CrossRef](#)]