

Article

# Structure of Bacterial Communities in Phosphorus-Enriched Rhizosphere Soils

Yuwei Hu <sup>1,2,3</sup> , Changqun Duan <sup>1,4,\*</sup> , Denggao Fu <sup>1,4,\*</sup>, Xiaoni Wu <sup>1,4</sup>, Kai Yan <sup>5</sup>, Eustace Fernando <sup>6</sup>, Samantha C. Karunaratna <sup>2</sup> , Itthayakorn Promputtha <sup>7,8</sup>, Peter E. Mortimer <sup>2</sup> and Jianchu Xu <sup>2</sup>

<sup>1</sup> School of Ecology and Environmental Sciences & Yunnan Key Laboratory for Plateau Mountain Ecology and Restoration of Degraded Environments, Yunnan University, Kunming 650091, China; huyuwei@mail.kib.ac.cn (Y.H.); wuxiaoxiaoni@163.com (X.W.)

<sup>2</sup> CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China; samantha@mail.kib.ac.cn (S.C.K.); peter@mail.kib.ac.cn (P.E.M.); jxu@mail.kib.ac.cn (J.X.)

<sup>3</sup> Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

<sup>4</sup> Yunnan International Joint Research Center of Plateau Lake Ecological Restoration and Watershed Management, Yunnan University, Kunming 650091, China

<sup>5</sup> College of Resources and Environment, Yunnan Agricultural University, Kunming 650201, China; ecoyankai@126.com

<sup>6</sup> Department of Biological Sciences, Faculty of Applied Sciences, Rajarata University, Mihintale 50300, Sri Lanka; eustace6192@as.rjt.ac.lk

<sup>7</sup> Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand; ppam118@hotmail.com

<sup>8</sup> Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

\* Correspondence: chqduan@ynu.edu.cn (C.D.); ddfu@ynu.edu.cn (D.F.); Tel.: +86-871-65032753 (C.D.)

Received: 29 August 2020; Accepted: 9 September 2020; Published: 14 September 2020



**Abstract:** Although phytoremediation is the main method for P-removal and maintaining ecosystem balance in geological phosphorus-enriched soils (PES), little is known about the structure and function of microbial communities in PES. Interactions between plants and soil microorganisms mainly occur in the rhizosphere. The aim of this work was to investigate the composition and diversity of bacterial communities found in rhizosphere soils associated with the following three dominant plant species: *Erianthus rufipilus*, *Coriaria nepalensis*, and *Pinus yunnanensis*. In addition, we compared these rhizosphere bacterial communities with those derived from bulk soils and grassland plots in PES from the Dianchi Lake basin of southwestern China. The Illumina MiSeq platform for high-throughput sequencing of 16S rRNA was used for the taxonomy and the analysis of soil bacterial communities. The results showed higher bacterial diversity and nutrient content in rhizosphere soils as compared with bulk soils. Rhizosphere bacteria were predominantly comprised of *Proteobacteria* (24.43%) and *Acidobacteria* (21.09%), followed by *Verrucomicrobia* (19.48%) and *Planctomycetes* (9.20%). A comparison of rhizosphere soils of the selected plant species in our study and the grassland plots showed that *Acidobacteria* were the most abundant in the rhizosphere soil of *E. rufipilus*; *Bradyrhizobiaceae* and *Rhizobiaceae* in the order *Rhizobiales* from *C. nepalensis* were found to have the greatest abundance; and *Verrucomicrobia* and *Planctomycetes* were in higher abundance in *P. yunnanensis* rhizosphere soils and in grassland plots. A redundancy analysis revealed that bacterial abundance and diversity were mainly influenced by soil water content, soil organic matter, and total nitrogen.

**Keywords:** phosphorus-enriched rhizosphere soils; phosphate; phytoremediation; bacterial communities; high-throughput sequencing

## 1. Introduction

Soil microbial communities are responsible for many ecosystem functions, including decomposition, nutrient cycling, nitrogen fixation, and soil formation [1–3]. Microorganisms that inhabit different soil types exhibit astonishing diversity and wide genetic variability even within species, particularly with respect to their metabolic pathways and host-interactive capabilities [4]. The majority of microbial populations in soils are concentrated in nutrient-rich niches such as rhizospheres that offer a constant supply of easily utilizable nutrients [5,6]. Recent advances in soil ecosystem microbial ecology have highlighted the interactions that occur among distinct microbial groups, now known as the “microbiome” [7].

Soil microbial communities are influenced by a series of biotic and abiotic factors [8], and specific microbial groups show high levels of diversity in different soil ecosystems [9,10]. Exogenous nutritional inputs can change structures of soil bacterial communities, promoting plant phosphorus uptake in rhizospheres [11,12]. Moreover, plant species’ richness and diversity affect phosphorus uptake in rhizospheres through interactions between plant and soil bacterial communities [13]. Rhizospheric effects influence bacterial communities in rhizospheres, and therefore the diversity of rhizosphere microbial communities differs from that of the bulk soil [14].

Several studies have reported that phosphorus is a limiting factor responsible for shaping structures of soil bacterial communities across different soil habitats [15,16]. Phosphorus-rich mountains are also vulnerable and face severe degradation due to mining and other human disturbances [17]. Phosphorus removal from phosphorus-enriched soils (PES), such as in agricultural lands, grasslands, and phosphate mining regions is a major cause of eutrophication in aquatic environments and an increasing environmental problem worldwide [18]. The Dianchi Lake basin is an important industrial and agricultural region in southwest China, home to numerous PES [19,20]. Dianchi Lake is a typical plateau lake in the basin currently undergoing severe eutrophication [21]. Previous studies regarding the restoration of PES have mainly focused on the development of dominant plant communities which is a cost-effective method [17,22].

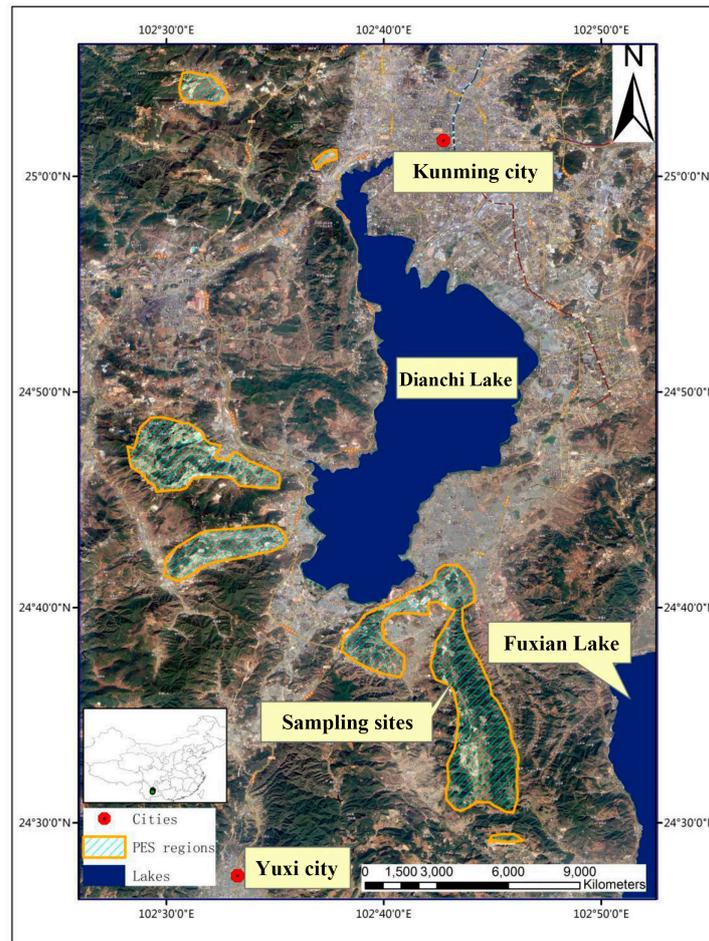
Rhizosphere microbes play important roles for sustaining ecosystems. For example, they accelerate phytoremediation processes and enhance plant ecosystem productivity [23]. Microbes inhabiting rhizospheres help plants grow and function more effectively through bolstering water retention, disease resistance, and nutrient acquisition [24]. Many studies have also shown that rhizosphere microbes promote soil remediation and restoration. Crops have been observed to reduce salt content and increase bacterial rhizosphere diversity in coastal saline soils, improving soil quality [25]. In addition, the inoculation of plant growth-promoting bacteria can change rhizosphere bacterial communities and accelerate restoration processes in eroded desert soils [26]. Rhizosphere bacteria foster arsenic bioaccumulation by plants and then improve phytoremediation of arsenic-contaminated soils [27]. However, little is known about microbes in PES. Previous studies have investigated functional bacteria taxa in PES and have identified 377 isolates that exhibited phosphate-solubilizing potential [28], although biodiversity and distribution of bacteria still remain unclear. Understanding bacterial diversity and distribution is the first step towards harnessing functional taxa to improve phytoremediation and recalibrate restoration efforts. This work highlights the urgency of studying microbial community structures in rhizospheres to support the restoration of PES.

The main objectives of this study were to investigate divergences in structures of soil bacterial communities between rhizosphere and bulk soils of plants in PES as well as understand the environmental factors that affect bacterial communities. In previous studies on phosphorus-rich mountains, efforts have centered around understanding phytoremediation. This work reports for the first time the structure of PES microbial communities influenced by dominant plants of PES.

## 2. Materials and Methods

### 2.1. Study Area

The sampling was conducted on phosphorus-rich mountains near Dianchi Lake in central Yunnan, China (Figure 1). In this study area, the average annual temperature is 14.7 °C, and the annual temperature difference is 10–15 °C. The average annual rainfall is 985.5 mm, and the rainy season lasts from May to October, accounting for 84–90% of the annual rainfall. In this study area, the variation range of the total phosphorus concentration in the soil and the nitrogen/phosphorus ratio are 1.15–80.2 g/kg and 0.006–0.98, respectively, [29] both of which are typical in a phosphorus-rich region.



**Figure 1.** Geological phosphorus-rich regions around Dianchi Lake basin and sampling sites.

Most vegetation in the research area comprises natural secondary forests, and the original subtropical semi-moist evergreen broad-leaved forest has been almost completely destroyed [30]. The dominant vegetation species identified for the study were *Pinus yunnanensis*, *Coriaria nepalensis*, *Eupatorium adenophora*, *Erianthus rufipilus*, *Rumex hastatus*, *Keteleeria evelyniana*, *Pinus armandii*, and *Eucalyptus urophylla*. This study selected three dominant plants for research, i.e., *E. rufipilus*, *C. nepalensis*, and *P. yunnanensis*, based on the investigation of previous research. *E. rufipilus* belongs to the family *Gramineae*, is a drought-tolerant herbaceous plant growing between elevation 1300–2400 m, has a plant density of 2610 plant/ha and a P removal potential of 22.78 kg/ha in PES. *C. nepalensis* belongs to the family *Coriariaceae*, is an actinorhizal N-fixing species growing between elevation 400–3200 m, has a plant density of 670 plant/ha and a P removal potential of 16.91 kg/ha in PES. *P. yunnanensis* belongs to the family *Pinaceae* growing between elevation 400–3500 m, has a plant density

of 497 plant/ha, a P removal potential of 27.69 kg/ha in PES, and features secondary communities that suffer from disturbance and invasion [17,22].

## 2.2. Soil Sampling

Fieldwork was conducted in March 2016. This study was conducted during the dry season, when rhizosphere soil acquisition is feasible, while also limiting the occurrence of rain erosion on bacterial communities. A total of thirteen sites were selected for the study area. Sampling sites were selected according to target plant communities. The soil samples in thirteen sites (Table 1) included the following: (1) rhizosphere and bulk soil of *E. rufipilus* from three sites (Er-R and Er-B), (2) rhizosphere and bulk soil of *C. nepalensis* from three sites (Cn-R and Cn-B), (3) rhizosphere and bulk soil of *P. yunnanensis* from three sites (Py-R and Py-B), and (4) soils from grassland plots from four sites (CK) with removal of surface grass debris at a random 0–20 cm soil depth in the absence of vegetation coverage. We selected plots of 10,000 m<sup>2</sup> (100 × 100 m) for each site. Five subplots (5 × 5 m, with at least one target plant in each plot) were chosen for soil sampling using an S-shaped sampling method [31] within sampling sites. Rhizosphere soils were collected from the fine roots of *E. rufipilus*, *C. nepalensis*, and *P. yunnanensis*. Bulk soil samples were taken at a depth of 0–20 cm in the same plots as rhizosphere soils, and then mixed thoroughly. Rhizosphere soils were collected by gently shaking off the bulk soil that adhered to the roots at a depth of 0–20 cm, and then mixed well. Each soil sample was sieved through a 2 mm mesh to remove plant roots and other plant materials. Sampling equipment including mesh, scalpels, and spoons was cleaned with 95% alcohol, followed by heating using an alcohol lamp for 30 s. Soil collections among samples were conducted after all sampling equipment was sterilized and cooled down. All soil samples were tagged and sealed in valve bags from fine roots at 0–20 cm depth. At the same time, the soil temperature (Tem) of each plot was measured using soil temperature instruments for five replicates. Next, all 22 soil samples were manually homogenized and divided into three parts. The first portion was preserved within sterile centrifuge tubes (2 mL) and put in liquid nitrogen for DNA extraction and molecular analysis; the second portion was kept in plastic bags for water content measurement; and the third portion was stored at 4 °C refrigerator for 24 h, and then dried at 25 °C for chemical analysis.

**Table 1.** Information of soil samples.

Abbreviation	Vegetation	Soil Location	Number of Samples
Er-R	<i>Erianthus rufipilus</i>	Rhizosphere	3
Er-B		Bulk	3
Cn-R	<i>Coriaria nepalensis</i>	Rhizosphere	3
Cn-B		Bulk	3
Py-R	<i>Pinus yunnanensis</i>	Rhizosphere	3
Py-B		Bulk	3
CK	Grassland plots	Bulk	4

## 2.3. Soil Physicochemical Analysis

Chemical properties for all the 22 soil samples from the study area were analyzed based on the following method: Soil water content (SWC) was determined using the oven-drying method [32]. The pH value (pH) was determined in a 1:2 (soil to water ratio) using a pH meter with a standard combination of electrodes [33]. Soil organic matter content (SOM) was determined through the potassium dichromate sulfuric acid oxidation method [34]. Total nitrogen (TN) was measured using Kjeldahl's method as modified by Bremner and Mulvaney [35]. Alkali solution nitrogen (AN) was determined using the alkali soluble diffusion method [36]. Total phosphorus (TP) content was measured using the ammonium molybdate spectrophotometric method [37]. Concentrations of

available phosphorus (AP) were measured using the ammonium molybdate spectrophotometric method, following hydrochloric and sulfuric acid leaching. The value of nitrogen/phosphorus (N/P) was calculated by the ratio of total nitrogen to total phosphorus. All 22 soil samples from the four parts were used in chemical tests. Lab analyses were repeated three times, and final data were averaged.

#### 2.4. Illumina MiSeq for High-Throughput Sequencing of 16S rRNA

Soil samples stored in liquid nitrogen were used for DNA extraction. An Agilent High Sensitivity DNA Kit (Agilent Technologies Inc., Lexington, MA, USA) was used with 0.5 g of soil, according to the manufacturer's instructions. Extraction protocol followed the manufacturer's instructions with recommended modifications to enhance the efficiency of cell lysis. To assess the quality and purity of DNA, crude DNA extracts (2  $\mu$ L) of each sample were run on 2% agarose gel and analyzed using ultraviolet spectrophotometer (Eppendorf Corporation, Hamburg, Germany). Detection results of qualified DNA samples are shown in the Appendix A Table A1. Electrophoresis detection bands were single, indicating no degradation, and had no protein or RNA contamination.

Bacterial 16S rRNA genes were amplified using the following two primers: 520F (5-AYTGG GYDTAAAGNG-3) and 802R (5-TACNVGGGTATCTAATCC-3). PCR reactions were carried out in triplicate with 25  $\mu$ L of reaction mixture, comprising 8.75  $\mu$ L of sterilized ultrapure water, 5  $\mu$ L of Q5 reaction buffer, 5  $\mu$ L of Q5 GC high enhancer, 2  $\mu$ L of 2.5 mM dNTPs, 2  $\mu$ L of DNA template, 1  $\mu$ L of each primer, and 0.25  $\mu$ L Q5 polymerase. For the subsequent PCR cycling reaction, the following parameters were used: 98  $^{\circ}$ C and 2 min for initial denaturation, followed by 25 cycles of denaturation at 98  $^{\circ}$ C for 30 s, annealing at 50  $^{\circ}$ C for 30 s, and elongation at 72  $^{\circ}$ C for 30 s, followed by a final extension at 72  $^{\circ}$ C for 5 min. All the samples were amplified in triplicate and detected and quantified using a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen Corporation, Waltham, MA, USA). This step ensured that all detected DNA samples had obvious and single bands before the next experiments. After quantification, paired-end sequencing of amplicons from each reaction mixture was performed using the Illumina MiSeq platform [38]. Sequencing services were provided by Personal Biotechnology Co., Ltd. Shanghai, China. The raw sequencing data of bacteria were deposited in NCBI Sequence Read Archive (SRA) under accession number PRJNA662457.

#### 2.5. Data Analysis

At least three groups of repeats and 22 soil samples in total from the geological phosphorus-rich mountains were collected. There were seven sets of mixed samples (Er-R, Er-B, Cn-R, Cn-B, Py-R, Py-B and CK) (as seen in Table 1) for the comparative analysis of bacterial communities. The resulting data from each set of repeated samples were averaged. For the FASTQ format of paired-end sequences, we ensured the mean quality of the base was  $\geq$ Q20 and sequence length  $\geq$ 150 bp. FLASH was used to pair and connect the screened paired-end sequences in order to obtain high quality sequences [39]. UCLUST [40] was used to align sequences and call OTUs (operational taxonomic units) using 97% as the cut-off sequence similarity, showing the rarefaction curves, then removing OTUs in which the abundance value was lower than 0.001% of the total sequenced quantity [41]. Subsequent microbial classification and analyses were conducted based on classifying the information of each OTU. For the richness and diversity index of bacterial communities, the *Chao1* index (1) and *ACE* (2) index were used to describe the richness [42]:

$$chao1 = S_{obs} + \frac{f_1(f_1 - 1)}{2(f_2 + 1)} \quad (1)$$

$$ACE = \sum_{k=1}^{S_{obs}} f_k + \frac{\sum_{k=1}^{10} f_k}{1 - \frac{f_1}{\sum_{k=1}^{10} k f_k}} + \frac{f_1}{1 - \frac{f_1}{\sum_{k=1}^{10} k f_k}} \max \left[ \frac{\sum_{k=1}^{10} f_k}{\sum_{k=1}^{10} k f_k} \frac{\sum_{k=1}^{10} k(k-1) f_k}{(\sum_{k=1}^{10} k f_k)(\sum_{k=1}^{10} k f_k - 1)} - 1, 0 \right] \quad (2)$$

where  $S_{obs}$  is the total number of species observed in a sample and  $f_k$  is the number of species each represented by  $k$  individuals in a single soil sample.

The Shannon index (3) [43] and Simpson index (4) [44] were used to describe the evenness:

$$Shannon = -\sum_{i=1}^R p_i \ln p_i \quad (3)$$

$$Simpson = \frac{\sum_{i=1}^R n_i(n_i - 1)}{N(N - 1)} \quad (4)$$

where  $P_i = N_i/N$ ,  $N_i$  is the individual number of species ( $i$ ),  $N$  is the total number of organisms of a particular species, and  $P_i$  is the proportion of  $i$  species.

Raw physicochemical data properties of soil samples were processed with Excel 2007, and the results were calculated for three (rhizosphere and bulk soil of three dominant plants) or four replicate (CK) samples and expressed by the mean  $\pm$  standard deviation. Statistical significance was determined by one-way analysis of variance (ANOVA), followed by Dunnett's T3 post hoc test in SPSS ver. 19.0. To compare the structure of bacterial communities across all soil samples based on the relative abundance of bacterial phyla class, principal components analysis of soil properties using redundancy analysis (RDA) by R software package was conducted. Bacterial community composition data, for each classification level, were clustered according to their number of OTUs and taxonomic data using MEGAN [45], and a phylogenetic tree based on the sequenced result of each sample and NCBI database was constructed. Heatmap and Venn diagram were drawn by hierarchal clustering performed based on Ihaka and Gentleman [46]. GraPhlAn [47] was used to construct circular representations of the taxonomic phylogenetic tree, showing the dominant taxa of rhizosphere soils and soils from grassland plots.

### 3. Results

#### 3.1. Physicochemical Properties

There were significant differences among the rhizosphere soils of *E. rufipilus* (Er-R), *C. nepalensis* (Cn-R), *P. yunnanensis* (Py-R), and grassland plots (CK) (Table 1) with respect to most physicochemical properties (Table 2). The amounts of SOM, AN, TP, and AP were significantly higher in Py-R as compared with Er-R, Cn-R, and CK. Gathered data demonstrate that rhizosphere soils of *P. yunnanensis* possess a rich nutrient content

**Table 2.** Physicochemical properties (mean  $\pm$  SD) of rhizosphere and bulk soils from three dominant plants and grassland plots.

Soil Samples	Tem (°C)	SWC (%)	pH	SOM (g/kg)	TN (g/kg)	AN (mg/kg)	TP (g/kg)	AP (mg/kg)	N/P
Er-R	15.45 $\pm$ 0.87 <b>a</b>	30.70 $\pm$ 5.16 <b>a</b>	4.48 $\pm$ 0.28 <b>a</b>	52.45 $\pm$ 15.68 <b>b</b>	2.79 $\pm$ 0.58 <b>ab</b>	94.06 $\pm$ 14.62 <b>bc</b>	5.13 $\pm$ 0.79 <b>b</b>	117.34 $\pm$ 44.06 <b>b</b>	0.55 $\pm$ 0.14 <b>a</b>
Er-B	15.61 $\pm$ 1.49 <b>a</b>	29.27 $\pm$ 2.34 <b>a</b>	4.34 $\pm$ 0.02 <b>a</b>	36.83 $\pm$ 13.75 <b>bc</b>	2.22 $\pm$ 0.41 <b>ab</b>	65.55 $\pm$ 19.15 <b>c</b>	4.57 $\pm$ 0.65 <b>b</b>	72.96 $\pm$ 30.43 <b>b</b>	0.49 $\pm$ 0.10 <b>a</b>
Cn-R	14.61 $\pm$ 1.16 <b>a</b>	28.35 $\pm$ 2.98 <b>ab</b>	4.34 $\pm$ 0.04 <b>a</b>	34.21 $\pm$ 5.72 <b>bc</b>	2.24 $\pm$ 0.11 <b>ab</b>	72.58 $\pm$ 14.81 <b>c</b>	3.93 $\pm$ 0.22 <b>b</b>	64.20 $\pm$ 24.64 <b>b</b>	0.57 $\pm$ 0.06 <b>a</b>
Cn-B	15.22 $\pm$ 1.48 <b>a</b>	26.95 $\pm$ 3.33 <b>ab</b>	4.32 $\pm$ 0.09 <b>a</b>	27.82 $\pm$ 8.39 <b>c</b>	1.93 $\pm$ 0.40 <b>b</b>	58.43 $\pm$ 18.33 <b>c</b>	4.20 $\pm$ 0.22 <b>b</b>	59.07 $\pm$ 20.74 <b>b</b>	0.46 $\pm$ 0.11 <b>a</b>
Py-R	11.09 $\pm$ 0.22 <b>b</b>	33.47 $\pm$ 1.03 <b>a</b>	4.36 $\pm$ 0.12 <b>a</b>	74.66 $\pm$ 10.94 <b>a</b>	2.99 $\pm$ 0.43 <b>a</b>	166.30 $\pm$ 10.24 <b>a</b>	11.36 $\pm$ 1.01 <b>a</b>	253.10 $\pm$ 30.77 <b>a</b>	0.27 $\pm$ 0.05 <b>b</b>

Table 2. Cont.

Soil Samples	Tem (°C)	SWC (%)	pH	SOM (g/kg)	TN (g/kg)	AN (mg/kg)	TP (g/kg)	AP (mg/kg)	N/P
Py-B	10.88 ± 0.50 <b>b</b>	31.38 ± 0.95 <b>a</b>	4.35 ± 0.05 <b>a</b>	39.22 ± 1.57 <b>bc</b>	1.83 ± 0.41 <b>b</b>	115.51 ± 19.16 <b>b</b>	12.09 ± 1.09 <b>a</b>	287.92 ± 56.28 <b>a</b>	0.15 ± 0.04 <b>b</b>
CK	11.09 ± 0.35 <b>b</b>	22.88 ± 1.15 <b>b</b>	4.39 ± 0.19 <b>a</b>	22.26 ± 2.24 <b>c</b>	1.92 ± 0.21 <b>b</b>	67.83 ± 2.06 <b>c</b>	3.71 ± 0.43 <b>b</b>	59.22 ± 11.56 <b>b</b>	0.52 ± 0.06 <b>a</b>

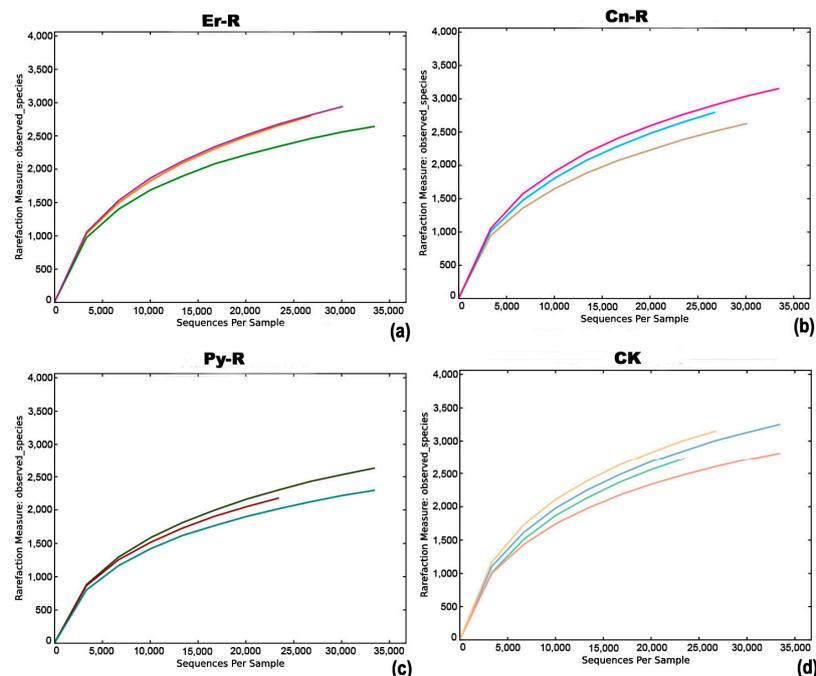
Notes: Value with different letters are significantly different under Dunnett's T3 post hoc test at  $p < 0.05$ . Er-R, rhizosphere soil of *Erianthus rufipilus*; Er-B, bulk soil of *Erianthus rufipilus*; Cn-R, rhizosphere soil of *Coriaria nepalensis*; Cn-B, bulk soil of *Coriaria nepalensis*; Py-R, rhizosphere soil of *Pinus yunnanensis*; Py-B, bulk soil of *Pinus yunnanensis*; CK, soil from grassland plots; Tem, temperature; SWC, soil water content; SOM, soil organic matter; TN, total nitrogen; AN, alkali solution nitrogen; TP, total phosphorus; AP, available phosphorus; N/P, total nitrogen/total phosphorus.

### 3.2. Sequencing Results

Analyses revealed an average of 40,667 valid sequences per sample, and subsequent screening analyzed 38,072 high quality sequences (accounting for 89.3–98.05%), with a read length of 200–250 bp.

### 3.3. Differences in Bacterial Diversity

The rarefaction curves (Figure 2) show that Py-R had the lowest bacterial diversity, and CK were more diverse as compared with the rhizosphere soils of Er-R and Cn-R. For richness and diversity estimations based on OTUs picker data, four indices of each sample group were analyzed using QIIME (Table 3). As shown in Table 3, scores for indices were as follows: Chao1 (1595) and ACE (2036) for Py-R; Chao1 (2012) and ACE (2511) for CK; the Simpson index was 0.981 and the Shannon index 8.11 for Py-R; and the Simpson index was 0.992 and the Shannon index 8.93 for Er-R. The data show that CK had the highest richness and Er-R had the highest diversity, while Py-R had the lowest richness and diversity.



**Figure 2.** Rarefaction curves show the number of operational taxonomic units (OTUs) from sequencing data. Notes: Er-R, rhizosphere soil of *Erianthus rufipilus*; Cn-R, rhizosphere soil of *Coriaria nepalensis*; Py-R, rhizosphere soil of *Pinus yunnanensis*; CK, soil from grassland plots. (a) observed species from Er-R, (b) observed species from Cn-R, (c) observed species from Py-R, (d) observed species from CK.

**Table 3.** Comparison of richness and diversity indices among the rhizosphere soils and grassland plots.

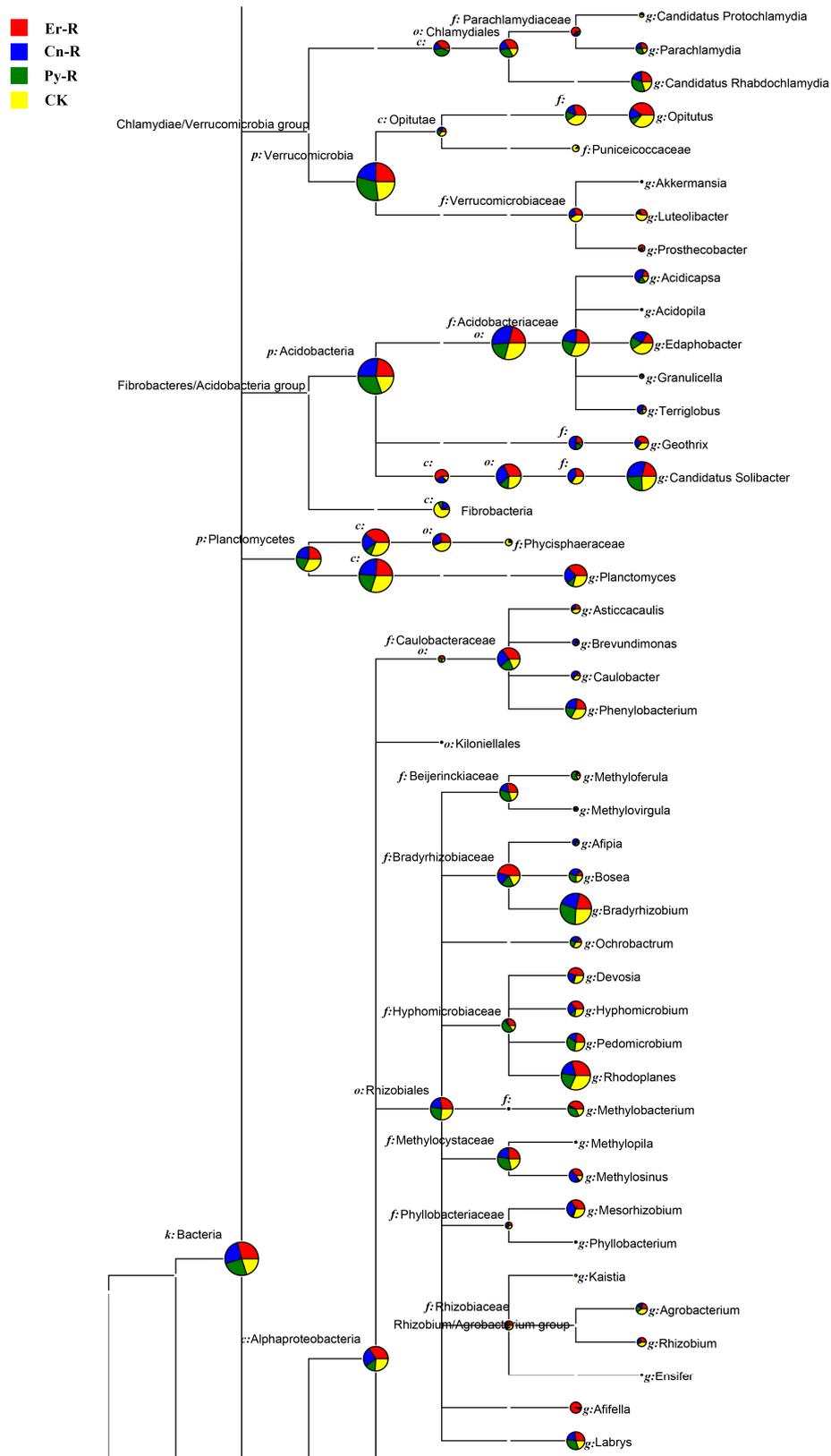
Soil Sample	Chao1	ACE	Simpson	Shannon
Er-R	1868(1748,1953)	2302(2135,2478)	0.99151443	8.93(8.85,9.02)
Cn-R	1817(1668,2068)	2209(2010,2503)	0.989189011	8.78(8.72,8.82)
Py-R	1595(1428,1745)	2036(1736,2193)	0.981008466	8.11(7.72,8.36)
CK	2012(1682,2333)	2511(1682,3530)	0.990400468	8.91(8.42,9.24)

Notes: The values in parentheses show the upper and lower limits; Er-R, rhizosphere soil of *Erianthus rufipilus*; Cn-R, rhizosphere soil of *Coriaria nepalensis*; Py-R, rhizosphere soil of *Pinus yunnanensis*; CK, soil from grassland plots.

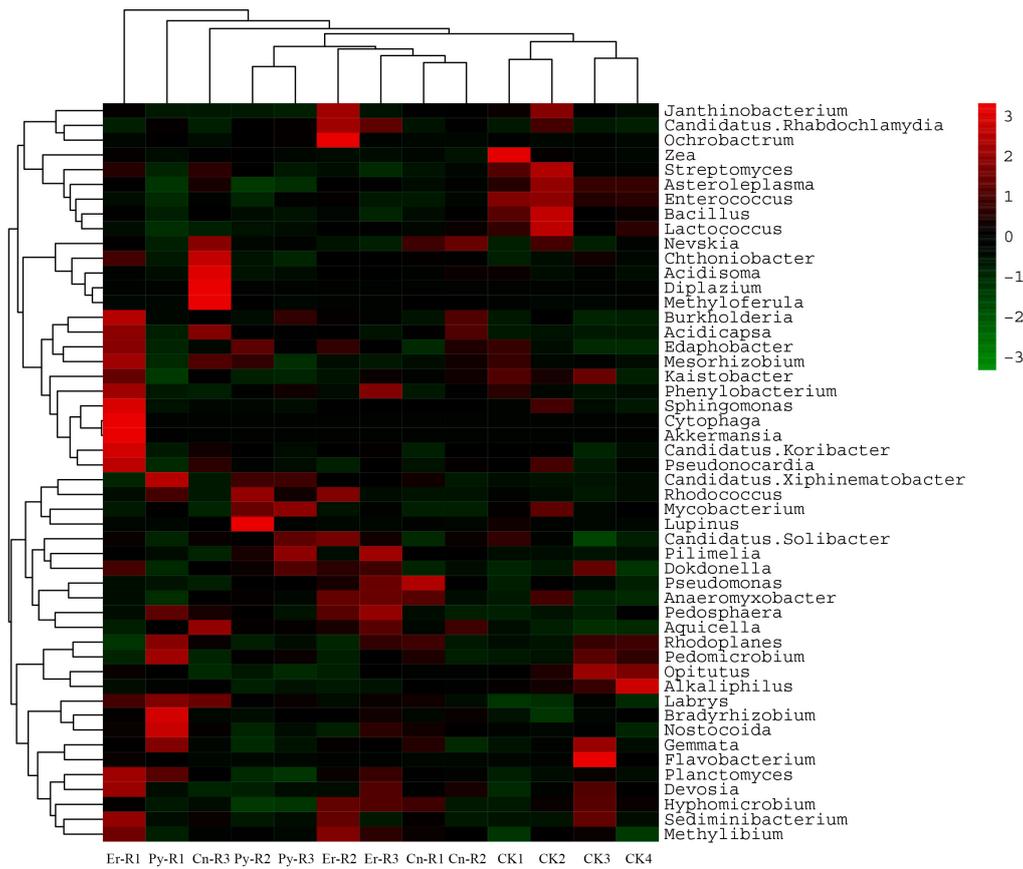
### 3.4. Bacterial Communities and Diversity

Relative bacterial OTU richness was present in four different soil samples across phylum, class, order, family, and genus (Figure 3). The dataset for phylogenetic analyses comprised 1 kingdom, 3 phyla, 7 classes, 7 orders, 16 families and 40 genera. The heatmap plot depicted the relative abundance of each dominant genera (variables clustering on the Y-axis) within soil samples (X-axis clustering) (Figure 4). As shown in Figure 4, four samples from CK grouped together; Cn-R2, Cn-R1, Er-R3, Er-R2 grouped together; and Py-R2 and Py-R3 grouped together. The 50 most abundant genera were depicted. The results show that the majority of sequences belonging to *Asteroleplasma*, *Enterococcus*, *Opitutus*, and *Alkaliphilus* were presented in soil from the CK group. *Dokdonella*, *Pseudomonas*, *Snaeromyxobacter*, and *Hyphomicrobium* were presented in soil from the Er-R and Cn-R groups. *Candidatus Xiphinematobacter*, *Mycobacterium*, *Bradyrhizobium*, and *Nostocoida* were presented in soil from the Py-R groups. We observed that the class *Proteobacteria* had the highest abundance (24.43%) in the rhizosphere soils of the three plants and soil samples from grassland plots, followed by *Acidobacteria* (21.09%), *Verrucomicrobia* (19.48%), *Planctomycetes* (9.20%), and *Anctinobacteria* (5.53%) (Figure 5).

A Venn diagram (Figure 6) illustrates that the bacterial communities of *E. rufipilus* (Er) shared 4095 OTUs between bulk (Er-B) and rhizosphere soils (Er-R) (Figure 6a); the bacterial communities of *C. nepalensis* (Cn) shared 3824 OTUs between bulk (Cn-B) and rhizosphere soils (Cn-R) (Figure 6b); the bacterial communities of *P. yunnanensis* (Py) shared 2817 OTUs between bulk (Py-B) and rhizosphere soils (Py-R) (Figure 6c); and the bacterial communities among the four groups (Er-R, Cn-R, Py-R, and CK) shared 2516 OTUs (Figure 6d). The OTUs derived from the soil associated with the three plant species showed that rhizosphere soils had more OTUs than bulk soils (Figure 6a–c).

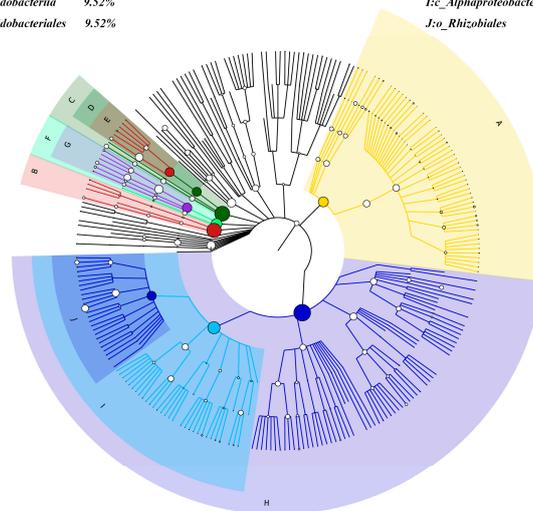


**Figure 3.** The phylogenetic tree of partial 16S rRNA gene read information based on NCBI Taxonomy by MEGAN. Notes: Er-R, rhizosphere soil of *Erianthus rufipilus*; Cn-R, rhizosphere soil of *Coriaria nepalensis*; Py-R, rhizosphere soil of *Pinus yunnanensis*; CK, soil from grassland plots.

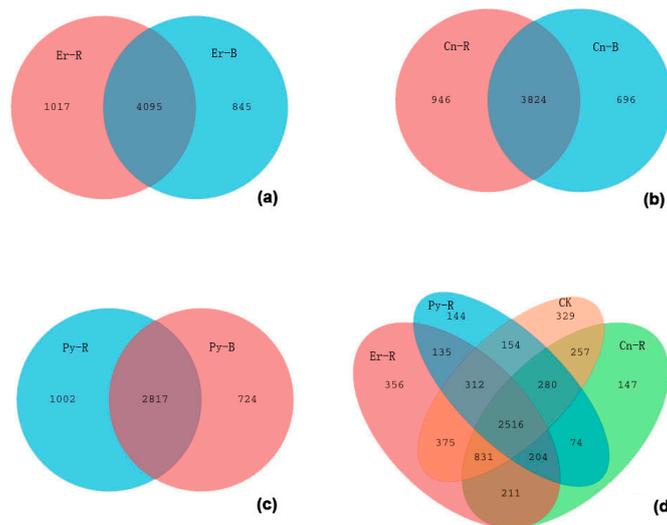


**Figure 4.** Hierarchically clustered heatmap of rhizosphere bacterial communities of dominant plants at the genus level. Soil samples are clustered laterally based on their bacterial similarity and bacterial taxa are clustered vertically. The red color represents the genus with higher abundance and green color represents the genus with lower abundance. Notes: Er-R, rhizosphere soil of *Erianthus rufipilus*; Cn-R, rhizosphere soil of *Coriaria nepalensis*; Py-R, rhizosphere soil of *Pinus yunnanensis*; CK, soil from grassland plots.

<i>Ap_Actinobacteria</i>	5.53%	<i>Fp_Planctomyces</i>	9.20%
<i>Bp_Verrucomicrobia</i>	19.48%	<i>Gc_Planctomyces</i>	7.57%
<i>Cp_Acidobacteria</i>	21.09%	<i>Hp_Proteobacteria</i>	24.43%
<i>Dc_Acidobacteria</i>	9.52%	<i>Ic_Alphaproteobacteria</i>	14.33%
<i>En_Acidobacteriales</i>	9.52%	<i>Jo_Rhizobiales</i>	8.34%



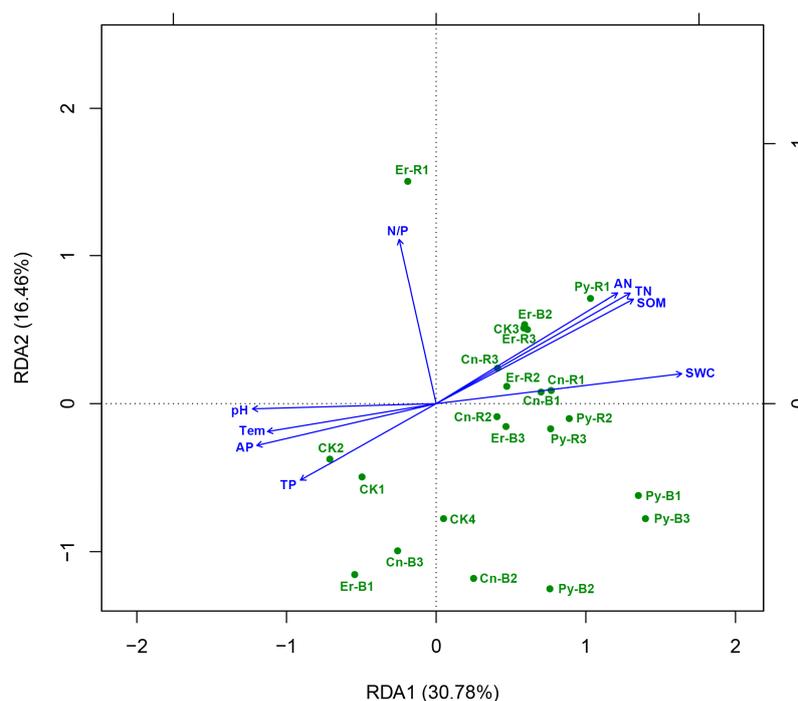
**Figure 5.** Hierarchical tree of dominant bacterial groups at classification level based on GraPhlAn software, it shows units of classification from phyla to genus (inner to outer rings). Size of nodes indicated the abundance.



**Figure 6.** Numbers of shared phylotypes (OTUs) observed between soil samples of different treatments. Notes: Er-R, rhizosphere soil of *Erianthus rufipilus*; Er-B, bulk soil of *Erianthus rufipilus*; Cn-R, rhizosphere soil of *Coriaria nepalensis*; Cn-B, bulk soil of *Coriaria nepalensis*; Py-R, rhizosphere soil of *Pinus yunnanensis*; Py-B, bulk soil of *Pinus yunnanensis*; CK, soil from grassland plots. (a) observed OTUs in Er-R and Er-B. (b) observed OTUs in Cn-R and Cn-B. (c) observed OTUs in Py-R and Py-B. (d) observed OTUs in Er-R, Cn-R, Py-R and CK.

### 3.5. Impact of Soil Properties on the Relative Abundances of Bacterial Taxa

We found a significant association among bacterial community structure and physicochemical properties in the soils (Figure 7). Bacterial communities from Py-R, Py-B, and Cn-R tend to group together, whereas Er-R, Er-B, Cn-B, and CK are scattered. Soil water content (SWC), soil organic matter (SOM), and total nitrogen (TN) were the main limiting factors, and the first two axes in RDA analysis explained 47.24% of variance for the relationship between soil bacterial community composition and physicochemical factors.



**Figure 7.** Redundancy analysis (RDA) of bacterial communities as affected by the environmental and

chemical properties factors, based on the relative abundance and similarity of bacterial phyla. RDA1 explained 30.78% and RDA2 16.46% variability. Each point represents a sample, the closer the distance between two points, the higher the bacterial communities' similarity. The arrow shows the influence factor, the longer the arrow, the greater the effect. The angle between the factors for acute angle shows that the two factors are positively corrected; an angle that is an obtuse angle is a negative correlation. Notes: Er-R, rhizosphere soil of *Erianthus rufipilus*; Er-B, bulk soil of *Erianthus rufipilus*; Cn-R, rhizosphere soil of *Coriaria nepalensis*; Cn-B, bulk soil of *Coriaria nepalensis*; Py-R, rhizosphere soil of *Pinus yunnanensis*; Py-B, bulk soil of *Pinus yunnanensis*; CK, soil from grassland plots; Tem, temperature; SWC, soil water content; SOM, soil organic matter; TN, total nitrogen; AN, alkali solution nitrogen; TP, total phosphorus; AP, available phosphorus; N/P, total nitrogen/total phosphorus.

#### 4. Discussion

Results of this study demonstrate the relationships among soil physicochemical properties and the bacterial communities associated with three selected plants (*E. rufipilus*, *C. nepalensis*, and *P. yunnanensis*), as well as grassland plots in phosphorus-rich mountains in southwestern China. The associations among soil nutrients and bacterial community structure of rhizosphere and bulk soils has been demonstrated in several previous studies [48–52]. Plant species play an important role in shaping rhizosphere microbial communities through their root exudates [24,53–55]. In this study, we found that the content of SOM, TN, and AN was present in higher concentrations in rhizosphere soils as compared with bulk soils among three plants in PES. The contents of SOM, TN, AN, TP, and AP in rhizosphere soils were elevated 55.31%, 34.11%, 39.02%, –2.11%, and 3.50% relative to bulk soil, consistent with soils from other studies and regions [56,57]. The rhizosphere soils of *P. yunnanensis* have higher TP content (elevated 121.44% and 189.06% relative to *E. rufipilus* and *C. nepalensis*, respectively), AP content (elevated 115.70% and 294.24% relative to *E. rufipilus* and *C. nepalensis*, respectively), and lower nitrogen/phosphorus (N/P) (declined 50.91% and 52.63% relative to *E. rufipilus* and *C. nepalensis*, respectively) than the other two dominant plants. Differences in soil phosphorus content among land-use types in PES could possibly be due to geological reasons; for example, this study site is home to geological phosphorus-rich mountains, and phosphate deposited in Yunnan Province accounts for 80% of high-grade phosphate ore in China [17]. According to previous studies on PES, phosphorus is found in high concentrations in soils with low soil nitrogen content [21,58]. Therefore, the results of this study are congruent with previous studies.

The 16S rRNA gene sequencing survey provides exhaustive information about the relative abundance, diversity, and composition of bacterial communities. This study was the first implementation of Illumina MiSeq high-throughput sequencing technology on soil microbial communities in phosphorus-rich mountains. The relative abundance of bacteria among the soil samples showed that *P. yunnanensis* had the lowest bacterial OTUs and CK had the highest. In contrast, soil nutrient properties revealed that *P. yunnanensis* had the highest concentration of SOM, AN, TP, and AP, whereas CK had the lowest SOM. This suggests that plant species shapes soil nutrient properties and bacterial community characteristics in rhizospheres and bulk soils.

The most abundant phylum of bacterial communities in the rhizosphere soil among plants was *Proteobacteria*. This result corroborates the results of several previous bacterial community studies done on the rhizosphere soils of crops [15,59], mining soils [60,61], and PES bacterial community research [28]. Furthermore, the class *Alphaproteobacteria* occupied the majority of the phylum *Proteobacteria*. *Rhizobiales* are the most abundant order in *Proteobacteria* across all soil samples. The families *Bradyrhizobiaceae* and *Rhizobiaceae* in the order *Rhizobiales* from *C. nepalensis* have higher abundance than *E. rufipilus*, *P. yunnanensis*, and grassland plots; this is because *C. nepalensis* forms symbiotic relationships with nitrogen-fixing bacteria [62,63].

The phylum *Acidobacteria* is the second most abundant community among the rhizosphere of the plants and grassland plots in this study. *Acidobacteria* is the predominant phylum of bacteria in semi-arid and other mature soil environments [64]. *Acidobacteria* exists most abundantly in the rhizosphere soil

of *E. rufipilus*; this is likely because *E. rufipilus* is highly drought resistant and suitable to grow in arid regions [65,66]. As a dominant species, *E. rufipilus* is widely distributed in phosphorus-rich regions and has a high phosphorus tolerance [63]. The next phyla in highest abundance were *Verrucomicrobia* and *Planctomycetes*. These two phyla were abundant in the rhizosphere soil of *P. yunnanensis* and grassland plots. *Planctomycetes* represented a small minority of aquatic bacteria found in seawater and freshwater environments [67,68]. However, the dry season occurs in central Yunnan, China from November to April [69], and *P. yunnanensis* exhibits drought tolerance [70], which may explain why *Planctomycetes* was present in soils linked to *P. yunnanensis* in study areas. According to the rhizosphere bacterial communities of *P. yunnanensis* reported in other regions of Yunnan Province where phosphorus is deficient, the dominant bacterial taxa are *Acitinobacteria*, *Alphaproteobacteria*, and *Acidobacteria*, whereas *Verrucomicrobia* and *Planctomycetes* were found in low abundance [71]. In addition to the plants reported in this study, phosphorus also alters bacterial community composition in soybean rhizosphere soils, and phosphorus fertilization decreased the relative abundance of *Bacillales* and *Pseudomonadales* [72].

Plant root exudates also shape rhizosphere bacterial structures [73–75]. Previous studies about phosphorus-enriched soils have concluded that phytoremediation is an effective measure for nutrient (nitrogen and phosphorus) removal [17,76]. Fewer studies, however, has been focused on the biodiversity of soil microbial communities based on vegetation regeneration. This study showed that bacterial communities in PES are strongly influenced by the rhizospheres of different plant species and physicochemical soil parameters. The distribution of soil bacteria was affected by multiple environmental factors. Soil macro-environmental conditions, such as soil water content, has the most notable impacts on the distribution of bacteria, followed by soil organic matter and total nitrogen. Although phosphate content was high in PES, total phosphorus was found to have the lowest impact as compared with other nutrient factors on bacterial communities. The findings of this study verified the results of a previous study on low nitrogen levels in the region [19].

On a larger scale, in the phosphorus-rich and nitrogen-deficient environments of PES, most vegetation is secondary, and habitats are relatively fragile. *E. rufipilus*, *C. nepalensis*, and *P. yunnanensis* are widely grown in phosphorus-enriched soils of China because of their high tolerance to drought and arid conditions. The *P. yunnanensis* plant communities have higher plant diversity in PES, whereas bacterial communities tend to feature lower diversity under the forest soil of *P. yunnanensis*. Higher plant diversity and lower litter decomposition efficiency [77], as well as litter inhibitory effect on bacterial communities such as production of tannin [78] occurring under *P. yunnanensis* as compared with *E. rufipilus* and *C. nepalensis* likely explains this lower level of diversity. In addition, *P. yunnanensis* is a common native secondary coniferous species growing in low temperatures. Because of its rapid growth and higher plant community diversity beneath trees, it is a preferred species in Yunnan [37]. On the basis of the above findings, follow-up research in plant rhizosphere microbial compositions, and diversity could be fruitful. Nutrient properties and microbial communities can provide guidance for understanding biochemical processes and ecosystem functionalities in the soils of phosphorus-rich mountains.

## 5. Conclusions

The richness and diversity of the bacterial communities reported in our study reveal prospective uncultured bacteria derived from rhizosphere soils of dominant plants in PES for the first time. Differences in the physicochemistry and structures of soil bacterial communities among the dominant plants form useful guidelines for examining how tree species and soil physicochemical parameters influence local soil environments in phosphorus-rich regions. The results from our study show that both bacterial communities and soil nutrients show Rhizospheric effects; rhizosphere bacteria are mainly composed of *Proteobacteria* and *Acidobacteria*, followed by *Verrucomicrobia* and *Planctomycetes*; bacteria abundance and diversity in PES are mainly influenced by soil water content, soil organic matter, and total nitrogen. Our results indicate that rhizosphere microbial communities could serve as

an important index for phytoremediation. This is of great significance for the restoration of disturbed ecosystems in PES.

**Author Contributions:** Conceptualization, C.D. and D.F.; Formal analysis, Y.H. and E.F.; Funding acquisition, C.D. and D.F.; Investigation, X.W. and K.Y.; Methodology, Y.H., C.D., and D.F.; Resources, C.D. and D.F.; Software, K.Y. and S.C.K.; Supervision, C.D., P.E.M., and J.X.; Writing—original draft, Y.H. and D.F. Writing—review and editing, I.P., P.E.M., and J.X. All authors have read and agree to the published version of the manuscript.

**Funding:** This research is supported by the National Science Foundation of China (NSFC) project codes 31670522 to Changqun Duan, NSFC project codes 31860133 to Denggao Fu, NSFC41807524 and 31861143002 to Kai Yan. Peter E. Mortimer thanks NSFC project codes 41761144055 and 41771063 for support. Samantha C. Kaunaratna thanks CAS President’s International Fellowship Initiative (PIFI) for funding his postdoctoral research (no. 2018PC0006) and NSFC project code 31851110759.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A

**Table A1.** Basic information of UV spectrophotometry test on DNA extraction products among soil samples.

No	Samples	DNA Concentration (ng/μL)	260/280 Value	260/230 Value	Volume (μL)	Total Amount (μg)
1	Er-R1	13.84	1.80	0.56	50.00	0.69
2	Er-R2	23.54	1.65	0.62	50.00	1.18
3	Er-R3	22.43	1.79	0.65	50.00	1.12
4	Er-B1	12.87	1.92	0.53	50.00	0.64
5	Er-B2	26.76	1.76	0.74	50.00	1.34
6	Er-B3	11.39	1.64	0.45	50.00	0.57
7	Cn-R1	12.26	1.72	0.43	50.00	0.61
8	Cn-R2	14.58	1.69	0.58	50.00	0.73
9	Cn-R3	24.89	1.82	0.73	50.00	1.24
10	Cn-B1	11.73	1.69	0.46	50.00	0.59
11	Cn-B2	5.57	1.71	0.24	50.00	0.28
12	Cn-B3	5.75	1.53	0.25	50.00	0.29
13	Py-R1	15.96	1.52	0.51	50.00	0.80
14	Py-R2	16.00	1.55	0.49	50.00	0.80
15	Py-R3	17.73	1.64	0.55	50.00	0.89
16	Py-B1	10.41	1.51	0.40	50.00	0.52
17	Py-B2	9.87	1.45	0.35	50.00	0.49
18	Py-B3	10.92	1.54	0.40	50.00	0.55
19	CK1	5.44	1.39	0.29	50.00	0.27
20	CK2	10.35	1.79	0.48	50.00	0.52
21	CK3	9.49	1.72	0.40	50.00	0.47
22	CK4	11.41	1.71	0.38	50.00	0.57

## References

1. McGuire, K.L.; Treseder, K.K. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. *Soil Biol. Biochem.* **2010**, *42*, 529–535. [[CrossRef](#)]
2. Bhatti, A.A.; Haq, S.; Bhat, R.A. Actinomycetes benefaction role in soil and plant health. *Microb. Pathog.* **2017**, *111*, 458–467. [[CrossRef](#)] [[PubMed](#)]
3. Ravi, R.K.; Anusuya, S.; Balachandar, M.; Muthukumar, T. Microbial Interactions in Soil Formation and Nutrient Cycling. In *Mycorrhizosphere and Pedogenesis*; Varma, A., Choudhary, D.K., Eds.; Springer: Singapore, 2019; pp. 368–382. [[CrossRef](#)]
4. Berg, G.; Grube, M.; Schloter, M.; Smalla, K. Unraveling the plant microbiome: Looking back and future perspectives. *Front. Microbiol.* **2014**, *5*, 148. [[CrossRef](#)] [[PubMed](#)]
5. Dessaux, Y.; Hinsinger, P.; Lemanceau, P. Rhizosphere: So many achievements and even more challenges. *Plant Soil* **2009**, *321*, 1–3. [[CrossRef](#)]
6. Jacoby, R.P.; Kopriva, S. Metabolic niches in the rhizosphere microbiome: New tools and approaches to analyse metabolic mechanisms of plant–microbe nutrient exchange. *J. Exp. Bot.* **2019**, *70*, 1087–1094. [[CrossRef](#)]
7. Pii, Y.; Penn, A.; Terzano, R.; Crecchio, C.; Mimmo, T.; Cesco, S. Plant-microorganism-soil interactions influence the Fe availability in the rhizosphere of cucumber plants. *Plant Physiol. Biochem.* **2015**, *87*, 45–52. [[CrossRef](#)]
8. Fierer, N. Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* **2017**, *15*, 579. [[CrossRef](#)]
9. O'Brien, S.L.; Gibbons, S.M.; Owens, S.M.; Hampton–Marcell, J.; Johnston, E.R.; Jastrow, J.D.; Gilbert, J.A.; Meyer, F.; Antonopoulos, D.A. Spatial scale drives patterns in soil bacterial diversity. *Environ. Microbiol.* **2016**, *18*, 2039–2051. [[CrossRef](#)]
10. Zhang, Y.; Dong, S.; Gao, Q.; Liu, S.; Ganjurjav, H.; Wang, X.; Su, X.; Wu, X. Soil bacterial and fungal diversity differently correlated with soil biochemistry in alpine grassland ecosystems in response to environmental changes. *Sci. Rep.* **2017**, *7*, 43077. [[CrossRef](#)]
11. Shen, P.; Murphy, D.V.; George, S.J.; Lapis-Gaza, H.; Xu, M.; Gleeson, D.B. Increasing the size of the microbial biomass altered bacterial community structure which enhances plant phosphorus uptake. *PLoS ONE* **2016**, *11*, e0166062. [[CrossRef](#)]
12. Wang, Q.; Wang, C.; Yu, W.; Turak, A.; Chen, D.; Huang, Y.; Ao, J.; Jiang, Y.; Huang, Z. Effects of nitrogen and phosphorus inputs on soil bacterial abundance, diversity, and community composition in Chinese fir plantations. *Front. Microbiol.* **2018**, *9*, 1543. [[CrossRef](#)] [[PubMed](#)]
13. Wu, H.; Xiang, W.; Ouyang, S.; Forrester, D.I.; Zhou, B.; Chen, L.; Ge, T.; Lei, P.; Chen, L.; Zeng, Y.; et al. Linkage between tree species richness and soil microbial diversity improves phosphorus bioavailability. *Funct. Ecol.* **2019**, *33*, 1549–1560. [[CrossRef](#)]
14. Philippot, L.; Raaijmakers, J.M.; Lemanceau, P.; Van Der Putten, W.H. Going back to the roots: The microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* **2013**, *11*, 789–799. [[CrossRef](#)] [[PubMed](#)]
15. Samaddar, S.; Chatterjee, P.; Truu, J.; Anandham, R.; Kim, S.; Sa, T. Long-term phosphorus limitation changes the bacterial community structure and functioning in paddy soils. *Appl. Soil Ecol.* **2019**, *134*, 111–115. [[CrossRef](#)]
16. Xie, J.; Xue, W.; Li, C.; Yan, Z.; Li, D.; Li, G.; Chen, X.; Chen, D. Water-soluble phosphorus contributes significantly to shaping the community structure of rhizospheric bacteria in rocky desertification areas. *Sci. Rep.* **2019**, *9*, 18408. [[CrossRef](#)] [[PubMed](#)]
17. Yan, K.; Ranjitkar, S.; Zhai, D.; Li, Y.; Xu, J.; Li, B.; Lu, Y. Current re-vegetation patterns and restoration issues in degraded geological phosphorus-rich mountain areas: A synthetic analysis of Central Yunnan, SW China. *Plant Divers.* **2017**, *39*, 140–148. [[CrossRef](#)]
18. Sharpley, A.N.; McDowell, R.W.; Kleinman, P.J. Phosphorus loss from land to water: Integrating agricultural and environmental management. *Plant Soil* **2001**, *237*, 287–307. [[CrossRef](#)]
19. Yan, K.; Duan, C.; Fu, D.; Li, J.; Wong, M.H.G.; Qian, L.; Tian, Y. Leaf nitrogen and phosphorus stoichiometry of plant communities in geochemically phosphorus-enriched soils in a subtropical mountainous region, SW China. *Environ. Earth Sci.* **2015**, *74*, 3867–3876. [[CrossRef](#)]

20. Yan, K.; Xu, J.-C.; Gao, W.; Li, M.-J.; Li, Y.-J.; Zhao, Z.-X.; Yuan, Z.-W.; Zhang, F.-S.; Elser, J. *Human Perturbation on Phosphorus Cycles of Lake Dianchi in Southwest China*; Yunnan Agricultural University: Kunming, China, 2020; Unpublished work.
21. Yan, K.; Yuan, Z.; Goldberg, S.; Gao, W.; Ostermann, A.; Xu, J.; Zhang, F.; Elser, J. Phosphorus mitigation remains critical in water protection: A review and meta-analysis from one of China's most eutrophicated lakes. *Sci. Total Environ.* **2019**, *689*, 1336–1347. [[CrossRef](#)]
22. Yan, K.; Wong, M.H.G.; Zhang, L.; Xiong, H.; Fu, D.; Duan, C.; Guo, Z.; Wang, C. Is Phytoextraction Efficient for Remediating Phosphorus-Enriched Soils in Mountainous Region? A Case Study of Lake Dianchi Watershed of Southwestern China. *Appl. Mech. Mater.* **2014**, *448–453*, 488–493. [[CrossRef](#)]
23. Ahkami, A.H.; White III, R.A.; Handakumbura, P.P.; Jansson, C. Rhizosphere engineering: Enhancing sustainable plant ecosystem productivity. *Rhizosphere* **2017**, *3*, 233–243. [[CrossRef](#)]
24. Olanrewaju, O.S.; Ayangbenro, A.S.; Glick, B.R.; Babalola, O.O. Plant health: Feedback effect of root exudates–rhizobiome interactions. *Appl. Microbiol.* **2019**, *103*, 1155–1166. [[CrossRef](#)] [[PubMed](#)]
25. Shao, T.; Gu, X.; Zhu, T.; Pan, X.; Zhu, Y.; Long, X.; Shao, H.; Liu, M.; Rengel, Z. Industrial crop Jerusalem artichoke restored coastal saline soil quality by reducing salt and increasing diversity of bacterial community. *Appl. Soil Ecol.* **2019**, *138*, 195–206. [[CrossRef](#)]
26. Galaviz, C.; Lopez, B.R.; de-Bashan, L.E.; Hirsch, A.M.; Maymon, M.; Bashan, Y. Root growth improvement of mesquite seedlings and bacterial rhizosphere and soil community changes are induced by inoculation with plant growth–promoting bacteria and promote restoration of eroded desert soil. *Land Degrad. Dev.* **2018**, *29*, 1453–1466. [[CrossRef](#)]
27. Mesa, V.; Navazas, A.; González-Gil, R.; González, A.; Weyens, N.; Lauga, B.; Gallego, J.L.R.; Sánchez, J.; Peláez, A.I. Use of endophytic and rhizosphere bacteria to improve phytoremediation of arsenic–contaminated industrial soils by autochthonous *Betula celtiberica*. *Appl. Environ. Microbiol.* **2017**, *83*. [[CrossRef](#)]
28. Yang, P.-X.; Ma, L.; Chen, M.-H.; Xi, J.-Q.; He, F.; Duan, C.-Q.; Mo, M.-H.; Fang, D.-H.; Duan, Y.-Q.; Yang, F.-X. Phosphate solubilizing ability and phylogenetic diversity of bacteria from P-rich soils around Dianchi Lake drainage area of China. *Pedosphere* **2012**, *22*, 707–716. [[CrossRef](#)]
29. Tang, C.Q.; Zhao, M.-H.; Li, X.-S.; Ohsawa, M.; Ou, X.-K. Secondary succession of plant communities in a subtropical mountainous region of SW China. *Ecol. Res.* **2010**, *25*, 149–161. [[CrossRef](#)]
30. Tang, C.Q.; Hou, X.; Gao, K.; Xia, T.; Duan, C.; Fu, D. Man-made versus natural forests in mid-Yunnan, southwestern China. *Mt. Res. Dev.* **2007**, *27*, 242–249. [[CrossRef](#)]
31. Zhu, A.X.; Yang, L.; Li, B.; Qin, C.; Pei, T.; Liu, B. Construction of membership functions for predictive soil mapping under fuzzy logic. *Geoderma* **2010**, *155*, 164–174. [[CrossRef](#)]
32. Jarrell, W.M.; Armstrong, D.E.; Grigal, D.F.; Kelly, E.F.; Monger, H.C.; Wedin, D.A. Soil water and temperature status. In *Standard Soil Methods for Long-Term Ecological Research*; Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P., Eds.; Oxford University Press: New York, NY, USA, 1999; Volume 2, pp. 55–73.
33. Robertson, G.P.; Sollins, P.; Ellis, B.G.; Lajtha, K. Exchangeable ions, pH, and cation exchange capacity. In *Standard Soil Methods for Long-Term Ecological Research*; Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P., Eds.; Oxford University Press: New York, NY, USA, 1999; Volume 2, pp. 106–114.
34. Nelson, D.W.; Sommers, L.E. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis, Part 3: Chemical Methods*; Sparks, D.L., Page, A.L., Helmke, P.A., Loepfert, R.H., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 1996; Volume 14, pp. 961–1010.
35. Bremner, J.M. Nitrogen—Total. In *Methods of Soil Analysis, Part 3: Chemical Methods*; Sparks, D.L., Page, A.L., Helmke, P.A., Loepfert, R.H., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 1996; Volume 14, pp. 1085–1121.
36. Mulvaney, R.L.; Khan, S.A.; Stevens, W.B.; Mulvaney, C.S. Improved diffusion methods for determination of inorganic nitrogen in soil extracts and water. *Biol. Fertil. Soils* **1997**, *24*, 413–420. [[CrossRef](#)]
37. Fu, D.; Wu, X.; Duan, C.; Zhao, L.; Li, B. Different life-form plants exert different rhizosphere effects on phosphorus biogeochemistry in subtropical mountainous soils with low and high phosphorus content. *Soil Tillage Res.* **2020**, *199*, 104516. [[CrossRef](#)]
38. Fadrosch, D.W.; Ma, B.; Gajer, P.; Sengamalay, N.; Ott, S.; Brotman, R.M.; Ravel, J. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome* **2014**, *2*, 6. [[CrossRef](#)]
39. Magoč, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963. [[CrossRef](#)] [[PubMed](#)]

40. Edgar, R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **2010**, *26*, 2460–2461. [[CrossRef](#)]
41. Bokulich, N.A.; Subramanian, S.; Faith, J.J.; Gevers, D.; Gordon, J.I.; Knight, R.; Mills, D.A.; Caporaso, J.G. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat. Methods* **2013**, *10*, 57–59. [[CrossRef](#)] [[PubMed](#)]
42. Gotelli, N.J.; Colwell, R.K. Estimating species richness. In *Biological Diversity: Frontiers in Measurement and Assessment*; Magurran, A.E., McGill, B.J., Eds.; Oxford University Press: New York, NY, USA, 2011; pp. 39–54.
43. Shannon, C.E. A mathematical theory of communication. *Bell Syst. Tech. J.* **1948**, *27*, 379–423. [[CrossRef](#)]
44. Simpson, E.H. Measurement of Diversity. *Nature* **1949**, *163*, 688. [[CrossRef](#)]
45. Huson, D.H.; Mitra, S.; Ruscheweyh, H.J.; Weber, N.; Schuster, S.C. Integrative analysis of environmental sequences using MEGAN4. *Genome Res.* **2011**, *21*, 1552–1560. [[CrossRef](#)]
46. Ihaka, R.; Gentleman, R. R: A language for data analysis and graphics. *J. Comput. Graph. Stat.* **1996**, *5*, 299–314.
47. Asnicar, F.; Weingart, G.; Tickle, T.L.; Huttenhower, C.; Segata, N. Compact graphical representation of phylogenetic data and metadata with GraPhlAn. *PeerJ* **2015**, *3*, e1029. [[CrossRef](#)]
48. Dotaniya, M.L.; Meena, V.D. Rhizosphere effect on nutrient availability in soil and its uptake by plants: A review. *Proc. Natl. Acad. Sci. India Sect B Biol. Sci.* **2015**, *85*, 1–12. [[CrossRef](#)]
49. Pii, Y.; Mimmo, T.; Tomasi, N.; Terzano, R.; Cesco, S.; Crecchio, C. Microbial interactions in the rhizosphere: Beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biol. Fertil. Soils* **2015**, *51*, 403–415. [[CrossRef](#)]
50. Sun, X.; Zhou, Y.; Tan, Y.; Wu, Z.; Lu, P.; Zhang, G.; Yu, F. Restoration with pioneer plants changes soil properties and remodels the diversity and structure of bacterial communities in rhizosphere and bulk soil of copper mine tailings in Jiangxi Province, China. *Environ. Sci. Pollut. Res.* **2018**, *25*, 22106–22119. [[CrossRef](#)] [[PubMed](#)]
51. Wang, Q.; Jiang, X.; Guan, D.; Wei, D.; Zhao, B.; Ma, M.; Chen, S.; Li, L.; Cao, F.; Li, J. Long-term fertilization changes bacterial diversity and bacterial communities in the maize rhizosphere of Chinese Mollisols. *Appl. Soil Ecol.* **2017**, *125*, 88–96. [[CrossRef](#)]
52. Cheng, J.; Lee, X.; Tang, Y.; Zhang, Q. Long-term effects of biochar amendment on rhizosphere and bulk soil microbial communities in a karst region, southwest China. *Appl. Soil Ecol.* **2019**, *140*, 126–134. [[CrossRef](#)]
53. Haichar, F.E.Z.; Marol, C.; Berge, O.; Rangel-Castro, J.I.; Prosser, J.I.; Balesdent, J.; Heulin, T.; Achouak, W. Plant host habitat and root exudates shape soil bacterial community structure. *ISME J.* **2008**, *2*, 1221–1230. [[CrossRef](#)]
54. Eisenhauer, N.; Lanoue, A.; Strecker, T.; Scheu, S.; Steinauer, K.; Thakur, M.P.; Mommer, L. Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Sci. Rep.* **2017**, *7*, 1–8. [[CrossRef](#)]
55. Zhalnina, K.; Louie, K.B.; Hao, Z.; Mansoori, N.; Da Rocha, U.N.; Shi, S.; Cho, H.; Karaoz, U.; Loqué, D.; Bowen, B.P.; et al. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nat. Microbiol.* **2018**, *3*, 470–480. [[CrossRef](#)]
56. Wang, Z.; Li, T.; Wen, X.; Liu, Y.; Han, J.; Liao, Y.; DeBruyn, J.M. Fungal communities in rhizosphere soil under conservation tillage shift in response to plant growth. *Front. Microbiol.* **2017**, *8*, 1301. [[CrossRef](#)]
57. Liu, J.; Ha, V.N.; Shen, Z.; Zhu, H.; Zhao, F.; Zhao, Z. Characteristics of bulk and rhizosphere soil microbial community in an ancient *Platykladus orientalis* forest. *Appl. Soil Ecol.* **2018**, *132*, 91–98. [[CrossRef](#)]
58. Li, M.; Hu, Z.; Zhu, X.; Zhou, G. Risk of phosphorus leaching from phosphorus-enriched soils in the Dianchi catchment, Southwestern China. *Environ. Sci. Pollut. Res.* **2015**, *22*, 8460–8470. [[CrossRef](#)] [[PubMed](#)]
59. Mendes, L.W.; Kuramae, E.E.; Navarrete, A.A.; Van Veen, J.A.; Tsai, S.M. Taxonomical and functional microbial community selection in soybean rhizosphere. *ISME J.* **2014**, *8*, 1577–1587. [[CrossRef](#)] [[PubMed](#)]
60. Li, Y.; Chen, L.; Wen, H. Changes in the composition and diversity of bacterial communities 13 years after soil reclamation of abandoned mine land in eastern China. *Ecol. Res.* **2015**, *30*, 357–366. [[CrossRef](#)]
61. Wei, Z.; Hao, Z.; Li, X.; Guan, Z.; Cai, Y.; Liao, X. The effects of phytoremediation on soil bacterial communities in an abandoned mine site of rare earth elements. *Sci. Total Environ.* **2019**, *670*, 950–960. [[CrossRef](#)] [[PubMed](#)]
62. Bargali, K.; Tewari, A. Growth and water relation parameters in drought-stressed *Coriaria nepalensis* seedlings. *J. Arid Environ.* **2004**, *58*, 505–512. [[CrossRef](#)]

63. Fu, D.; He, F.; Guo, Z.; Yan, K.; Wu, X.; Duan, C. Assessment of ecological restoration function of the *Coriaria nepalensis*-*Erianthus rufipilus* community in the phosphorus-enriched degraded mountain area in the Lake Dianchi Watershed, Southwestern China. *Chin. J. Plant Ecol.* **2013**, *37*, 326–334. (In Chinese) [[CrossRef](#)]
64. Tarlera, S.; Jangid, K.; Ivester, A.H.; Whitman, W.B.; Williams, M.A. Microbial community succession and bacterial diversity in soils during 77 000 years of ecosystem development. *FEMS Microbiol. Ecol.* **2008**, *64*, 129–140. [[CrossRef](#)]
65. Wang, X.-H.; Yang, Q.-H.; Li, F.-S.; He, L.-L.; He, S.-C. Characterization of the chromosomal transmission of intergeneric hybrids of *Saccharum* spp. and *Erianthus fulvus* by genomic in situ hybridization. *Crop Sci.* **2010**, *50*, 1642–1648. [[CrossRef](#)]
66. Evans, D.L.; Joshi, S.V.; Wang, J. Whole chloroplast genome and gene locus phylogenies reveal the taxonomic placement and relationship of *Tripidium* (Panicaceae: Andropogoneae) to sugarcane. *BMC Evol. Biol.* **2019**, *19*, 33. [[CrossRef](#)]
67. Kohn, T.; Heuer, A.; Jogler, M.; Vollmers, J.; Boedeker, C.; Bunk, B.; Rast, P.; Borchert, D.; Glöckner, I.; Freese, H.M.; et al. *Fuerstia marisgermanicae* gen. nov., sp. nov., an unusual member of the phylum Planctomycetes from the German Wadden Sea. *Front. Microbiol.* **2016**, *7*, 2079. [[CrossRef](#)]
68. Andrei, A.S.; Salcher, M.M.; Mehrshad, M.; Rychtecký, P.; Znachor, P.; Ghai, R. Niche-directed evolution modulates genome architecture in freshwater Planctomycetes. *ISME J.* **2019**, *13*, 1056–1071. [[CrossRef](#)]
69. Zhang, Y.; Huang, K.; Yu, Y.; Hu, T.; Wei, J. Impact of climate change and drought regime on water footprint of crop production: The case of Lake Dianchi Basin, China. *Nat. Hazards* **2015**, *79*, 549–566. [[CrossRef](#)]
70. Ma, F.; Zhao, C.; Milne, R.; Ji, M.; Chen, L.; Liu, J. Enhanced drought-tolerance in the homoploid hybrid species *Pinus densata*: Implication for its habitat divergence from two progenitors. *New Phytol.* **2010**, *185*, 204–216. [[CrossRef](#)] [[PubMed](#)]
71. Fan, Z.; Lu, S.; Liu, S.; Guo, H.; Wang, T.; Zhou, J.; Peng, X. Changes in Plant Rhizosphere Microbial Communities under Different Vegetation Restoration Patterns in Karst and Non-karst Ecosystems. *Sci. Rep.* **2019**, *9*, 1–12. [[CrossRef](#)]
72. Tian, J.; Lu, X.; Chen, Q.; Kuang, X.; Liang, C.; Deng, L.; Lin, D.; Cai, K.; Tian, J. Phosphorus fertilization affects soybean rhizosphere phosphorus dynamics and the bacterial community in karst soils. *Plant Soil* **2020**, 1–16. [[CrossRef](#)]
73. Cortois, R.; De Deyn, G.B. The curse of the black box. *Plant Soil* **2012**, *350*, 27–33. [[CrossRef](#)]
74. Weidner, S.; Koller, R.; Latz, E.; Kowalchuk, G.; Bonkowski, M.; Scheu, S.; Jousset, A. Bacterial diversity amplifies nutrient-based plant–soil feedbacks. *Funct. Ecol.* **2015**, *29*, 1341–1349. [[CrossRef](#)]
75. Emmett, B.D.; Buckley, D.H.; Drinkwater, L.E. Plant growth rate and nitrogen uptake shape rhizosphere bacterial community composition and activity in an agricultural field. *New Phytol.* **2020**, *225*, 960–973. [[CrossRef](#)]
76. Xiao, G.; Li, T.; Zhang, X.; Yu, H.; Huang, H.; Gupta, D.K. Uptake and accumulation of phosphorus by dominant plant species growing in a phosphorus mining area. *J. Hazard. Mater.* **2009**, *171*, 542–550. [[CrossRef](#)]
77. Fu, D.; Wu, X.; Huang, N.; Duan, C. Effects of the invasive herb *Ageratina adenophora* on understory plant communities and tree seedling growth in *Pinus yunnanensis* forests in Yunnan, China. *J. For. Res.* **2018**, *23*, 112–119. [[CrossRef](#)]
78. De Marco, A.; Esposito, F.; Berg, B.; Zarrelli, A.; Virzo De Santo, A. Litter Inhibitory Effects on Soil Microbial Biomass, Activity, and Catabolic Diversity in Two Paired Stands of *Robinia pseudoacacia* L. and *Pinus nigra* Arn. *Forests* **2018**, *9*, 766. [[CrossRef](#)]

