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Article

Rhizosphere-Associated Microbiome Profile of Agriculture Reclaimed Lands in Egypt

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Abstract: Plants especially in their natural habitat are considered part of a rich ecosystem that includes many various microorganisms in the soil. The current study aimed to identify the bacterial profile of agriculture-related soil samples using the metabarcoding technique to compare and explore relevant rhizosphere bacteria associated with plant cultivations in newly reclaimed land and habitual cultivated ones. Total environmental DNA was extracted from rhizosphere and non-cultivated samples derived from three land types in Egypt. The bacterial 16S rDNA was amplified and sequenced by NGS technology to profile each sample. The microbial profile was characterized by statistical and literature-based methods. Among all samples, the most identified phyla were Actinobacteriota (28%), followed by Proteobacteriota (26%), Firmicutes (14%), Acidobacteriota and Chloroflexi (7%), Gemmatimonadota (5%), Bacteriodota and Crenarchaeota (3%), and Myxococcota (2%), in addition to 37 other phyla with <1% counts. A total of 74 OTU was unique to the plant rhizosphere area and classified as Bacteriodota (5.1%:0.3%), Firmicutes (2.4%:0.1%), and Proteobacteria (3.5%:2%) phyla in agriculture and reclaimed lands, respectively. Moreover, the rhizosphere profile included a large portion of uncultured and unidentified bacterial species, which opened a window to further analysis. Our analysis provides a key Knowledge about the rhizosphere microbiome and highlights its possible use to create microbial-based biofertilizers targeting plant performance in contrast to traditional fertilizers and their side effect on the agriculture sector.

Keywords: Microbiome; Rhizosphere; Metabarcoding; 16S rDNA; Agriculture lands; Reclaimed lands

1. Introduction

Plants especially in their natural habitat are considered part of a rich ecosystem that includes many various microorganisms in the soil [1], in addition to microbes' that are functioning as a community that forms ecological niches [2]. Soil is the ultimate reservoir of culturable and nonculturable microorganisms and provides different environments and nutrients for their survival [3], where microbial populations are instrumental to fundamental processes that drive stability and productivity of agroecosystems [4]. Soil microorganisms (i.e., bacteria, fungi, actinomycetes, and total microorganisms), help in the safe growth of plants in addition to microbes simultaneously [3]. And are correlated with soil physicochemical properties (soil pH, soil moisture, soil temperature, soil carbon, and nitrogen contents) [5]. Microorganisms play a dichotomous role in the soil nitrogen cycle through mineralization and immobilization, therefore contributing to the maintenance of soil fertility and mitigation of global warming [eg., 5]. They catalyze various biochemical processes (decomposition, nutrient turnover, degradation of pesticides, and toxic metabolites of the soil) and can be used to assess soil quality and health [eg., 3]. In the last few years, great progress has been made in the knowledge of the composition of rhizosphere microbiomes and their dynamics [1]. The rhizosphere microbiome plays a key role in plant nutrient provision [6]. Rhizosphere microorganisms offer to host plants the essential as-

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similable nutrients, stimulate the growth and development of host plants, and induce antibiotics production [7]. Recent advances in microbe–plant interactions research revealed that plants have the ability to shape their rhizosphere microbiome, as proved by the fact that specific microbial communities were hosted in different plant species even when they grow on the same soil [8], and the growth of rhizosphere micro-organisms is regulated by the phytoproducts excreted from plant roots, this excretion of phytochemicals alters the chemistry of rhizosphere soil, and also commands the fate of linked organisms and vice versa [2].

Beneficial microbes in the microbiome of plant roots improve plant health. Induced systemic resistance (ISR) emerges as an important appliance by which selected plant growth-promoting bacteria (PGPB) in the rhizosphere stimuli the plant for improved defense versus a wide range of insect herbivores and pathogens, for example, two genera, Azospirillum and Azotobacter, were found in virtually in abundance in soil [eg., 9]. A wide variety of root-associated mutualists, including Pseudomonas, Bacillus, Trichoderma, and Mycorrhiza species sensitize the plant immune system to enhance defense without directly activating costly defenses [9].

Using standard methods for DNA extraction often results in poor quality and low yield making them inappropriate for the analysis of community through polymerase chain reaction (PCR) because of the presence of humic substances and the formation of chimeric products with smaller template DNAs [10], so the requirement for development of metagenomic information of provincially important crops, their plant interactions with microorganisms and agricultural performs for narrowing down important information from huge databases have been recommended. The role of functional and taxonomical diversity of soil microorganisms in understanding soil repression and the portion played by the microbial metabolites in the process has been evaluated and discussed in the context of the 'omics' approach, 'Omics' studies have discovered significant data about microbial variety, their responses to numerous biotic and abiotic stimuli, in addition to the physiology of disease repression [11]. Currently, NGS methods such as 16S rDNA/rRNA metagenomics or amplicon sequencing are intended for the taxonomic profiling of the soil microbial communities. Although 16S rDNA/rRNA NGS-based microbial data are not suited for the analysis of the functional potential of the recognized operational taxonomic units as compared to shotgun metagenomics, developments in nowadays the bioinformatics discipline allow the performance of such studies [12]. Advances in next-generation sequencing (NGS) platform, gene editing technologies, metagenomics, and bioinformatics approaches allow us to unravel the entangled webs of interactions of holobionts and core microbiomes for efficiently deploying the microbiome to increase crops nutrient acquisition and resistance to abiotic and biotic stress [13], in addition to the microbial diversity analysis from these environments will help identify new microorganisms having specificity for unique applications [14].

The current study aimed to identify the bacterial profile of agriculture-related soil samples using the metabarcoding technique to compare and explore relevant rhizosphere bacteria associated with plant cultivations in newly reclaimed land and habitual cultivated ones.

2. Materials and Methods

2.1 Sampling locations

Field sampling used in this study was conducted during February 2021. Fifteen soil samples were collected from three different locations in Egypt namely, agricultural land in Qalubiya governorate (30° 7' 42 '' N and 31° 14' 32'' E), horticulture farm on Cairo-Alexandria Road (31° 12' 20'' N and 29° 55' 28'' E), and a desert land (29° 20' 32'' N and 25° 5' 19'' E). Plants with the same growth status were randomly selected from similar plots. For each plant species, a soil sample was taken in the root rhizosphere area (i.e., soils adjacent

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to the plant roots at ~ 5 cm depth), and the area between two plants of the same type. The soil of the same site was mixed to obtain a composite sample from the same location. Triplicate composite samples were collected for each plot, and the replicates were approximately 2 m apart from each other (Table 1; Fig. 1).

Table 1. A list of the soil sample collection describing the lands type, plantation, and the area for each sample, samples are identified by a specific sample code.

Land type	Plantation Type	Sample Type	Sample Code
Agriculture Lands (A)	Citrus trees	Rhizosphere area	AP1
		Uncultivated area	AC1
	Olives trees	Rhizosphere area	AP2
		Uncultivated area	AC2
	Fava beans	Rhizosphere area	AP3
		Uncultivated area	AC3
Reclaimed Lands (R)	Apricot trees	Rhizosphere area	RP1
		Uncultivated area	RC1
	Pear trees	Rhizosphere area	RP2
		Uncultivated area	RC2
	Eggplants	Rhizosphere area	RP3
		Uncultivated area	RC3
Desert Lands (D)	No plantations	Uncultivated area	DC1
		Uncultivated area	DC2
		Uncultivated area	DC3

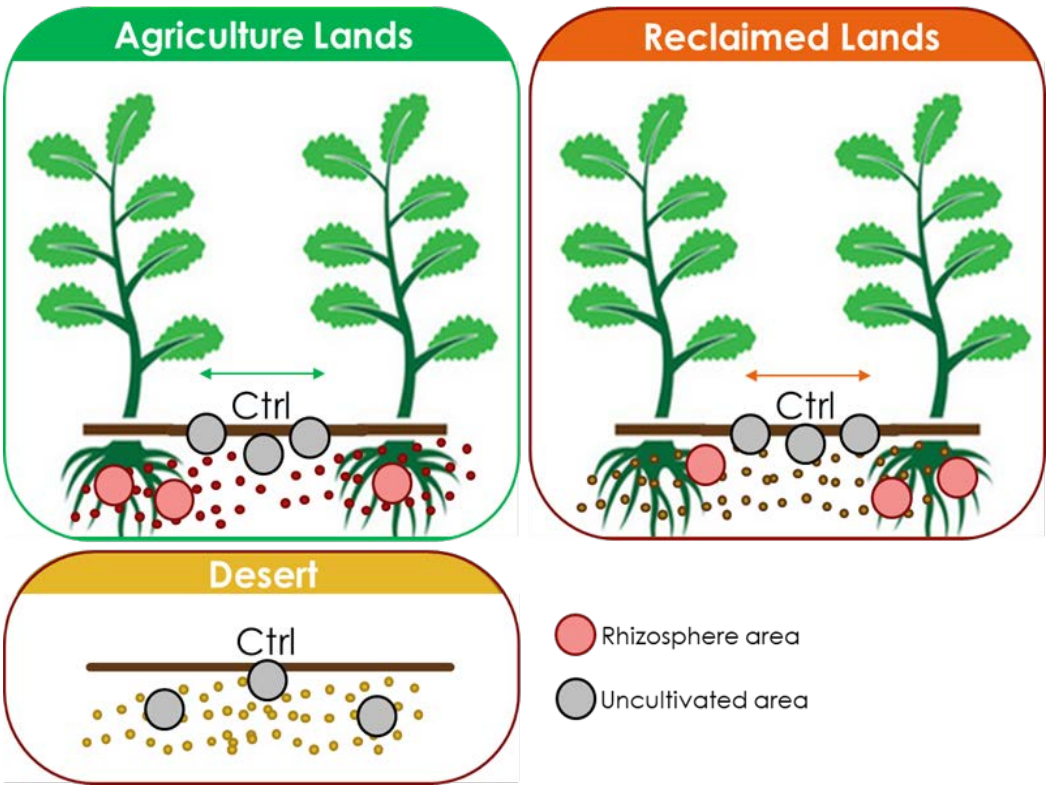

Figure 1. A schematic representation of the sampling process from three land types (Agriculture, Reclaimed, and Desert), at each land type samples around plant roots (i.e., Rhizosphere) and from the uncultivated area at the same depth were collected in triplicates.

2.2. Sampling technique & soil physico-chemical characterization

Samples of soil were taken with a sterile spatula into Falcon™ 50 mL sterile plastic tubes from the surface layer (0–15 cm depth), labeled with a site code, and kept at room temperature until examination. The samples were divided into two subsamples: one was air-dried and then stored at 25 °C to determine the chemical properties, and the other was stored at −80 °C for DNA extraction. The underlying soil physical-chemical properties, including soil texture [15], pH [16], and organic matter [17], were done using the standard laboratory protocols.

2.2. DNA extraction

Genomic DNA was extracted from soil microorganisms via chemical and mechanical lysis using the Power Soil MoBio DNA Isolation Kit (MO BIO Laboratories Inc., CA, USA), according to the manufacturer's instructions, with a final elution volume of 100 ml. Subsequently, the DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined with Quantus™ Fluorometer (Promega, USA). Extracted DNA was stored at -20 °C until required for PCR.

2.3. 16S rRNA amplicon-based sequencing

The bacterial communities in the soil were assessed by sequencing amplicons of the V3–V4 variable region of the 16S rRNA gene, with primer pair 338F (5'-ACT CCT ACG GG AGG CAG CAG-3') and 806R (5'- 489 GGA CTA CHV GGG TWT CTA AT-3'; Ref). The PCR reaction was performed using TransStart FastPfu DNA Polymerase mixture. The reaction mixture (20 μL) was composed of 4 μl of 5x FastPfu Buffer, 2 μl of 2.5 mM (each) dNTPs, 0.8 μl of 5 μM Bar-PCR primer F, 0.8 μl of 5 μM primer R, 0.4 μl of FastPfu polymerase, 0.2 μl of BSA and 10 ng of template DNA. Amplification conditions for PCR was as follows: 3 min at 98 °C to denature the DNA, followed by 27 cycles of denaturation at 98 °C for 10 sec, primer annealing at 60 °C for 30 sec, and strand extension at 72 °C 45 s, followed by 7 min at 72 °C on an ABI GeneAmp 9700 thermocycler (IET, USA). Electrophoresis on a 2% agarose gel was used to check the quality of the PCR products and purified using Agencourt AMPure XP beads (Beckman, USA). The pooled DNA product was used to construct an Illumina Pair-End library followed by Illumina-adapter ligation and sequencing by Illumina (MiSeq, PE 2 x 300 bp mode), following the manufacturer's instructions.

2.4. Metabarcode data processing and analysis

Paired-end data were demultiplexed into each sample based on the index sequences downloaded from the Illumina MiSeq platform. Hence, paired-end sequences of each sample were trimmed based on their quality and length using Trimmomatic [18] and FLASH [19] software with the following criteria: (I) reads were trimmed at any site that obtained an average quality score of <20 over a 50-bp sliding window, and the truncated reads shorter than 50 bp were discarded; (II) reads with any mismatch in the barcode, more than two nucleotide mismatches in the primer or containing ambiguous characters were removed; and (III) only sequences that overlapped by more than 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded. The metabarcoding analysis was performed using the online Majorbio Cloud Platform (http://en.majorbio.com/). *De novo* and reference-based chimera detection and removal were performed using Uparse V7.1 (http://drive5.com/uparse/). Richness inference and library comparisons were performed using the Mothur v.1.9.0 software [20]. Alignments were performed by Mothur using the SILVA bacteria database, with OUT sequence similarity of 0.97. The taxonomy of each 16S rRNA gene sequence was analyzed by the RDP classifier algorithm (http://rdp.cme.msu.edu/) against the Silva 16S rRNA database (Release138 http://www.arb-silva.de) using a confidence threshold of 70%. The microbiome shared by the different microbial samples was compared and visualized through a Venn diagram plot (R package; https://github.com/vegandevs/vegan). Circos

plots showing microbial structures were performed in Circos -0.67 (http://circos.ca/). A polygenetic tree was generated by FastTree package (V2.1.3, http://www.microbesonline.org/fasttree/). PICRUSt (http://huttenhower.sph.harvard.edu/galaxy/) and FUNGuild (http://www.funguild.org/) were employed to decipher microbial communities and functions. A heatmap plot was constructed to visualize the functional feature abundance profiles using Orange V3.24.1 (https://orange.biolab.si/).

3. Results

3.1. Species composition analysis

3.1.1. Taxonomical representation

Complete diversity analysis for 15 soil samples yielded a total of 1,062,960 optimized sequences (i.e., 450,976,362 bases) with an average sequence length of 424 ± 15 bp. A total of 801,936 sequences were valid, and 5,490 OTU were classified and sorted in 46 phylum, 131 classes, 322 orders, 535 families, 1015 genera, and 1935 species quantified by 801,936 total counts across all samples. The average Shannon index for replicates per location was applied to estimate the detected diversity within each sample (i.e., alpha diversity). The index ranged between 0.61 to 6.08, where the highest index was recorded for the rhizosphere area sampled from the agricultural lands, in contrast to the uncultivated area sampled from the reclaimed lands.

Among all samples, the most identified phyla were Actinobacteriota (28%), followed by Proteobacteriota (26%), Firmicutes (14%), Acidobacteriota and Chloroflexi (7%), Gemmatimonadota (5%), Bacteriodota and Crenarchaeota (3%), and Myxococcota (2%), in addition to 37 other phyla with <1% counts. At the family level, the most represented families were Bacillaceae, Nitrosphaeraceae, Nocardiaceae, Sphingomonadaceae, Burkholderiaceae, Rhizobiaceae, Psuedonocardiacea, Gemmatimonadaceae, and Vicinamibacteriaceae, in addition to 33 other families with counts <1% in at least one of the sampled areas, and 493 families with counts <1% in all samples. Regardless of the land type or the sampled area, the family Bacillaceae was the most presented family among all with almost an equal distribution among different categories (land or sample types), followed by the families Nocardioidaceae, Sphingomonadaceae that were highly presented in agriculture, and reclaimed lands, specifically more in the rhizosphere than the uncultivated area. The families Burkholderiaceae and Nitrosphaeraceae were highly presented in a desert land and subsequently ranked lower than the other families when the sample area was studied. However, the presented species of this family were presented in the uncultivated area more than the rhizosphere area (Fig. 1).

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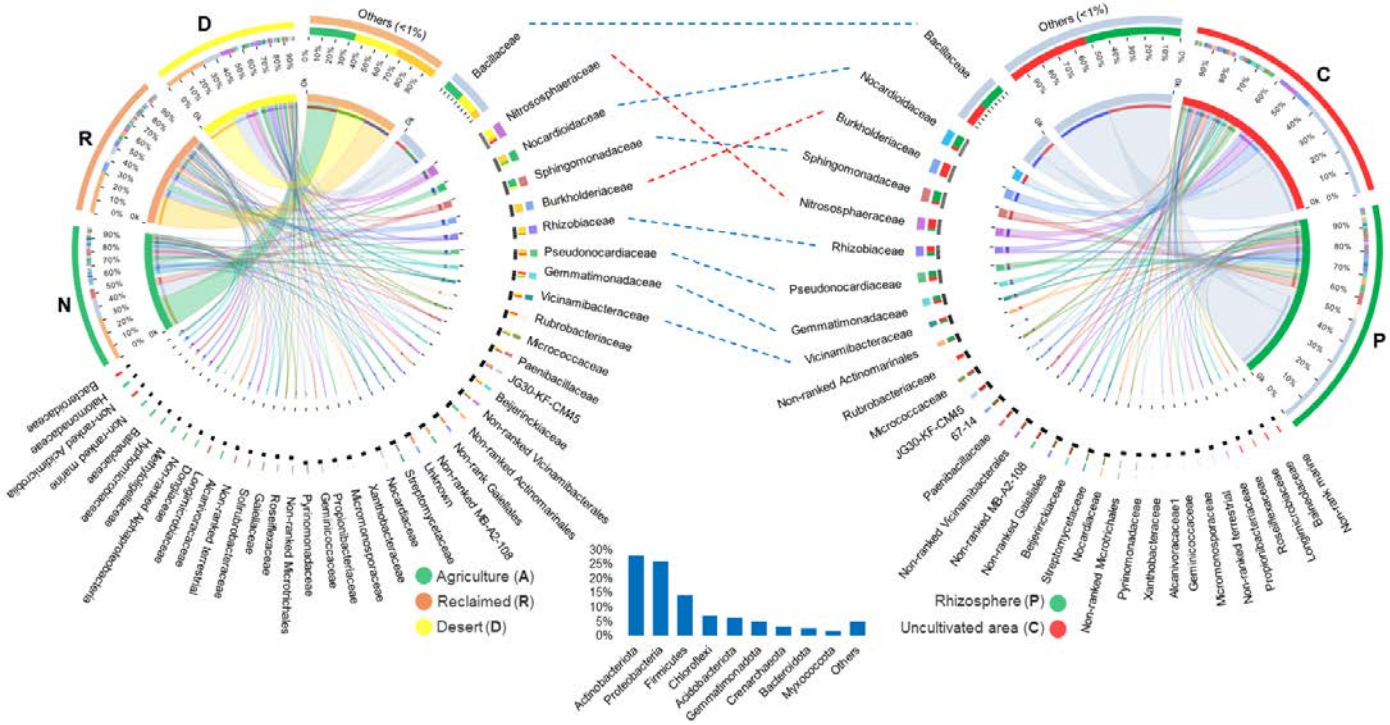

Figure 1. Comparative circos plot showing the two contrasting comparisons (land types on left and sample types on right). Below is a histogram of the presented phyla in the analyzed samples.

3.1.2. OTUs inhabiting rhizosphere areas

The total OTUs counts was 4033, 3575, and 3327 for agriculture, reclaimed, and desert lands, respectively. A total of 1984 OTUs co-occurred among the three types of lands regardless of the sampling area, while 779, 1055, and 159 were unique OTUs for agriculture, reclaimed and desert lands, respectively. The desert lands shared 977 and 243 for agriculture and reclaimed lands separately. All the unique and shared OTUs between desert lands and the other land types were discarded, except for the common shared OTUs that were subject to statistical refining along with the 329 shared OTUs between agriculture and reclaimed lands (Fig. 2).

Based on the t-test analysis, on one hand, the common shared OTUs among the three land types revealed significant differences in seven microbial phyla, the significant direction was indicated by the positive sign "+" when the phyla are significantly presented in cultivated lands (agriculture and/or reclaimed) *vice versa*. In details, two phyla (Acidobacteriota and Actinobacterioda) contained two groups per each where the assigned species were significantly presented in deserted lands versus the cultivated ones. Besides the phylum Myxoccota, the other phyla were significantly presented in both cultivated lands, namely are ranked and non-ranked Chloroflexi, non-ranked Gemmatimonadota, and Proteobacteria (Fig. 2). On the other hand, the shared OTUs between agriculture and reclaimed lands revealed significant differences in nine phyla and a non-ranked bacteria group, the significant direction was indicated by the positive sign "+" when the phyla are significantly presented in the rhizosphere area and *vice versa*. In details, Actinobacteria, Firmicutes, non-ranked Acidobacteriota, non-ranked Chloroflexi, non-ranked Myxococcota, Placentomycetota, and Proteobacteria, in contrast to non-ranked Gemmatimonadota and Verrucomicrobiota. By comparing both significant profiles, the negatively significant Actinobacteriota and Acidobacteriota were not present in the rhizosphere, while the non-ranked Gemmatimonadota significantly presented in agriculture and reclaimed lands were absent in the rhizosphere area in contrast to Myxococcota (Fig. 2).

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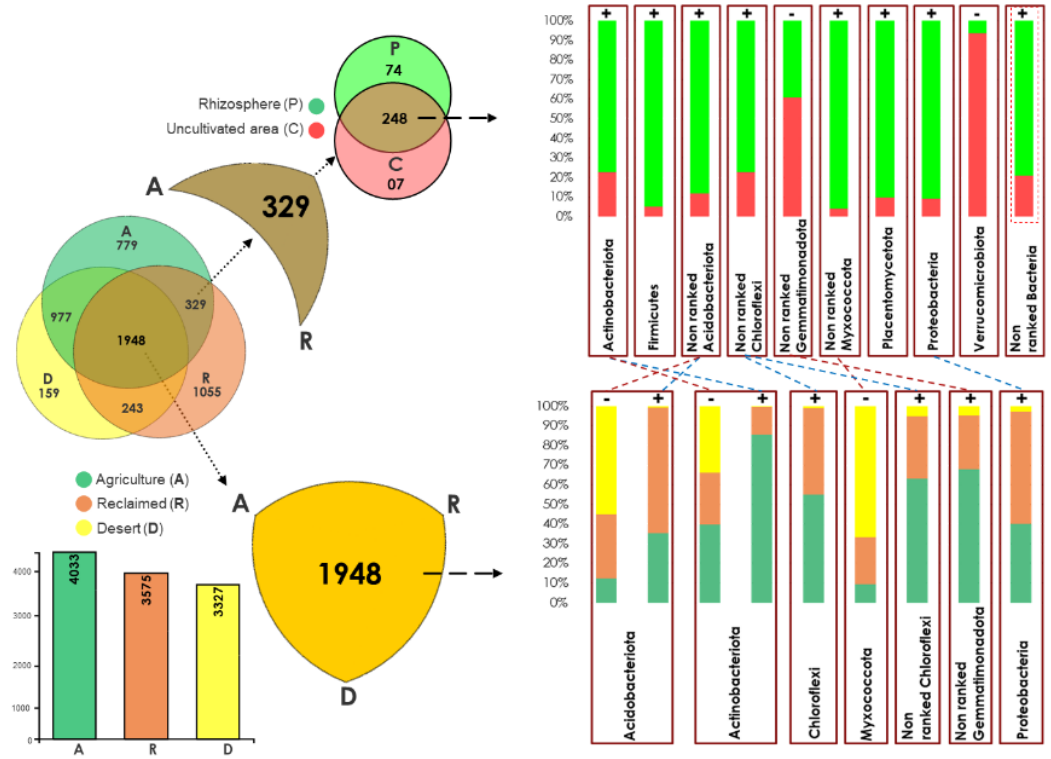

Figure 2. A comparative Venn diagram between three land types based on species counts and the total count for each land type is shown as a histogram (left). The common OTUs were compared statistically and shown (right). the significance direction as indicated by the positive sign "+" when the phyla are significantly presented in cultivated lands (agriculture and/or reclaimed; rhizosphere area) and *vice versa*.

The 74 OTUs unique to the rhizosphere area were quantified below 0.5% of the total samples counts. The assigned OTUs recorded ≥10 folds were quantified relative to the total of each rhizosphere group (agriculture *versus* reclaimed). The most represented phyla were Bacteriodota (5.1%:0.3%), Firmicutes (2.4%:0.1%), and Proteobacteria (3.5%:2%) in agriculture and reclaimed lands, respectively. The most represented species >0.1% in at least one land type, were *Bacteroides coprocola* DSM17136, unclassified Staphylococcus, unclassified Enterococcus, *Brevundimonas vesicularis*, the uncultured bacterium of the family Longimicrobiaceae, an uncultured bacterium of family Muribaculaceae, *Nafulsella turpanensis* ZLM-10, uncultured Epsilonproteobacteria of the order Saccharimonadales, *Rhodococcus rhodochrous*, Non-ranked Saccharimonadales, and uncultured bacterium of family Cellvibrionaceae.

3.2. Prediction analysis

3.2.1. Phenotypic prediction

Based on the recorded metadata for microbial species in databases, seven categories are defined. The most represented categories among all samples were biofilms forming, gram negative, and aerobic bacteria, respectively. The phenotypic profiles of the rhizosphere and uncultivated areas were compared and controlled by the land types. The aerobic and biofilm forming bacteria were found to be highly present in the rhizosphere and were present in the cultivated lands (agriculture & reclaimed) higher than the desert land. While the bacteria contained mobile elements, potentially pathogenic or tolerant to stress was highly present in the rhizosphere than in the uncultivated area, however, they were only high in the reclaimed lands rather than the agriculture or desert lands (Fig. 3A).

When the species-phenotype contribution was revised at the genus level, the species belonging to order Actinomarinales (non-ranked species), and the genus Sphingomonas

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were highly contributing to both phenotypes (aerobic and biofilms forming bacteria) in the rhizosphere in contrast to the uncultivated area. Equally, the species belonging to the genus Nocardioides were highly contributing as aerobic bacteria only. For the species belonging to genera, Ralostonia and Rubrobacter were the most contributing aerobic and biofilms forming bacteria in the uncultivated areas and were absent in the rhizosphere areas. Additionally, species belonging to the family Vicinamibacteraceae (non-ranked) were one of the most contributing biofilms forming bacteria in both rhizosphere and uncultivated areas (Fig. 3B).

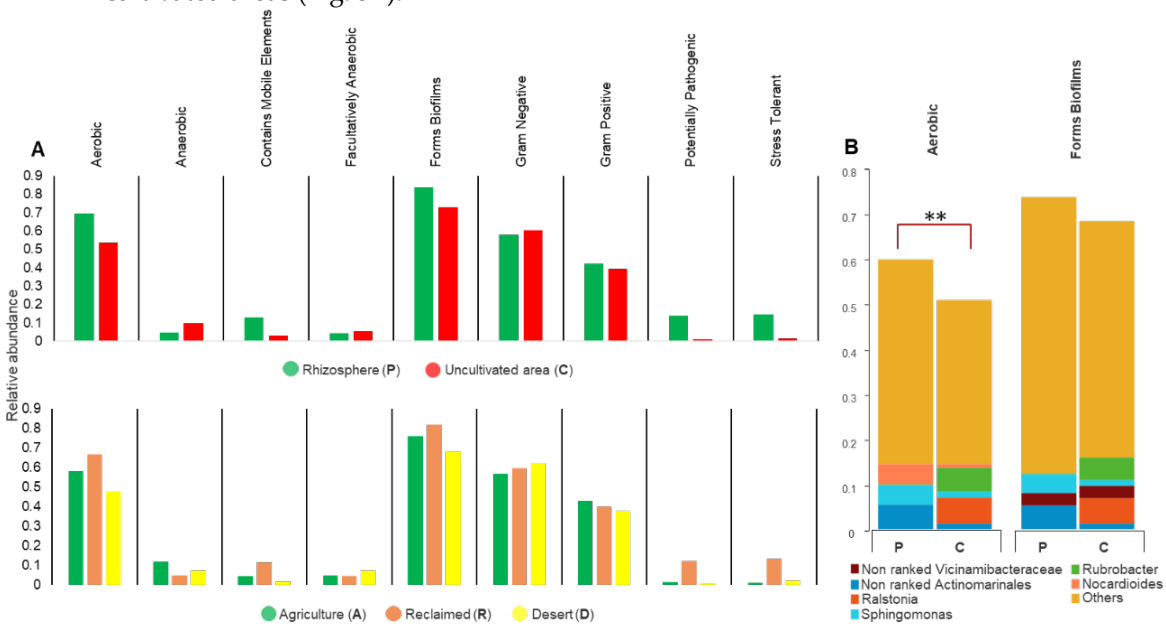

Figure 3. Histogram of the predicted phenotypes for the microbial community detected among all samples (land and sample types; A), the species representing the significant phenotypic profiles are presented comparing rhizosphere to the uncultivated area (B).

3.2.2. Functional prediction

Among all samples, the most presented cluster of ortholog genes (COG) was the amino acid transportation and metabolism, counting an average abundance of 30 M genes. Followed by the cluster of genes with unknown function, the genes of general function prediction. The energy production and conversion genes in addition to transcription genes were among the highly abundant clusters (Fig. 4A). Particularly, the gene cluster described as dehydrogenase reductase (COG1028), transcriptions regulators (COG1309), and major facilitator (COG2814) were the most abundant genes in the rhizosphere area samples compared to the uncultivated samples. With less abundancy, the histidine kinase (COG0642), alpha-beta hydrolase (COG0596), RNA Polymerase (COG1595), acyl-CoA dehydrogenase (COG1960), transcriptional regulator (COG0583), glycosyl transferase (COG0438), the transcriptional regulator "LuxR family" (COG2197) and Methyltransferase required for the conversion of demethylmenaquinone (DMKH2) to menaquinone (MKH2) (COG2226), were detected.

By comparing the gene enrichment results between desert land *versus* the cultivated lands (agriculture and reclaimed), the most enriched genes were forming part of the Citrate cycle (TCA cycle, Krebs cycle; M00009), followed by the reductive citrate cycle (Arnon-Buchanan cycle; M00173), NADH: quinone oxidoreductase (M00144), Glycolysis (Embden-Meyerhof pathway; M00001), Citrate cycle, second carbon oxidation (M00011), Phenylacetate degradation (M00878) Dicarboxylate-hydroxybutyrate cycle (M00374) and beta-Oxidation (M00087; Fig. 4B).

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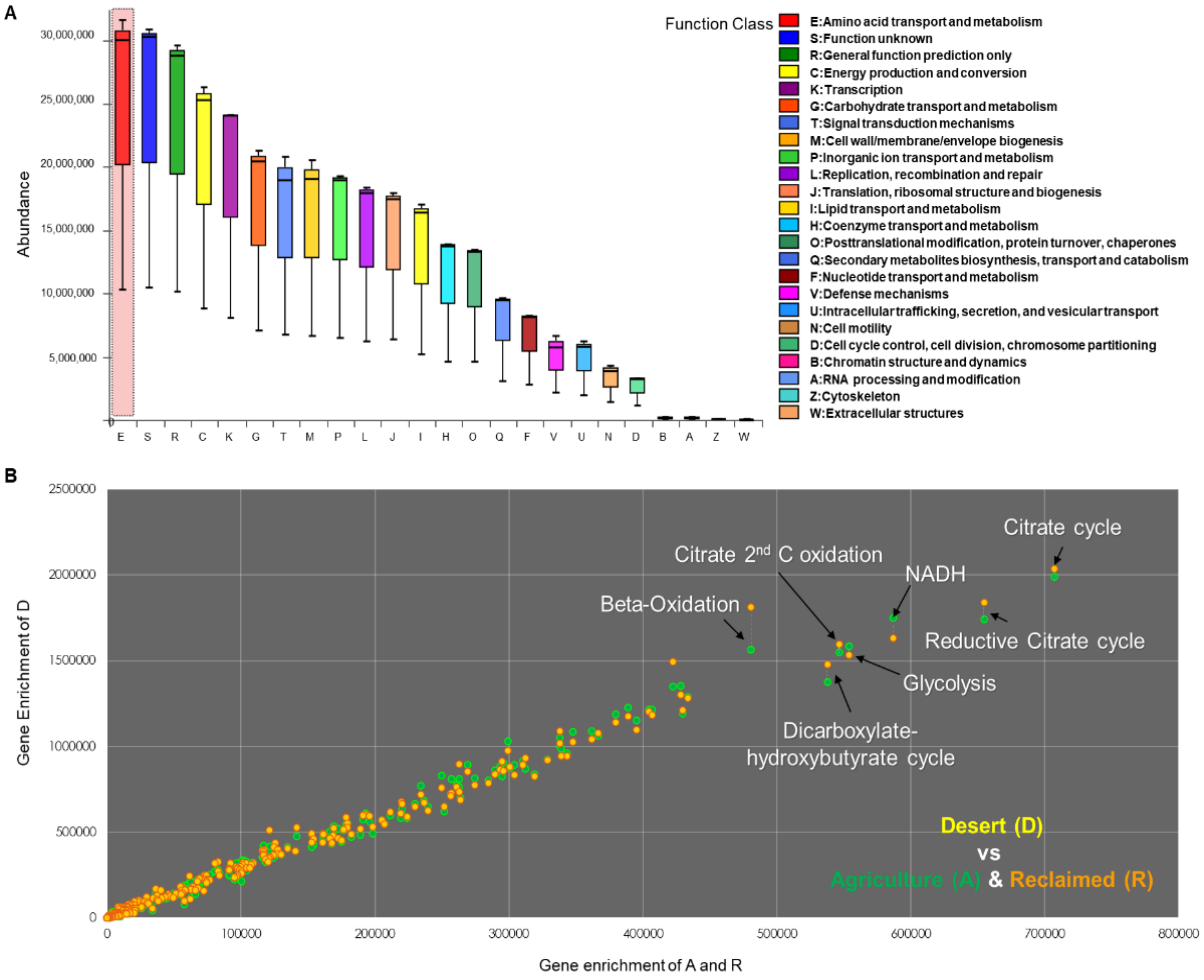

Figure 4. The functional prediction charts include the clusters of orthologue genes (COGs) for all samples (A) and the gene enrichment dot plot of the cultivated lands versus the desert land.

3.3. Association analysis

3.3.1. Co-occurrence correlation network

The correlation-based distance matrix revealed several correlation blocks of positively and negatively correlated bacterial genera. The blocks were defined by a descending letter by the block size. The D block was represented by species belonging to the order Actinomarinales (non-ranked species) correlated to species belonging to genera Chelativorans and Halomonas, as well as non-ranked species of the PAUC43f marine group, this block along with species of the genera Rhodococcus and Ralstonia were negatively correlated to blocks A, B, and C as marked and labeled as E and F. The A correlation block represents the highest group of correlated species, for example, species belonging to the genera Sphingomonas, Rubrobacter, and species belonging to the family Vicinamibacteraceae (non-ranked) and family Gemmatimonadaceae (non-ranked). The B block included species of the families Nitrososphaeraceae (non-ranked), Roseiflexacea (non-ranked), along with species belonging to the genera Nitrospira, Aeromicrobium, and Dongia, Nocardioides, and Pseudonocardia. The C correlation block was formed by species belonging to the genera Ammoniphilus, Streptomyces, Arthrobacter, Paenibacillus, and Bacillus (Fig. 5).

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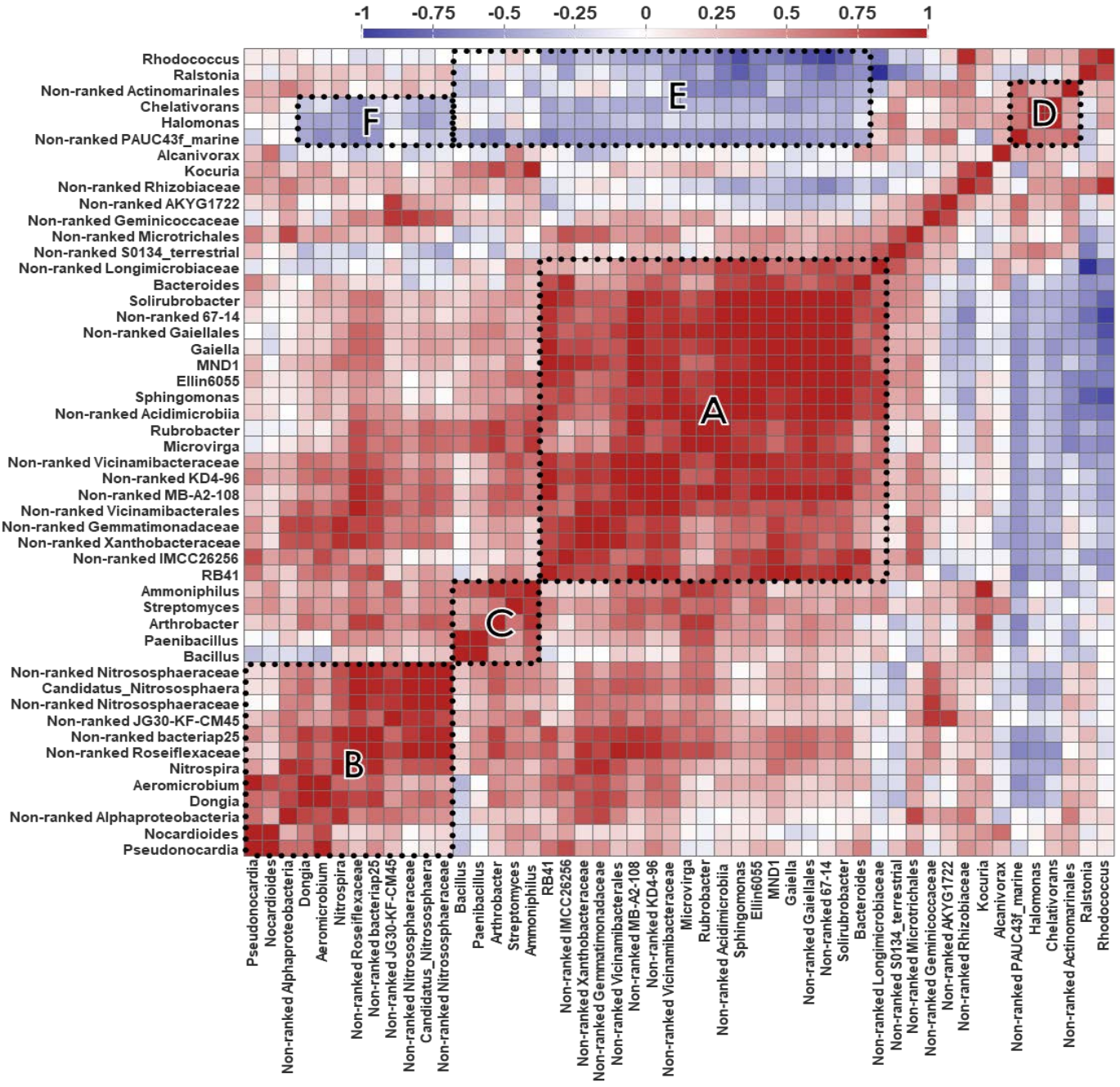

Figure 5. Heatmap correlation distance matrix among all the recorded species. Correlation blocks are defined by letters, as A – D represents the positively correlated blocks (0> r-value ≥1), while E and F represent the negatively correlated blocks (-1≥ r-value >0).

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3.3.2. Association to soil properties

Major elements were detected from the sampling area, including P, Fe, K, Mn, Zn, and Cu in addition to the soil organic matter (OM). The K was the highest major component among all with an average of 277.21±76.82. The followed major component of the soil was Fe and Mn with an average of 9.54±2.75 and 9.28±4.08, respectively. The averages of P, Zn, and Cu were 5.18±4.67, 3.59±1.29, and 4.34±71, respectively. While the lowest average was found for the soil organic matter 0.06±0.04 among all land types. The reclaimed land soil profile contained the highest in P (10.55), Fe (12.71), Zn (5.08), Cu (4.95), and moderate in Mn (8.07), and the lowest in K (194.31). The agricultural land was approximately equal to desert lands in P (~2.5), Fe (~8), Zn (~3), and Cu (~4). The agricultural land was only the highest in the OM (0.09) equal to the reclaimed one (0.08). The desert lands were remarkably the highest in K (345.99) and Mn (13.84). The Mn and Fe profiles were reversely proportional in the desert (i.e., Fe was lower in the desert than Mn) compared to cultivated lands regardless of the quantity (i.e., Fe was higher in agriculture and reclaimed lands than Mn; Fig. 6A).

The species statistical association to soil elements was analyzed, where the P was the most influential element to the number of associated species (14 species). Followed by Mn and K associated with unknown and uncultured species of the genus Nocadriodes and Psuedonocardia, respectively. *Arthrobacter globiformis* was associated with both Cu and OM values. Additionally, uncultured archaea of the genus Nitrososphaera and uncultured bacteria of the genus Marmoricola were associated with OM and Cu, respectively. The K was associated with an uncultured bacteria of the family Rhizobiaceae, while Fe with uncultured bacteria of the family Geminicoccaceae. The Zn was not associated with any of the detected microorganisms (Fig. 6B).

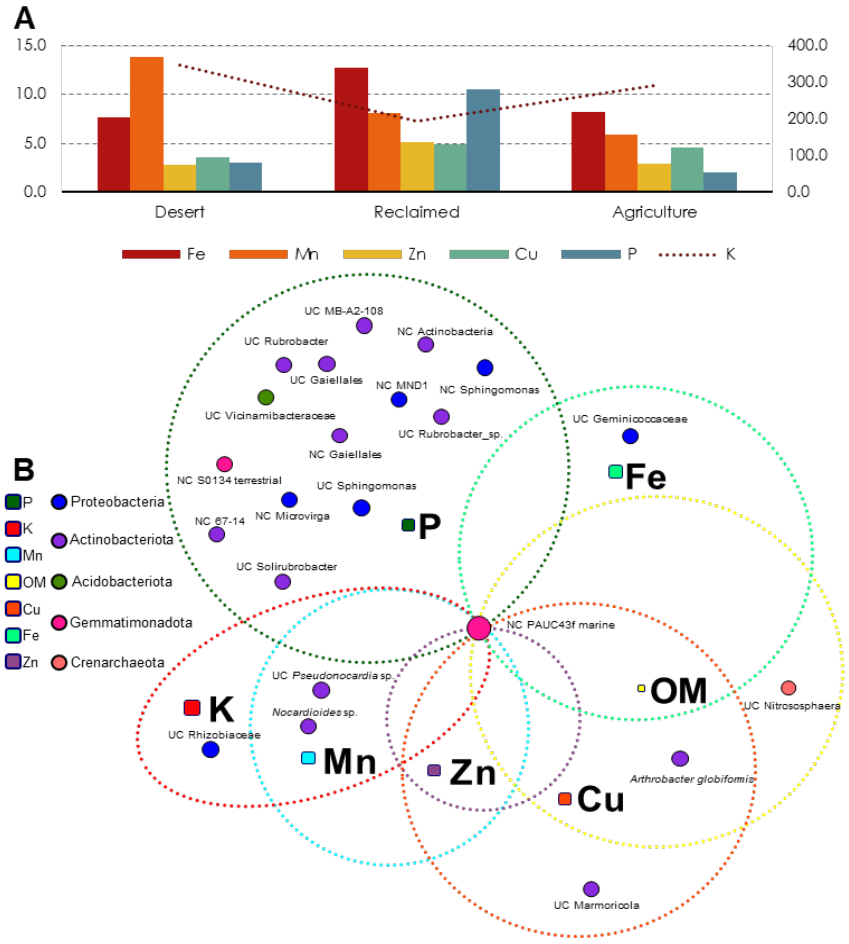

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Figure 6. The metal composition measured per land type is shown (A) and the association of species to each metal is shown (B).

4. Discussion

The current study aimed to identify the rhizosphere associated bacteria by following a specific sampling design. The multi-plant sampling from different land types was the main feature of our analysis, using the desert as general control to omit and discard desert related and significantly presented species from our analysis. Additionally, the rhizosphere areas in the cultivated lands (whether the usual agriculture or newly reclaimed lands) were controlled by the uncultivated areas. The application of different statistical approaches was meant to resolve as many relevant species as possible associated with the plant rhizosphere. The general observation was the species taxonomical status of the relevant species, in which the majority were unidentified, unknown, unranked, and/or uncultivated bacteria. Even though the lack of this knowledge would fog our survey, it also opens an interesting window for traditional isolation of the relevant species for future studies. Thus, the interpretations at the genus level were the dominating aspect of this discussion.

Carbon fixation is considered one of the main biochemical processes in the biosphere, providing the carbon constructing blocks for entirely living organisms [21]. The species belonging to the order vicinamibacterales were recently recorded as potential carbon fixation bacteria [eg., 22]. Nitrification consists of the biological oxidation of ammonia (NH_3) to nitrite (NO_2^-) that is carried out by NH3-oxidizing archaea (AOA) and bacteria (AOB), combined with the oxidation of NO_2^- to nitrate (NO_3^-; a form that can be used by plants) are carried out by phylogenetically diverse NO_2^--oxidizing bacteria (NOB) [eg., 23]. Species belonging to the phylum Chloroflexi, order Rhizobiales, the family Nitrosomonadaceae, and genus Nocardioides are known NOBs that gain energy from the oxidation of nitrite to nitrate and some are capable to convert the atmospheric N_2 to NH_3 [eg., 24]. Actinomarinales are well-known aquatic-system-associated Actinobacteria, particularly in nutrient-limited locations that require high surface-to-volume ratios, with a potential capability to reduce carbonate and nitrate [25]. Cyclobacteriaceae is considered a member of the class "Sphingobacteriia" that belongs to the phylum Bacteroidetes. The members of the family were reported from diverse inland and off-land habitats [26]. Under aerobic conditions, they can oxidize carbohydrates (one species ferments glucose) but do not produce indole, while some taxa reduce nitrate to nitrite [eg., 27]. The phylum Planctomycetota is highly abundant in sediments from diverse geographical ubiquity, they are reported to be the dominant group in oxygen minimum zones (OMZ). The members of one of this phyla family, namely Phycisphaeraceae can oxidize ammonium under oxygen minimum conditions and are collectively named anammox [eg., 28]. Iron is a prevalent redox-active metal element present on the Earth and occurs in two oxidation states (Fe^{2+} and Fe^{3+}) in nature. Some microorganisms drive the oxidation of Fe^{2+} to gain energy for growth, with molecular oxygen or NO_3^- like the electron acceptor [29]. Acidimicrobiia is a deep-rooting lineage that belongs to the phylum Actinobacteria, which can oxidize ferrous iron at comparatively fast rates as well as responsible for the regeneration of ferric iron in the acidic ecosystem [30]. On the contrary, Bryobacter is Chemoheterotrophs, Aerobes, and facultative anaerobes and is capable of reducing Fe (III). Members that belong to these genera were usually isolated from acidic wetlands but grow better through mildly acidic conditions [31]. In the current analysis, despite the presence of alternative electron transports in soil (e.g., Fe and Mn), and bacterial species with metal oxidation and reduction capabilities that may promote the anaerobic respiration [32], the most enriched functional orthologue cluster of genes were related to the aerobic respiratory bacterial phenotypic profile. A phenotype that was significantly present in the rhizosphere area when compared to uncultivated areas.

Many of the surveyed species belonging to the rhizosphere area were previously reported as decomposers of soil organic matter, producers of secondary metabolites, soil

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remediators, and plant growth-promoting bacteria. Plant-promoting bacteria can act on the regulation of plant metabolism by producing or stimulating the production of various phytohormones that enhance intensive growth for seedlings such as auxins Indole-3-Byturic Acid [IBA], (Indole-3-acetic acid [IAA], and Phenyl Acetic Acid [PAA]; [33].

Species belonging to the phylum Actinomycetota were the most represented in the plant rhizosphere, especially the class Actinobacteria. This class represents an enormous group of microorganisms that can produce a varied range of secondary metabolites, involving surfactants. They also contribute to the rotation of soil components into organic components through the decomposition of a complex combination of organic matter in lifeless plants, and animals, in addition to fungal material [eg., 34]. *Rhodococcus rhodochrous* species that belongs to the family Nocardiaceae is well-known to co-metabolize difficult to degrade hydrocarbons [35]. Members of the family Gaiellaceae are naturally producing many different antibiotics and contribute to global carbon cycling through the decomposition of soil organic matter, increase plant productivity, and are widely known as prolific producers of bioactive compounds essential for humans and animal health [eg., 36]. The genus Streptomyces, which is known as the most abundant and certainly the most important actinomycetes, is considered a good source of antibiotics, bioactive compounds, and extracellular enzymes. It plays a major role in nutrient cycling and, more significantly, because of the general propensity of members of the genus to produce secondary metabolites of biotechnological and clinical importance. The importance of Streptomyces through its biocontrol, plant growth-promoting, and being efficient as a biofertilizer [eg., 37]. Members of the genus Actinoplanes which belong to the family Micromonosporaceae are prolific sources of novel enzymes, antibiotics, and other bioactive compounds [eg., 38]. The genus Micromonospora within the same family has a great potential for producing secondary metabolites. Micromonospora species function in biocontrol, plant growth promotion, root ecology, and the breakdown of plant cell wall material [eg., 39]. Functions that predicted the order Microtrichales were related to gluconeogenesis and/or glycolysis, chlorophyll and porphyrin metabolism, transcription factors, and photosynthetic proteins [40], while the relative abundances of the Solirubrobacteraceae family were found positively correlated with cultivated plant growth [41]. Within the family Nocardioidaceae, the genus Nocardioides is an aerobic, motile, or non-motile genus that plays an important role in the degradation of di-2-Ethylhexyl phthalate (DEHP) in natural soil environments [eg., 42], and the decomposition of various pollutants such as alkanes, pyridine, phenols, phenanthrene, etc [43]. Many organisms within the genus Nocardioides show biodegradative activities, exhibiting the capacity to metabolize recalcitrant and toxic environmental pollutants, in addition to secreting a range of extracellular enzymes [44]. The genus Aeromicrobium produces a wide diversity of secondary metabolites as major compounds with antibacterial activity, and they could be potential indicators for disease repression [eg., 45]. Pseudonocardia in the family Pseudonocardiaceae, are well-known to degrade 1,4-dioxane as the sole carbon and energy source in addition to degrading tetrahydrofuran (THF). It has an important role in biotechnology due to the production of secondary metabolites, some of which have anti-bacterial and anti-fungal effects and helps in the decomposition of the organic matter of dead organisms [eg., 46].

Within the phylum, Pseudomonadota (synonym Proteobacteria), the second most represented phylum, genus Roseomonas is an aerobic, motile bacteria of the family Acetobacteraceae known as acetic acid bacteria that could produce specific secondary metabolites, i.e., gentamycin, and asukamycic [eg., 47]. The species that belong to the Sphingomonas genus have many functions ranging from remediation of environmental contaminations to producing highly beneficial phytohormones, for example, sphingan and gellan gum. The degradation of organometallic compounds as well as improve plant growth during stress conditions such as salinity, drought, and heavy metals in agricultural soil by producing plant growth hormones e.g., gibberellins and indole acetic acid [48]. Bacteria that belong to the genus Novosphingobium regularly participate in the biodegradation of aromatic compounds such as aniline, phenol, 4-chlorobenzene, nitrobenzene, and pyrene,

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phenanthrene, dibenzofuran, carbofuran, and estrogen [49]. Cellvibrionaceae have a terrestrial origin, related to soil and decaying plant materials; however, Cellvibrionaceae are marine bacteria and display a slightly halophilic behavior. Most species in this family possess a large variety of polysaccharide-degrading abilities and their genomes contain dozens of CAZyme (carbohydrate-active enzyme) genes, enabling the hydrolysis of cellulose, agar, carrageenan, xylan, starch, chitin, and several other polysaccharides [50].

Additional phyla were detected as part of the rhizosphere microbial community. In the phylum Acidobacteriota, the bacterial species have genes that probably help in survival as well as competitive colonization in the rhizosphere, leading to the establishment of beneficial relationships with plants, regulation of biogeochemical cycles, decomposition of biopolymers, exopolysaccharide secretion, and plant growth promotion [51]. Blastocatella is important in pharmaceutical wastewater treatment plants, and it contributes to ammonium nitrogen removal [52]. Members that belong to the family Anaerolineaceae within the phylum Chloroflexi are anaerobic microbes, that can co-exist with methane metabolism microbes and are important organic matter degraders under anoxic conditions, while methane metabolism is used for bioremediation of soil Cd contamination and promotes the precipitation of soluble Cd [53]. The Bacillales of the phylum Firmicutes enhance plant growth through the production of ACC deaminase and pathogen suppression [54]. Within the phylum Myxococcota, the members of the family Myxococcaceae are broadly distributed in soil and also existed in freshwater in addition to the marine environment with the ability to produce diverse secondary metabolites acting as antimicrobials, antiparasitics, antivirals, cytotoxins, and anti-blood coagulants [55]. Members of the class Polyangia are well-known for their extraordinary social lifestyle and diverse novel gene clusters of secondary metabolites in soil [56]. Saccharimonadales that belongs to the Patescibacteria phylum, had small genomes with supposed parasitic or symbiotic lifestyle. Saccharimonadales were known as candidate bioindicators of high P availability and considered the dominant bacteria in salt stress or organic enriched sludge which might degrade plastics and also show synergistic effects through the nitrogen cycling-related genes. Saccharimonadales are fast-growers and use sugars for energy metabolism [57]. Within the Verrucomicrobiota phylum, the members of the Pedosphaeraceae family were found to tolerate Cd bio-toxicity and are used for the optimization of phytoremediation in Cd-contaminated sediment [58].

Gemmatimonadota is known as the eighth-most abundant bacterial phylum in soils, representing about 1–2% of the soil bacteria worldwide. They are typically short rods and are rich in soils, wherever they seem to be frequently associated with the plants and the rhizosphere as well as freshwaters, wastewater treatment plants, biofilms, and sediments and are capable of anoxygenic photosynthesis [59]. Longimicrobiaceae are found in Mediterranean forest soil and is a member of the order Longimicrobiales within the class Longimicrobia. They are considered nonmotile, gram-negative, short to long rod-shaped bacteria with anaerobic chemoorganoheterotrophic metabolism. Members of the family Longimicrobiaceae as well as the family members of the family Cyclobacteriaceae (the phylum Bacteroidota) facilitate phosphate solubilization in the soil [60]. Some of these bacterial taxa were also frequently found in other semi-arid soils with low organic matter content and adapted to extreme conditions, such as Blastococcus or uncultured members of the family Longimicrobiaceae and the genus Staphylococcus, which appeared exclusively in no-organic matter control soils [eg., 60].

5. Conclusions

Carbon fixation and nitrification capable bacteria were abundant in the rhizosphere in addition to other species with the ability to reduce and oxidize carbonate and reduce nitrate, oxidize ammonium under oxygen minimum conditions, and oxidize Fe^{2+} to gain energy with molecular oxygen or NO^{3-} like the electron acceptor. Many of the surveyed species in the rhizosphere area were previously reported as aerobes, and known as decom-

posers of soil organic matter, soil remediators to produce highly beneficial phytohormones, and producers of a varied range of secondary metabolites, involving surfactants, fengymycin, and asukamycic. Additional bacteria were detected that can produce diverse secondary metabolites acting as antimicrobials, anti-parasitic, antivirals, cytotoxins, and anti-blood coagulants. Besides bacteria are capable of degradation of organometallic compounds as well as improving plant growth during stress conditions such as salinity, drought, and heavy metals in agricultural soil by producing plant growth hormones e.g., gibberellins and indole acetic acid. Additional bacteria were highlighted for their biodegradative activities, exhibiting the capacity to metabolize recalcitrant and toxic environmental pollutants, in addition to secreting a range of extracellular enzymes. More bacteria were identified as bioindicators of high P availability and considered the dominant bacteria in salt stress or organic enriched sludge which might degrade plastics and show synergistic effects through the nitrogen cycling-related genes. At the functional level, the genes enriched gluconeogenesis and/or glycolysis pathway, chlorophyll and porphyrin metabolism, transcription factors, and photosynthetic proteins characterized the microbial community of the rhizosphere area, as well as genes that probably help in the survival and competitive rhizosphere colonization. In the rhizosphere, even though the surveyed microbial list included several unknown species, it opens a prospect for in vitro isolation and cultivation of new species, to identify and characterize bacteria capable of establishing beneficial relationships with plants (i.e., promoting growth) and provide a potential candidate as biofertilizers.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, S.M.R., M.M., and M.A.R.; methodology, M.M.; software, M.M. and Y.G.F.; validation, X.X., Y.Y., and Z.Z.; formal analysis, X.X.; investigation, X.X.; resources, X.X.; data curation, X.X.; writing—original draft preparation, X.X.; writing—review and editing, X.X.; visualization, X.X.; supervision, X.X.; project administration, X.X.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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