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Effect of Pyroligneous Acid on the Microbial Community Composition and Plant Growth-Promoting Bacteria (PGPB) in Soils

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Abstract: Pyroligneous acid (PA) is often used in agriculture as a plant growth and yield enhancer. However, the influence of PA application on soil microorganisms is not often studied. Therefore, in this study, we investigated the effect of PA (0.01–5% *w*/*w* in soil) on the microbial diversity in two different soils. At the end of eight weeks of incubation, soil microbial community dynamics were determined by Illumina-MiSeq sequencing of 16S rRNA gene amplicons. The microbial composition differed between the lower (0.01% and 0.1%) and the higher (1% and 5%) concentration in both PA spiked soils. The lower concentration of PA resulted in higher microbial diversity and dehydrogenase activity (DHA) compared to the un-spiked control and the soil spiked with high PA concentrations. Interestingly, PA-induced plant growth-promoting bacterial (PGPB) genera include *Bradyrhizobium*, *Azospirillum*, *Pseudomonas*, *Mesorhizobium*, *Rhizobium*, *Herbaspiriluum*, *Acetobacter*, *Beijerinckia*, and *Nitrosomonas* at lower concentrations. Additionally, the PICRUSt functional analysis revealed the predominance of metabolism as the functional module's primary component in both soils spiked with 0.01% and 0.1% PA. Overall, the results elucidated that PA application in soil at lower concentrations promoted soil DHA and microbial enrichment, particularly the PGPB genera, and thus have great implications for improving soil health.

Keywords: pyroligneous acid; microbial community; dehydrogenase activity; Illumina Miseq; PGPB

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1. Introduction

Pyroligneous acid (PA) or wood vinegar is an acidic reddish-brown aqueous liquid obtained from the pyrolysis of wood and other lignocellulosic raw material [1]. The carbonization of the wood and wood products results in charcoal, non-condensable gases (NCG), tar, and PA. The raw materials for making the PA are abundant, such as wood, wood residues, and other biomaterials such as corn cobs, pine cones, fruit shells, and even weeds [2]. The chemical constituents and PA yield from the pyrolysis process vary depending on the source and the pyrolysis conditions. PA consists of different chemical compounds, primarily acetic acid, phenols, methanol, and formaldehyde [3].

PA has a high degree of antifungal, termiticidal, and antimicrobial activities [4–6]. These attributes of the PA make it a safe alternative for synthetic and chemical pesticides and a better choice for sustainable farming. In saline soil, PA application was reported to reduce leaching of the soluble salts by decreasing soil pH [7]. Additionally, studies on the co-application of wood vinegar and biochar reported enhancement of soil fertility and plant growth by increasing seed germination and seedling growth, preventing leaching

of harmful chemical herbicides into the aquatic environment, and in the degradation and remediation of heavy metals [8–10].

Generally, bacteria belonging to the genera *Acetobacter*, *Azospirillum*, *Bacillus*, and *Pseudomonas* that can stimulate root growth and reduce disease or damage by insects are termed plant growth-promoting bacteria (PGPB) [11]. Several PGPB are used in agriculture as biocontrol agents and biological fertilizers. PGPB promote plant growth and development mainly by (1) producing plant growth regulators such as Indole acetic acid (IAA), gibberellic acid, cytokinins, and ethylene; (2) increasing the availability of soil nutrients to plants through solubilization of mineral phosphate and other nutrients; (3) asymbiotic nitrogen fixation; (4) demonstrating antagonism against phytopathogenic microorganisms; and (5) producing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase to promote plant growth and development under adverse environmental conditions [12–14]. Currently, biological approaches for improving crop production are gaining importance in sustainable agriculture systems. In this context, there is ongoing research globally to explore the use of microorganisms with novel traits to improve crop growth and yield even under adverse environmental conditions [15]. However, there is a deficiency of information on the impact of PA on soil microbial activities, especially related to PGPB, which limits their application and usage in agriculture.

PA contains readily degradable organic compounds that microbes could use for their metabolism, resulting in increased beneficial microbial biomass, population growth, and microbial efficiency [16]. Recently, high throughput culture-independent techniques such as 16S rRNA-based next-generation sequencing/pyrosequencing have enabled the analysis of a vast number of sequences to visualize and characterize a microbial community [17]. Moreover, the high resolving power of this technique provides significant access to uncultured bacterial groups that are otherwise not detected by other microbial fingerprinting methods [18].

Therefore, the objective of this study was to analyze the impact of PA on soil physicochemical properties, microbial composition, and their effectiveness on plant growth, with a particular focus on PGPB. We hope the knowledge gained in this study will promote the utilization of PA in sustainable agriculture.

2. Materials and Methods

2.1. Chemicals, Experimental Soil, and Treatment Description

The refined pyroligneous acid (PA) product (PyroAg®) was obtained from Northside Industries Pty Ltd., Cromer, Australia. All the reagents and solvents used in the study were analytical grade purchased from Sigma-Aldrich, Castle Hill, Australia. The working solutions of PA were prepared freshly using sterile deionized water (Milli-Q, 18 Ω cm^{-1}, ELGA Lab Water, High Wycombe, UK). Two soils with no history of pesticide application collected from Lovedale, (Latitude: −32°74′41.16″ S and Longitude: 151°35′69.99″ E) Hunter Valley, NSW (soil A) and Boanbong Road (Latitude: −33°60′60.63″ S and Longitude: 151°32′66.65″ E), Palm Beach, NSW (soil B), were used in this study. The collected soils were air-dried and sieved (<2 mm) before use. Soil pH and EC were measured using a pH/conductivity meter in soil suspensions (1:5 of soil to water). The soil texture was measured with a micropipette method [19]. Soils A and B were spiked with five different concentrations of PA ranging from 0.01 to 5% (weight/weight in soil) in triplicates and incubated for eight weeks. Triplicate samples were taken from each spiked and control treatment.

2.2. Soil Dehydrogenase Activity (DHA)

The dehydrogenase activity (DHA) in soil was determined as per the method described in our previous study [20]. Briefly, the moist soil was treated with 2,3,5-triphenyl tetrazolium chloride (TTC), and the conversion of TTC to a colored product, triphenyl formazan (TPF) by microorganisms was analyzed at 485 nm in a microplate reader (Bio-Tek® SynergyTM HT equipped with KC4 software) [21].

2.3. Soil DNA Extraction

Following the manufacturer's protocol, DNA from each soil was extracted using a Powersoil DNA isolation kit (Mo Bio Labs Inc., Carlsbad, CA, USA). Briefly, the genomic DNA was extracted from 0.25 g soil sample. Mechanical cell lysis (bead-beating) was carried out with the PowerLyser® 24 homogenizer. The lysate was centrifuged at 13,400× *g* for 1 min, and the resulting supernatant was used for the DNA extraction. The quality of the extracted DNA was checked by running the agarose gel and quantified further using the QuantiFlour® dsDNA system (Promega), Sydney, Australia.

2.4. Illumina Miseq Analysis

The composite sample of DNA extracted from triplicate of each treatment and control was sent to the Australian Genome Research Facility (AGRF), Melbourne, Australia. The extracted genomic DNA was amplified in the AGRF using the 16S 27F-519R (V1–V3) primer with the Forward Sequence: 5′AGAGTTTGATCMTGGCTCAG 3′ and Reverse Sequence: 5′GWATTACCGCGGCKGCTG 3′. The conditions for the primary polymerase chain reaction (PCR) were as follows: 95 °C for 7 min, 29 cycles of 94 °C for 45 s, 50 °C for 60 s, 72 °C for 60 s, and 72 °C for 7 min using AmpliTaq Gold 360 Master Mix (Life Technologies, Victoria, Australia). The primary PCR amplicons were purified using AMP pure XP magnetic beads (Beckman Coulter, Brea, CA, USA). A secondary 8-cycle PCR with Illumina Nextera XT V2 indices and High Fidelity Takara Taq indexed each amplicon. The resulting amplicons were AMPure purified and measured using fluorometry (Invitrogen PicoGreen, Waltham, MA, USA), and the obtained data were normalized. The equimolar pool was measured by qPCR (KAPA) on the AB QuantStudio, followed by sequencing on the Illumina MiSeq with two X 300 base pairs (bp) Paired-End V3 Chemistry.

2.5. Bioinformatics Analysis of Amplicon Sequences

The sequences from read one and read two were merged to improve the sequence quality. Following this, the raw sequencing reads were quality checked using FastQC (v0.11.9), and low-quality reads were filtered out from the subsequent analysis according to the following criteria: raw reads shorter than 110 nt and read with the length of the variable region shorter than 100 nt; reads lacking a perfect BLAST match to described barcodes; mismatches to at least one end of the 16S rRNA gene primers; and reads harboring more than 7% of low-quality bases (Phred score < 19). The DNA sequences were uploaded in Meta Genomics Rapid Annotation using subsystem technology (MG-RAST) pipeline server. The metagenome ID generated from MG-RAST for the samples submitted are mgm4843281.3 mgm4843282.3, mgm4843283.3, mgm4843284.3, mgm4843285.3, mgm4843286.3, mgm4843287.3, mgm4843288.3, mgm4843289.3, and mgm4843290.3. The reads were annotated using MG-RAST, employing the Greengenes as the annotation source [22]. The maximum E-value cutoff was set to 5, the minimum percentage identity cutoff was 97%, and the specified minimum alignment length cutoff was 15 bp. The MG-RAST metagenome overview was applied to examine the quality of sequences in terms of base-pair count, sequence length, sequence count, GC percentage, and identification of rRNA features. The Quantitative Insights Into Microbial Ecology (QIIME) data file in biome format obtained from the QIIME plugin in MG-RAST was used for the functional predictions. The Phylogenetic Communities by Reconstruction of Unobserved States (PICRUSt, v1.1.3) was applied to predict the functional features of the soil samples based on the OTU table [23]. The statistically significant differences in functional categories were performed using linear discriminant analysis (LDA) effect size (LEfSe). The LDA values > 2 at a p-value < 0.05 were considered significantly enriched. To estimate community diversity indices (Taxa, Chao-1, dominance, Shannon, alpha diversity, and Evenness), and for canonical correspondence analysis (CCA), the data obtained from the MG-RAST pipeline were imported into the PAST 3.06 [24] spreadsheet. They were calculated by default settings (Bootstrap N = 9999, Bootstrap type: percentile).

3. Results

3.1. Soil Physicochemical Properties and Dehydrogenase Activity

The experimental soils A and B were identified as loamy sand (Soil A—82.3% sand, 17.4% silt and 0.2% clay and Soil B—85.4% sand, 14.4% silt, 0.2% clay) with a pH of 5.9 and 6.6, respectively. The total carbon percentages in soils A and B were 0.51 and 1.48%, respectively. The changes in soil pH and EC after spiking with different concentrations of PA are shown in Table 1.

Table 1. Changes in soil pH and EC after spiking with different concentrations of PA.

Treatments	Soil A		Soil B	
	pH	EC (µS/cm)	pH	EC (µS/cm)
Control	5.9	39.5	6.6	145.7
0.01% PA	5.9	39.2	6.6	142.5
0.1% PA	5.5	44.2	6.5	137.1
1% PA	4.6	85.9	6.0	194.9
5% PA	4.0	146.7	5.1	373.7

Soil dehydrogenase activity was determined in the PA spiked and control soils after eight weeks of incubation, and the results are shown in Figure 1. Both soils, A and B, spiked with low PA concentrations (0.01% and 0.1%) exhibited an increase in the dehydrogenase activity over the control. A significant increase in the dehydrogenase activity (18.7%) over the control was measured in soil A spiked with 0.01% PA. A substantial reduction in the dehydrogenase activity compared to the control was measured in both soils spiked with 1 and 5% PA.

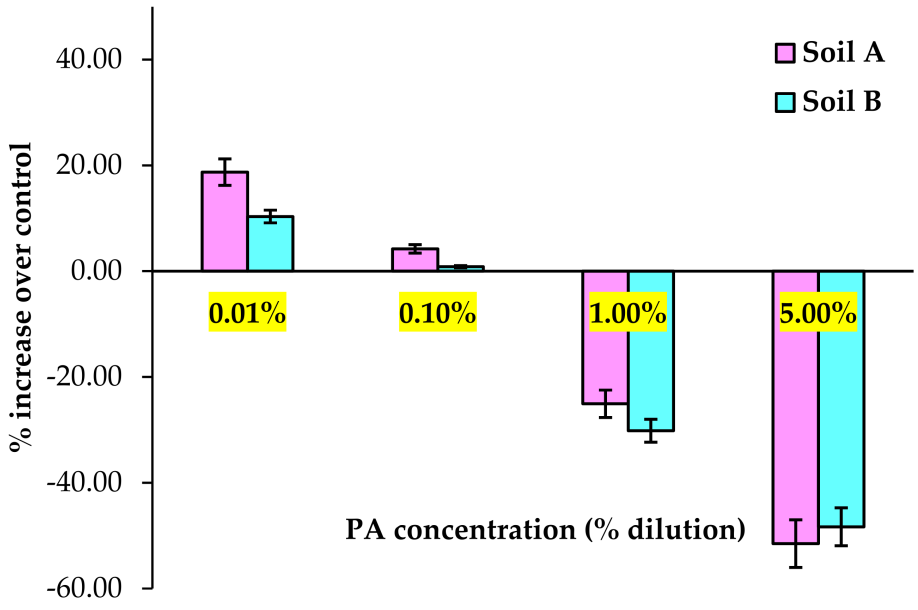

Figure 1. The effect of PA on soil dehydrogenase activity.

3.2. Soil Microbial Diversity

After eight weeks of incubation, 16S rRNA amplicon-based sequencing revealed a difference in the number of reads in PA-treated soils A and B and their corresponding control samples (Figure S1). The total obtained reads were assigned to classified and unclassified bacteria, Eukaryota, and unclassified and unassigned sequences. Only classified bacterial sequences were used for the sequencing analysis, and all others were omitted from further analysis. Several richness and diversity measures (Taxa_S, Alpha diversity, Chao-1 estimate of species richness, and Shannon index) were calculated for both soils, and

the results are presented in Table 2. In both cases, the 0.01% PA spiked soils exhibited the highest species richness.

Table 2. Estimated richness and diversity indices of Soil A and Soil B spiked with different concentrations of PA.

Treatment	Taxa_S		Alpha Diversity		Chao-1		Shannon Index	
	Soil A	Soil B	Soil A	Soil B	Soil A	Soil B	Soil A	Soil B
Control	1312	2167	259	404.9	1945	2933	3.15	4.66
0.01% PA	1734	2302	299.4	426.2	2349	3151	3.67	5.17
0.1% PA	1426	2174	253.5	423.5	2085	2938	3.34	4.89
1% PA	1375	1929	235.9	392.0	1947	2629	2.79	4.65
5% PA	1305	1535	216	263.5	1807	2225	2.51	4.38

3.3. Soil Bacterial Community Composition

In soil A, the total bacterial community contained 21 phyla and unclassified bacterial sequences, whereas, in soil B, 25 bacterial phyla and unclassified bacterial sequences were present (Figure 2). Among the bacterial phyla, the unclassified bacterial phyla constituted the largest population with an estimated relative abundance of 49, 54, and 48% in soil A with PA concentrations of 0.01, 0.1%, and control, respectively. The subsequent abundant bacterial phyla in this study are Proteobacteria, followed by Actinobacteria. In soil A spiked with 1% PA treatment, Actinobacteria was the highest (34%), followed by Proteobacteria (18%). Whereas for 5% PA treated soil A, Firmicutes was the highest (86%). For soil B, on the other hand, Actinobacteria was the highest in all the PA-treated soils and control, except for the 5% spiked case, where *Firmicutes* was the highest (63%), followed by Actinobacteria (30%).

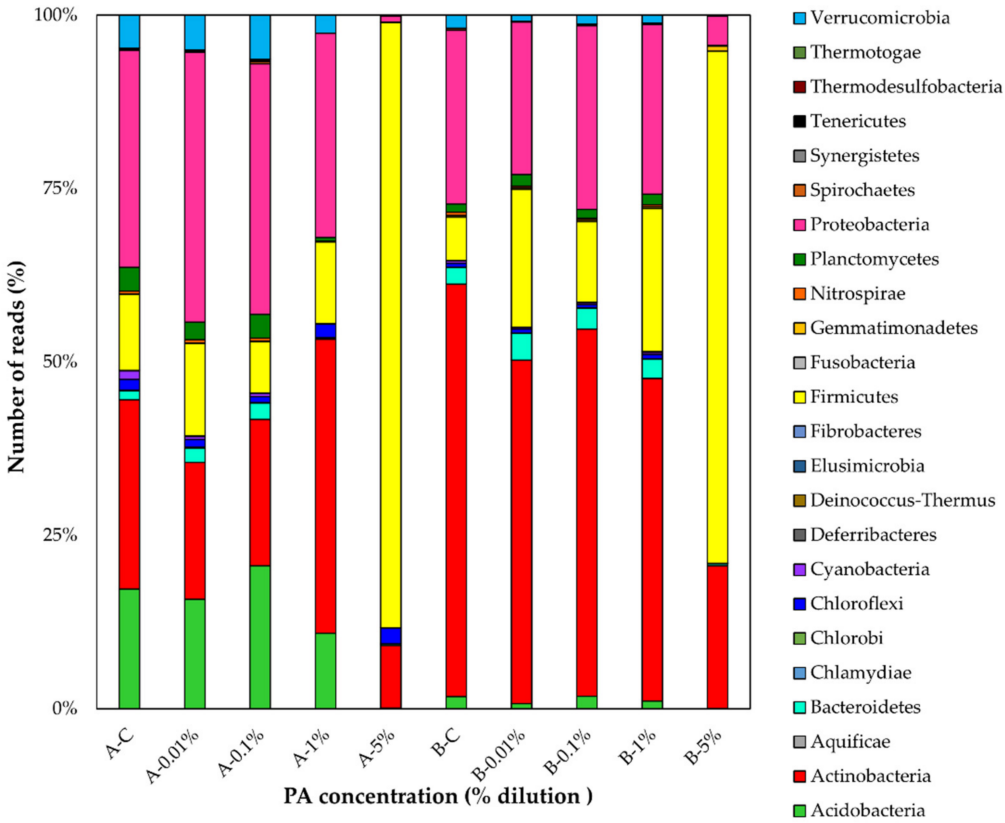

Figure 2. The relative abundance of the most abundant bacterial phyla in Soil A and B spiked with different concentrations of PA (control (C), 0.01%, 0.1%, 1%, and 5%).

3.4. Changes in Plant Growth Promoting Bacteria (PGPB) in Response to PA

We examined the effect of PA on the selected plant growth-promoting rhizobacterial genera that are reported as beneficial plant bacterial genera in Australia [25]. The results for soils A and B are presented in Figure 3. The relative abundance of plant growth-promoting bacterial genera such as *Acetobacter*, *Azospirillum*, *Bacillus*, *Beijerinckia*, *Bradyrhizobium*, *Corynebacterium*, *Herbaspirillum*, *Mesorhizobium*, *Micromonospora*, *Pseudomonas*, and *Rhizobium* was noted in both soils amended with 0.01% and 0.1% PA. The most abundant plant growth-promoting bacterial genera in soil A and soil B were *Bacillus* and *Bradyrhizobium*, which are important in agriculture. The most abundant genus *Bacillus* is a Gram-positive bacterium that is ubiquitous. The next most abundant bacterial genus *Bradyrhizobium* is Gram-negative and capable of fixing atmospheric nitrogen and making it available to plants. Among the plant growth-promoting bacterial genera, the number of reads percentage for the genus *Pseudomonas* was higher at 0.01% of PA than the control. The other PGPB genera, such as *Azospirillum*, *Acetobacter*, *Bradyrhizobium*, *Mesorhizobium*, *Pseudomonas*, and *Rhizobium*, increased in soil A with PA concentrations up to 1%. In soil B, the PGPB genera *Acetobacter*, *Bacillus*, *Herbaspirillum*, and *Pseudomonas* increased compared to the control at all PA levels.

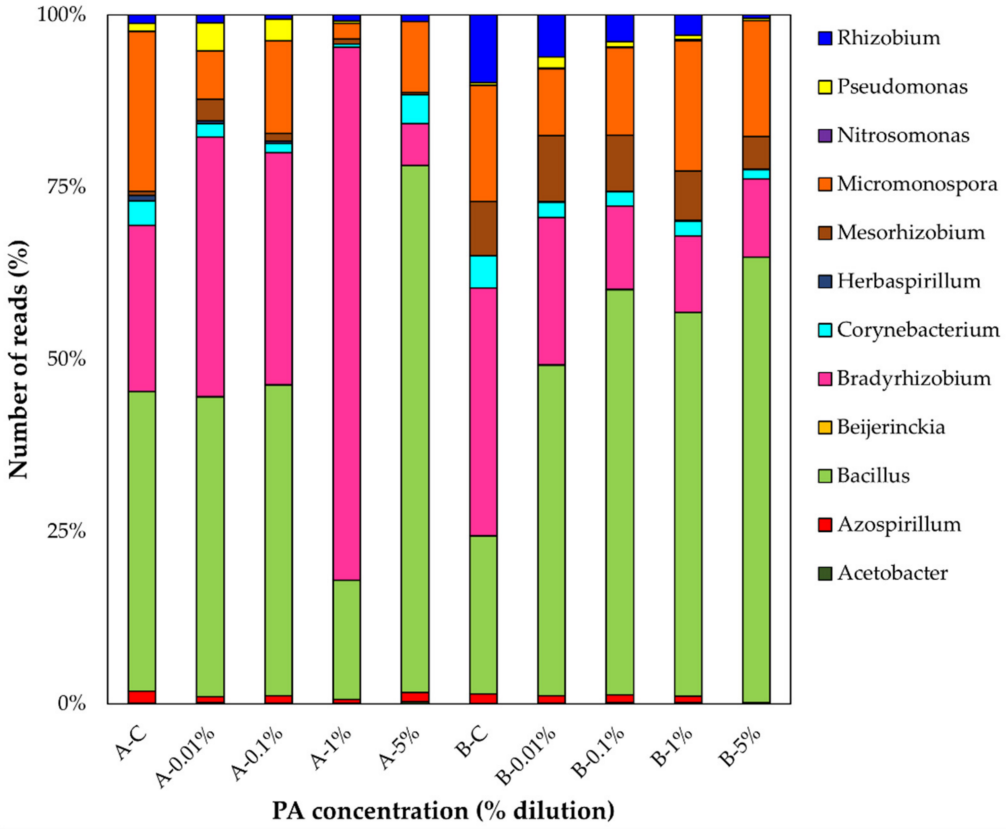

Figure 3. The relative abundance of the important plant growth-promoting rhizobacteria in Soil A and B spiked with different concentrations of PA (Control (C), 0.01%, 0.1%, 1%, and 5%).

3.5. Predicted Functional Features in PA Amended Soils

The functional capacity of the microbial communities in both soils with different PA concentrations were predicted by the PICRUSt, v1.1.3 based on OTUs. The predicted functional pathways were classified based on the cellular processes, environmental information processing, genetic information processing, and metabolism level 2 (Figure 4). The functional categories related to amino acid metabolism, energy metabolism, lipid metabolism, and the biosynthesis of other secondary metabolites were enriched in soils A and B spiked with 0.01% PA. Notably, the most metabolism-associated functional categories

were predominant in the PA spiked soil with 0.01% and 0.1%. Interestingly, the functional pathways—cell growth and death, and xenobiotic biodegradation and metabolism, were predominant in soil A, whereas this was not significant in soil B spiked with 5% PA.

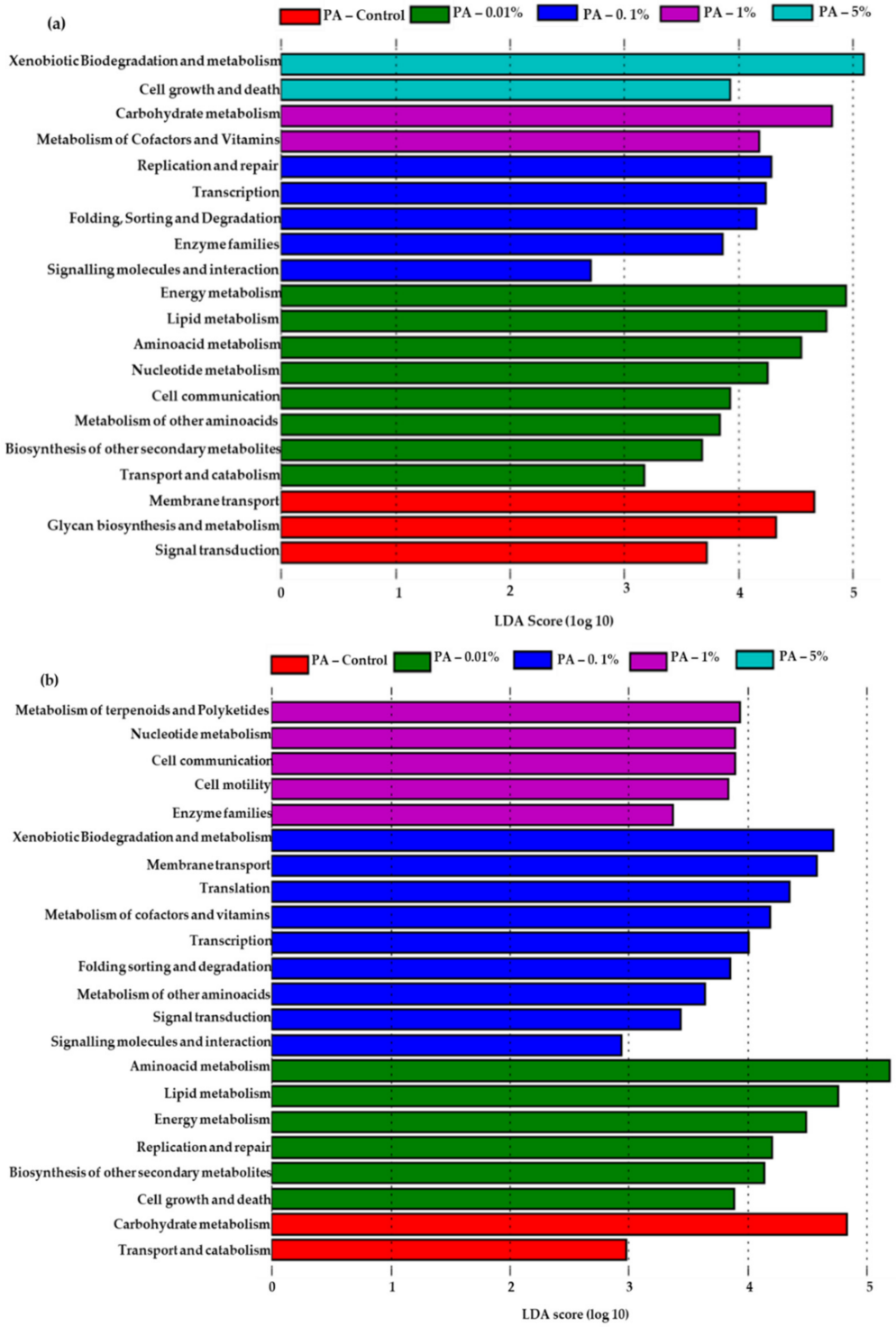

Figure 4. Prediction of the predominant functional potential of bacterial communities using LEfSe analysis for soil A (**a**) and soil B (**b**).

3.6. The Response of the Microbial Communities to Soil Properties

The response of bacterial phyla to PA amendment in soil and the soil properties were analyzed using CCA (Figure 5). Parameters including pH, EC, soil PA concentration, and bacterial phylae were selected for CCA based on the significance test. The analysis revealed that the chosen variables could explain 86% of the variation in bacterial phylae. The first canonical axis (Axis 1) positively correlated with pH and EC but negatively correlated with soil DHA. The second canonical axis (Axis 2) positively correlated with soil PA concentration and DHA. Therefore, this study shows that soil pH, EC, and PA concentration play a significant role in determining the bacterial community structure in the soil.

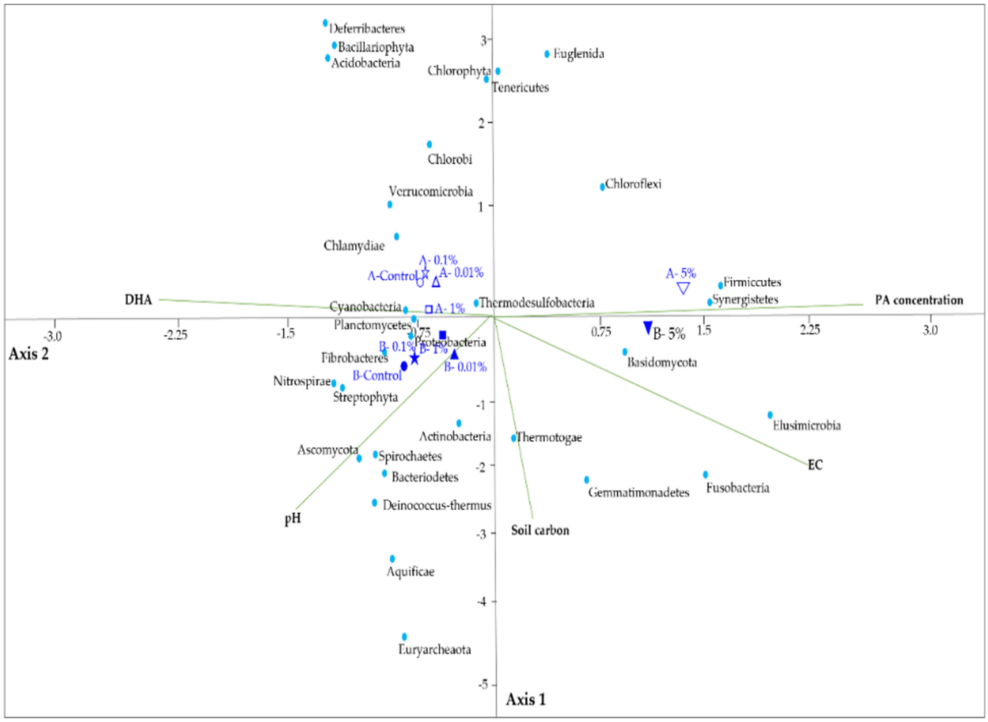

Figure 5. Canonical correspondence analysis (CCA) correlating environmental factors and bacterial phylum of all samples (Circle: control group, triangle: 0.01%, star: 0.1%, square: 1%, and inverted triangle: 5%). *Axis1* eigen value 0.54 (85.65%), $p = 0.004$; *Axis* 2 eigen value 0.08 (12.54%), $p = 0.006$.

3.7. Changes in the Microbial Community Structure in Response to PA Concentration

The principal component analysis (PCA) shows the difference in the microbial community structure in two different types of soils amended with a range of PA concentrations (Figure 6). The 5% PA spiked samples were well separated from the low PA concentration group in both soils. Among the low concentration spiked group, in soil A, the 0.01% PA sample was distinctly different from the rest, whereas in soil B, all samples (0.01, 0.1, and 1%) and the control were grouped. The results show that PA concentration in the soil impacted the bacterial community in both soils, though the extent of the impact appears different.

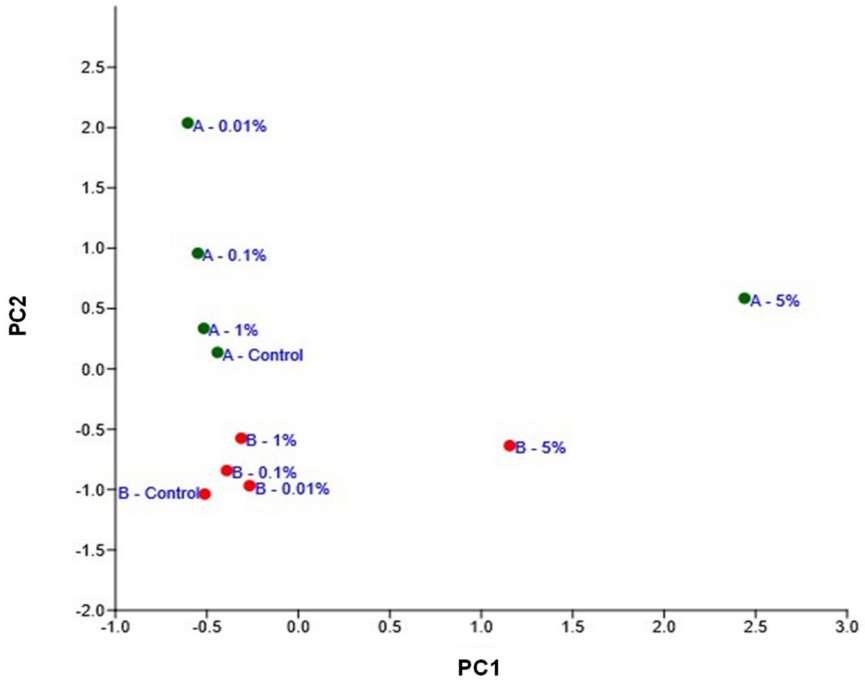

Figure 6. Principal component analysis (PCA) of microbial communities obtained from spiked and control samples from both soils. PC1 eigen value 1.46 (87%); PC2 eigen value 1.08 (6%).

4. Discussion

Many soil properties, such as pH and soil organic matter, have influenced soil microbial activities [26]. Additionally, soil pH is reportedly the dominant factor among soil parameters in determining plant growth and yield and the adsorption and transport of toxic heavy metals from soil to plants [27,28]. In the present study, no significant changes in soil pH were observed for 0.01 and 0.1% PA amendments; however, the pH reduced significantly at 1 and 5% PA. Additionally, the CCA biplot analysis suggests that soil pH and DHA had the most significant impact on the microbial community structure. Past studies have also reported the influence of soil pH on microbial composition [29,30]. The results from the present study on the impact of soil acidification were further expanded by measuring soil dehydrogenase activity, which is an excellent indicator of the microbial oxidative activity in soils [31]. A significant variation in soil dehydrogenase activity was observed between the soils spiked with lower PA concentrations (0.01 and 0.1%) and the higher PA spiked soils (1 and 5%). The soil spiked with high PA concentrations (1 and 5%) exhibited a significant reduction in pH, which presumably led to a substantial decrease in the bacterial community compared to their control (control for soil A and control for soil B). In contrast, PA spiked at lower concentrations (0.01 and 0.1%) improved the bacterial community composition compared to their control. These results show that PA concentration in the soil is an important factor in altering soil pH and determining microbial diversity.

The alpha diversity analysis was performed on the data to draw meaningful conclusions on the results obtained from the Illumina MiSeq sequencing. This analysis reveals the microbial community's correlations concerning the number of taxonomic groups and their distribution abundance [32]. In the present study, the calculated values of Alpha diversity, species richness Chao-1, Taxa_S, and Shannon index confirmed an increase in bacterial diversity in soils amended with low PA concentrations (0.01 and 0.1%). In contrast, in the soils spiked with 5% PA, a significant reduction in bacterial diversity was observed. This reduction in bacterial diversity is mainly due to the microorganisms' inability to survive under stress caused by PA application. This result is similar to the earlier studies on reducing bacterial diversity in response to environmental factors such as pH, temperature, humidity, and heavy metal contamination [33–35].

In this study, the relative abundance of bacterial phyla and the selected PGPB were analyzed at all test concentrations to obtain a deeper understanding of PA on soil health. Proteobacteria was the most abundant bacterial phylum, followed by *Actinobacteria* and *Acidobacteria* in soil A. This outcome is consistent with earlier findings. *Proteobacteria* was reported as the most abundant bacterial phyla in the soil, even when the diversity was analyzed by different techniques such as microarrays, clone libraries, and pyrosequencing [36]. *Proteobacteria* is the major phylum of Gram-negative aerobic or facultatively anaerobic bacteria, which includes *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* classes. Generally, the phylum *Proteobacteria* contains bacterial species that are known to thrive in low nutrient levels. In addition, the phylum *Proteobacteria* includes legume nodule endophytes [37], as well as rhizobial endosymbionts [38]. Additionally, Proteobacteria was the highest proportion of bacteria within the plant growth promotors. The agriculturally important plant growth-promoting bacterial genera, including *Bradyrhizobium, Azospirillum*, *Pseudomonas*, *Mesorhizobium*, *Rhizobium*, *Herbaspiriluum*, *Acetobacter*, *Beijerinckia*, and *Nitrosomonas*, were observed in this study.

The next abundant phylum is *Actinobacteria*, which is the major group of Gram-positive bacteria. This bacterial phylum is of greater importance in agriculture and forestry due to its contributions to the soil systems [39]. Most of the bacterial genera from the phylum *Actinobacteria* have high cellulose and hemicellulose degrading enzymes and thus help decompose organic matter and facilitate nutrient uptake by the plants [40]. The phylum *Firmicutes* contains spore-forming bacterial genera adapted to survive extreme conditions [41]. It was found in this study that the phylum *Firmicutes* was abundant in both soils with 5% PA concentration. These results suggest that the higher concentration of PA reduced soil pH (4.0 for soil A and 5.1 for soil B) and increased stress, which led to the abundance of phylum *Firmicutes.*

The PGPB is a group of bacterial communities that promotes plant growth and development through the production of various phytohormones such as Indole acetic acid (IAA), gibberellic acid (GA), and cytokinins [42]. The relative abundance of the selected PGPB genera in soil A and B was closely examined. The results revealed that PGPB genera *Bacillus* and *Bradyrhizobium* exhibited high relative abundance in both soils. Generally, the plant beneficial *Bacillus* genus is spore-forming, rod-shaped bacteria associated with roots or rhizosphere and develop biofilms to increase plant growth by enhancing plant-available form of nutrients in the rhizosphere, control disease-causing pathogenic microbial growth, and induce pest defense systems [43,44]. This study shows that the relative abundance of *Bacillus* genera was high in soils amended with 5% PA, which is in line with the reported ability of *Bacillus* to survive adverse conditions; in this case, the stress caused by higher PA concentration. *Bradyrhizobium*, which plays a vital role in fixing atmospheric nitrogen and enhancing plant growth and development [45], was higher in soil spiked with 0.01% PA. The genus *Pseudomonas* was reportedly linked to a wide range of processes involving plant growth promotion, disease control, nutrient cycling, nitrogen fixation, and bioremediation [46]. The increase in *Pseudomonas* genus compared to the control at lower concentrations in soils A and B suggests the positive effect of PA application in agricultural lands.

Typically, different microbial species could intervene in similar functions in a microbial community, which led to changes in the microbial structure and community over time [47]. Therefore, it is crucial to predict the functional capacity of microbial communities. To achieve this, the computational program PICRUSt has been used to predict the gene function from the 16S rRNA sequences [48,49]. Interestingly, in the present study, the PICRUSt analysis predicted that, at lower (0.01 and 0.1%) PA spiked concentrations, the soil bacterial communities had elevated metabolic potential with predominant pathways such as amino acid, energy, and lipid. This observation indicated that PA at lower concentrations stimulated the bacteria involved in increasing the bioavailability of nutrients and promoting plant growth. Several other studies using PICRUSt analysis revealed metabolism as the primary component of the functional categories in most of the rhizospheric microbial

communities that is primarily involved in plant growth and development [50–52]. The predominance of the cellular processes, such as cell growth and death, in soil A spiked with 5% PA, could be due to the stress response by the microbial community due to the significant reduction in soil pH. The results from the present study suggest that PA could be highly beneficial in agricultural applications as a means of controlling pathogens and pests at relatively high concentrations and effectively promoting plant growth and yield at lower concentrations. Further studies on the role of PA in enhancing soil health and crop productivity need to be conducted at the field level.

5. Conclusions

PA application caused a distinct change in the microbial composition between low (0.01 and 0.1%) and high (1 and 5%) PA amended soils. PA application up to 0.1% enhanced microbial diversity and the abundance of beneficial microbes such as *Bacillus*, *Bradyrhizobium*, *Azospirillum*, *Pseudomonas*, *Micromonospora Mesorhizobium*, *Rhizobium*, *Herbaspirillum*, *Acetobacter*, *Beijerinckia*, and *Nitrosomonas*. Such a high abundance of the beneficial PGPB at lower PA concentrations could positively relate to better soil quality, indicating enhanced plant growth and yield. In contrast, higher concentrations of PA (5%) promoted the abundance of spore-forming bacterial genera such as *Bacillus*, which is responsible for defensive action such as reducing the pathogenic bacteria and pest control. Overall, this study demonstrates PA's potential for improving soil biological health by enhancing the beneficial plant growth-promoting bacteria.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/soilsystems6010010/s1, Figure S1: Rarefaction curve of bacterial 16S rRNA genes from (a) soil A spiked with and without PA and (b) soil B spiked with and without PA.

Author Contributions: Conceptualization, A.K.S. and M.M.; methodology, A.K.S. and L.P.; validation, A.K.S., L.P. and M.M.; formal analysis, A.K.S. and L.P.; investigation, A.K.S. and L.P.; data curation, A.K.S., L.P. and K.M.; writing—original draft preparation, A.K.S., L.P., K.M. and M.M.; writing—review and editing, A.K.S., L.P., K.M. and M.M.; supervision, M.M. All authors have read and agreed to the published version of the manuscript.

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References

1. Zheng, H.; Sun, C.; Hou, X.; Wu, M.; Yao, Y.; Li, F. Pyrolysis of *Arundo donax* L. to produce pyrolytic vinegar and its effect on the growth of dinoflagellate *Karenia brevis*. *Bioresour Technol.* **2018**, *247*, 273–281. [CrossRef] [PubMed]
2. Wu, Q.; Zhang, S.; Hou, B.; Zheng, H.; Deng, W.; Liu, D.; Tang, W. Study on the preparation of wood vinegar from biomass residues by carbonization process. *Bioresour. Technol.* **2015**, *179*, 98–103. [CrossRef] [PubMed]
3. Pimenta, A.S.; Fasciotti, M.; Monteiro, T.V.C.; Lima, K.M.G. Chemical composition of pyroligneous acid obtained from Eucalyptus GG100 clone. *Molecules* **2018**, *23*, 426. [CrossRef] [PubMed]
4. de Souza Araújo, E.; Pimenta, A.; Feijó, F.; Castro, R.; Fasciotti, M.; Monteiro, T.; De Lima, K. Antibacterial and antifungal activities of pyroligneous acid from wood of *Eucalyptus urograndis* and *Mimosa tenuiflora*. *J. Appl. Microbiol.* **2018**, *124*, 85–96. [CrossRef]
5. Oramahi, H.A.; Yoshimura, T.; Diba, F.; Setyawati, D. Antifungal and antitermitic activities of wood vinegar from oil palm trunk. *J. Wood Sci.* **2018**, *64*, 311–317. [CrossRef]
6. Wei, Q.; Ma, X.; Dong, J. Preparation, chemical constituents and antimicrobial activity of pyroligneous acids from walnut-tree branches. *J. Anal. Appl. Pyrolysis* **2010**, *87*, 24–28. [CrossRef]

7. Lashari, M.S.; Liu, Y.; Li, L.; Pan, W.; Fu, J.; Pan, G.; Zheng, J.; Zheng, J.; Zhang, X.; Yu, X. Effects of amendment of biochar-manure compost in conjunction with pyroligneous solution on soil quality and wheat yield of a salt-stressed cropland from Central China Great Plain. *Field Crops Res.* **2013**, *144*, 113–118. [CrossRef]
8. Hagner, M.; Penttinen, O.P.; Tiilikkala, K.; Setälä, H. The effects of biochar, wood vinegar and plants on glyphosate leaching and degradation. *Eur. J. Soil Biol.* **2013**, *58*, 1–7. [CrossRef]
9. Li, Y.; Pei, G.; Qiao, X.; Zhu, Y.; Li, H. Remediation of cadmium contaminated water and soil using vinegar residue biochar. *Environ. Sci. Pollut. Res.* **2018**, *25*, 15754–15764. [CrossRef]
10. Luo, X.; Wang, Z.; Meki, K.; Wang, X.; Liu, B.; Zheng, H.; You, X.; Li, F. Effect of co-application of wood vinegar and biochar on seed germination and seedling growth. *J. Soils Sediment* **2019**, *19*, 3934–3944. [CrossRef]
11. Bhattacharyya, P.N.; Jha, D.K. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1327–1350. [CrossRef]
12. Ardakani, S.S.; Heydari, A.; Tayebi, L.; Mohammadi, M. Promotion of cotton seedlings growth characteristics by development and use of new bioformulations. *Int. J. Bot.* **2010**, *6*, 95–100. [CrossRef]
13. Pandey, S.; Gupta, S. ACC deaminase producing bacteria with multifarious plant growth-promoting traits alleviates salinity stress in French bean (*Phaseolus vulgaris*) plants. *Front. Microbiol.* **2019**, *10*, 1506.
14. Tariq, M.; Noman, M.; Ahmed, T.; Hameed, A.; Manzoor, N.; Zafar, M. Antagonistic features displayed by plant growth-promoting rhizobacteria (PGPR): A review. *J. Plant Sci. Phytopathol.* **2017**, *1*, 38–43.
15. Lin, Y.; Watts, D.B.; Kloepper, J.W.; Feng, Y.; Torbert, H.A. Influence of Plant Growth-Promoting Rhizobacteria on Corn Growth under Drought Stress. *Commun. Soil Sci. Plant Anal.* **2020**, *51*, 250–264. [CrossRef]
16. Steiner, C.; Das, K.C.; Garcia, M.; Förster, B.; Zech, W. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. *Pedobiologia* **2008**, *51*, 359–366. [CrossRef]
17. Cole, J.R.; Wang, Q.; Cardenas, E.; Fish, J.; Chai, B.; Farris, R.J.; Kulam-Syed-Mohideen, A.; McGarrell, D.M.; Marsh, T.; Garrity, G.M. The Ribosomal Database Project: Improved alignments and new tools for rRNA analysis. *Nucleic Acids Res.* **2009**, *37*, D141–D145. [CrossRef]
18. Uroz, S.; Buée, M.; Murat, C.; Frey-Klett, P.; Martin, F. Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. *Environ. Microbiol. Rep.* **2010**, *2*, 281–288. [CrossRef]
19. Miller, W.P.; Miller, D.M. A micro-pipette method for soil mechanical analysis. *Commun. Soil Sci. Plant Anal.* **1987**, *18*, 1–15. [CrossRef]
20. Sivaram, A.K.; Logeshwaran, P.; Subashchandrabose, S.R.; Lockington, R.; Naidu, R.; Megharaj, M. Comparison of plants with C3 and C4 carbon fixation pathways for remediation of polycyclic aromatic hydrocarbon contaminated soils. *Sci. Rep.* **2018**, *8*, 2100. [CrossRef]
21. Casida, L.; Klein, D.; Santoro, T. Soil dehydrogenase activity. *Soil Sci.* **1964**, *98*, 371–376. [CrossRef]
22. Meyer, F.; Bagchi, S.; Chaterji, S.; Gerlach, W.; Grama, A.; Harrison, T.; Paczian, T.; Trimble, W.L.; Wilke, A. MG-RAST version 4—Lessons learned from a decade of low-budget ultra-high-throughput metagenome analysis. *Brief. Bioinform.* **2019**, *20*, 1151–1159. [CrossRef]
23. Langille, M.G.; Zaneveld, J.; Caporaso, J.G.; McDonald, D.; Knights, D.; Reyes, J.A.; Clemente, J.C.; Burkepile, D.E.; Thurber, R.L.; Knight, R.; et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat. Biotechnol.* **2013**, *31*, 814–821. [CrossRef]
24. Hammer, Ø.; Harper, D.A.; Ryan, P.D. PAST-palaeontological statistics, ver. 1.89. *Palaeontol. Electron.* **2001**, *4*, 1–9.
25. Gupta, V.V.S.R. Beneficial microorganisms for sustainable agriculture. *Microbiol. Aust.* **2012**, *33*, 113–115. [CrossRef]
26. Malik, A.A.; Puissant, J.; Buckeridge, K.M.; Goodall, T.; Jehmlich, N.; Chowdhury, S.; Gweon, H.S.; Peyton, J.M.; Mason, K.E.; van Agtmaal, M. Land use driven change in soil pH affects microbial carbon cycling processes. *Nat. Commun.* **2018**, *9*, 3591. [CrossRef]
27. Sherene, T. Mobility and transport of heavy metals in polluted soil environment. *Biol. Forum—Int. J.* **2010**, *2*, 112–121.
28. Yang, X.; Liu, J.; McGrouther, K.; Huang, H.; Lu, K.; Guo, X.; He, L.; Lin, X.; Che, L.; Ye, Z. Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil. *Environ. Sci. Pollut. Res.* **2016**, *23*, 974–984. [CrossRef]
29. Lauber, C.L.; Hamady, M.; Knight, R.; Fierer, N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* **2009**, *75*, 5111–5120. [CrossRef]
30. Rousk, J.; Bååth, E.; Brookes, P.C.; Lauber, C.L.; Lozupone, C.; Caporaso, J.G.; Knight, R.; Fierer, N. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* **2010**, *4*, 1340–1351. [CrossRef]
31. Wolińska, A.; Stępniewska, Z. Dehydrogenase activity in the soil environment. In *Dehydrogenases*; Canuta, R.A., Ed.; IntechOpen: London, UK, 2012; pp. 183–210.
32. Willis, A.D. Rarefaction, alpha diversity, and statistics. *Front. Microbiol.* **2019**, *10*, 2407. [CrossRef] [PubMed]
33. Luo, L.; Xie, L.; Jin, D.; Mi, B.; Wang, D.; Li, X.; Dai, X.; Zou, X.; Zhang, Z.; Ma, Y. Bacterial community response to cadmium contamination of agricultural paddy soil. *Appl. Soil Ecol.* **2019**, *139*, 100–106. [CrossRef]
34. Mahbub, K.R.; Subashchandrabose, S.R.; Krishnan, K.; Naidu, R.; Megharaj, M. Mercury alters the bacterial community structure and diversity in soil even at concentrations lower than the guideline values. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 2163–2175. [CrossRef] [PubMed]

35. Mandakovic, D.; Rojas, C.; Maldonado, J.; Latorre, M.; Travisany, D.; Delage, E.; Bihouée, A.; Jean, G.; Díaz, F.P.; Fernández-Gómez, B. Structure and co-occurrence patterns in microbial communities under acute environmental stress reveal ecological factors fostering resilience. *Sci. Rep.* **2018**, *8*, 5875. [CrossRef]
36. Spain, A.M.; Krumholz, L.R.; Elshahed, M.S. Abundance, composition, diversity, and novelty of soil *Proteobacteria*. *ISME J.* **2009**, *3*, 992–1000. [CrossRef]
37. Sánchez-Cañizares, C.; Jorrín, B.; Durán, D.; Nadendla, S.; Albareda, M.; Rubio-Sanz, L.; Lanza, M.; González-Guerrero, M.; Prieto, R.I.; Brito, B. Genomic diversity in the endosymbiotic bacterium *Rhizobium leguminosarum*. *Genes* **2018**, *9*, 60. [CrossRef]
38. Jiménez-Zurdo, J.I.; Valverde, C.; Becker, A. Insights into the noncoding RNome of nitrogen-fixing endosymbiotic *α-proteobacteria*. *Mol. Plant-Microbe Interact.* **2013**, *26*, 160–167. [CrossRef]
39. Nemergut, D.R.; Cleveland, C.C.; Wieder, W.R.; Washenberger, C.L.; Townsend, A.R. Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. *Soil Biol. Biochem.* **2010**, *42*, 2153–2160. [CrossRef]
40. López-Mondéjar, R.; Zühlke, D.; Becher, D.; Riedel, K.; Baldrian, P. Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. *Sci. Rep.* **2016**, *6*, 25279. [CrossRef]
41. Teixeira, L.C.R.S.; Peixoto, R.S.; Cury, J.C.; Sul, W.J.; Pellizari, V.H.; Tiedje, J.; Rosado, A.S. Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. *ISME J.* **2010**, *4*, 989–1001. [CrossRef]
42. Kloepper, J.; Gutierrez-Estrada, A.; McInroy, J. Photoperiod regulates elicitation of growth promotion but not induced resistance by plant growth-promoting rhizobacteria. *Can. J. Microbiol.* **2007**, *53*, 159–167. [CrossRef]
43. García-Fraile, P.; Menéndez, E.; Rivas, R. Role of bacterial biofertilizers in agriculture and forestry. *AIMS Bioeng.* **2015**, *2*, 183–205. [CrossRef]
44. Kang, S.M.; Radhakrishnan, R.; Lee, I.J. *Bacillus amyloliquefaciens* subsp. *plantarum* GR53, a potent biocontrol agent resists Rhizoctonia disease on Chinese cabbage through hormonal and antioxidants regulation. *World J. Microbiol. Biotechnol.* **2015**, *31*, 1517–1527. [CrossRef]
45. Queiroz Rego, C.H.; Cardoso, F.B.; Carina da Silva Cândido, A.; Teodoro, P.E.; Alves, C.Z. Co-inoculation with *Bradyrhizobium* and *Azospirillum* increases yield and quality of soybean seeds. *J. Agron.* **2018**, *110*, 2302–2309. [CrossRef]
46. de Souza, J.T. Distribution, Diversity, and Activity of Antibiotic-Producing *Pseudomonas* spp. Ph.D. Thesis, Wageningen University, Wageningen, The Netherlands, 2002.
47. Louca, S.; Jacques, S.M.; Pires, A.P.; Leal, J.S.; Srivastava, D.S.; Parfrey, L.W.; Farjalla, V.F.; Doebeli, M. High taxonomic variability despite stable, functional structure across microbial communities. *Nat. Ecol. Evol.* **2016**, *1*, 0015. [CrossRef]
48. Douglas, G.M.; Beiko, R.G.; Langille, M.G. Predicting the functional potential of the microbiome from marker genes using PICRUSt. In *Microbiome Analysis*; Humana Press: New York, NY, USA, 2018; pp. 169–177.
49. Ortiz-Estrada, Á.M.; Gollas-Galván, T.; Martínez-Córdova, L.R.; Martínez-Porchas, M. Predictive functional profiles using metagenomic 16S rRNA data: A novel approach to understanding the microbial ecology of aquaculture systems. *Rev. Aquac.* **2019**, *11*, 234–245. [CrossRef]
50. Chen, Y.; Tian, W.; Shao, Y.; Li, Y.J.; Lin, L.A.; Zhang, Y.J.; Han, H.; Chen, Z.J. Miscanthus cultivation shapes rhizosphere microbial community structure and function as assessed by Illumina MiSeq sequencing combined with PICRUSt and FUNGUIld analyses. *Arch. Microbiol.* **2020**, *202*, 1157–1171. [CrossRef]
51. Mahoney, A.K.; Yin, C.; Hulbert, S.H. Community structure, species variation, and potential functions of rhizosphere-associated bacteria of different winter wheat (*Triticum aestivum*) cultivars. *Front. Plant Sci.* **2017**, *8*, 132. [CrossRef]
52. Pii, Y.; Borruso, L.; Brusetti, L.; Crecchio, C.; Cesco, S.; Mimmo, T. The interaction between iron nutrition, plant species, and soil type shapes the rhizosphere microbiome. *Plant Physiol. Biochem.* **2016**, *99*, 39–48. [CrossRef]