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Increased Diversity of Rhizosphere Bacterial Community Confers Adaptability to Coastal Environment for *Sapium sebiferum* Trees

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Abstract: *Sapium sebiferum* (L.) Roxb. is an economically important tree in eastern Asia, and it exhibits many traits associated with good forestation species in coastal land. However, scarce research has been conducted to elucidate the effects of rhizosphere bacterial diversity on the adaptability and viability of *S. sebiferum* trees grown in the coastal environment. Field trials were conducted, and rhizosphere soil samples were collected from typical coastal and forestry nursery environments. Rhizosphere bacterial communities were evaluated using 16S rRNA pyrosequencing. A total of 43 bacterial phyla were detected in all the coastal and nursery rhizospheric soil samples. Relatively higher rhizosphere community diversity was found in coastal field-grown trees. Proteobacteria, Acidobacteriota, Bacteroidota, Chloroflex, and Gemmatimonadota were dominant bacterial phyla in rhizosphere communities of tallow trees. However, the rare groups in the coastal rhizosphere soils, with a relative abundance lower than 1%, including Latescibacterota, Methylomirabilota, NB1-j, and Nitrospirota, were largely absent in the nursery field-grown tree's rhizosphere soils. LEfSe analysis identified a total of 43 bacterial groups that were more significantly abundant in the coastal rhizosphere environment than in that of forestry nursery grown trees. Further, our cladogram analysis identified Nitrospirota, Methylomirabilota, NB1-j, and Latescibacterota as biomarkers for the coastal environment at the phylum taxonomic level. These results suggested that the adaptability of *S. sebiferum* trees in coastal environment might be promoted by rhizosphere microbial interactions. Complex tree–microbe interactions might enhance the resistance of the trees to coastal environment, partially by recruiting certain bacterial microbiome species, which is of high saline-alkali resistance.

Keywords: coastal environment; *Sapium sebiferum*; rhizosphere bacteria; community diversity; adaptability

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1. Introduction

Sapium sebiferum (L.) Roxb., known as Chinese tallow tree, is an economically important tree, which has been cultivated for more than 14 centuries in eastern Asia [1]. The tree has long been a popular choice for landscaping and forest construction because of its brilliant color in autumn and the highly unsaturated oil in the seed [2]. *S. sebiferum* is drought-, flood-, and salt-tolerant, and has strong adaptability to a wide range of soil conditions. In the southeastern United States, at the same latitudes of its distribution as those in eastern Asia, *S. sebiferum* is considered to be an exotic, invasive tree species, which is a serious threat to the preservation of the coastal prairie ecosystem [3,4].

Many marsh lands in coastal ecosystems are bare without trees due to their high soil salinity and alkalinity, caused by frequent hydrothermal changes, such as flooding and

drought. *S. sebiferum* has many traits of good forestation species, such as rapid growth, high rates of reproduction, and a degree of saline-alkali stress tolerance [5,6]. Reportedly, salinity is an important factor limiting the distribution range of *S. sebiferum* as the tree is considered to be modestly tolerant to saline conditions [6]. The growth of *S. sebiferum* was not reduced by watering with 2 g L^{-1} saltwater, and the mortality was significantly higher in the trees watered with 10 g L^{-1} of saltwater [7]. In a comparative study on the effects of flooding and salinity on forested wetland species, *S. sebiferum* seedlings survived six weeks in 10 ppt saltwater, whereas tupelo and green ash survived only two weeks [8]. Under continuous flooding with polyhaline (27 g L^{-1}) salt water, 60% of the *S. sebiferum* trees survived, suggesting that this species had certain tolerance to infrequent periods of saltwater flooding in coastal forests [3,9]. In recent years, tallow trees have been used for ecological restoration (landscape construction and carbon fixation) in the eastern coastal regions of China. *S. sebiferum* grows well on the eastern coast of Jiangsu Province, with high rates of seed reproduction, indicating the action of tolerance acclimation. Large areas of tallow trees provide food and habitat for *Elaphurus davidianus* animals and improve restoration of the coastal ecological environment.

To understand the mechanism of high adaptability of *S. sebiferum* on the east coast of China, we evaluated the microbiome diversity of the bacterial communities in the rhizosphere of coastal soil- and nursery field-grown *S. sebiferum* trees using 16S rRNA pyrosequencing. The rhizosphere bacterial diversity, taxonomic distribution, and biomarkers of the bacterial microbiome of the two groups of trees were investigated. This study provides evidence for increased diversity of the rhizosphere bacterial community in coastal environments. The adaptability to coastal environment of *S. sebiferum* was found to be promoted, partially by increasing diversity of rhizosphere microbiome community.

2. Materials and Methods

2.1. Experimental Materials

In this study, we conducted field trials with *S. sebiferum*. Samples were collected from two fields located in Dafeng, Jiangsu Province, China. One of the fields had typical coastal fields (denoted as S in the figures), with salinity over 0.3%. The second field was situated in a forestry nursery (indicated as C in the figures), with soil salinity lower than 0.1%; this field was used as control for comparison with coastal soils. In April 2019, seeds were sown in the soil of each of the fields. *S. sebiferum* trees were sampled in June 2021 using the following protocol. In each field, trees were collected from four different locations spaced at least 8 m apart. Soil particles that had adhered to the roots of the trees were sampled as rhizosphere soil. The soil particles that were directly dislodged from the roots by shaking were sampled. Then, the samples were stored at −80 °C for DNA extraction.

2.2. DNA Extraction

Microbial DNA was extracted from rhizosphere soil samples using the EZNA Soil DNA Kit (Omega Bio-tek, Inc., Norcross, GA, USA) following the manufacturer's protocols. The V4–V5 regions of the bacterial 16S ribosomal RNA genes were amplified by PCR. Next, PCR with the following steps was conducted: incubation at 95 °C for 2 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. The primers used were 515F 5′-barcode-GTGCCAGCMGCCGCGG-3′ and 907R 5′-CCGTCAATTCMTTTRAGTTT-3′. PCR reactions were performed in triplicate. A sample of 20 μL mixture contained 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase (TransGen Biotech, Beijing, China), and 10 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) in accordance with the manufacturer's instructions.

2.3. Library Construction and Sequencing

Purified PCR products were quantified by the Qubit dsDNA HS Assay Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), and equal amplicon quantities were mixed. The pooled DNA product was used to construct Illumina paired-end library following the genomic DNA library preparation procedure recommended by Illumina, Inc. Then, the amplicon library was paired-end sequenced (2 × 250) on an Illumina MiSeq platform by Shanghai BIOZERON Biotechnology Co., Ltd. (Shanghai, China) according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRR17344535-SRR17344542).

2.4. Processing of Sequencing Data

Raw fastq files were first demultiplexed by in-house PERL scripts based on the barcode sequences information for each sample using the following criteria: (i) The 250 bp reads were truncated at any site receiving an average quality score < 20 over a 10 bp sliding window, discarding the truncated reads that were shorter than 50 bp; (ii) Exact barcode matching, 2-nucleotide mismatching in primer matching, and reads containing ambiguous characters were removed; (iii) Only sequences that had an overlap longer than 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded.

2.5. Alpha and Beta Diversity Analyses

We conducted rarefaction analysis with Mothur v.1.21.1 [10] to determine the diversity indices, including the Chao and Shannon diversity indices. Beta diversity analysis was performed using UniFrac [11] to compare the results of the principal component analysis (PCA) carried out with the community ecology package, R-forge (Vegan 2.0 package was used to generate a PCA figure). All statistical analyses were performed with R stats package. R (pheatmap package) and Cytoscape (http://www.cytoscape.org, Version 3.5.1 (accessed on 27 July 2021) were utilized to visualize the relationships through correlation heatmaps and network diagrams, respectively. Redundancy analysis (RDA) was employed to explore the relationship between environmental factors and bacterial communities. One-way analysis of variance (ANOVA) tests were performed to assess the statistical significance of the differences in the diversity indices. Differences were considered statistically significant at $p < 0.05$. Venn diagrams were then drawn using the online tool Draw Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn (accessed on 27 July 2021) to analyze the overlapped and unique OTUs established during the treatment processes. One-way permutational analysis of variance (PERMANOVA) was performed using the R vegan package to assess the statistically significant effects of treatment processes on bacterial communities.

2.6. LEfSe Analysis

For identification of the biomarkers for highly dimensional colonic bacteria, LEfSe (linear discriminant analysis effect size) analysis was performed [12]. Kruskal–Wallis sum-rank test was performed to examine the changes and dissimilarities among classes, followed by linear discriminant analysis (LDA) to determine the size effect of each distinctively abundant taxon.

3. Results

3.1. Quality Metrics of the Sequencing Analysis

Based on Illumina sequencing of 16S ribosomal RNA, sequencing of the amplicon library results in a total number of 341,920 reads after quality checking and trimming, and average read length was 417.02 bp. These sequences were grouped into 5417 OTUs, defined at a 97% sequence similarity cut-off value (Table 1).

Table 1. Quality metrics of the trimmed sequences.

Statistical Data of Reads after Quality Checking and Trimming						
Total of assigned reads after QC				341,920		
Average read length after QC				417.02		
Length distribution of valid sequences	Length	1–250	251–300	301–350	351–400	401–450
	Sequences	52	109	161	5030	336,568
	Percent	0.02%	0.03%	0.05%	1.47%	98.43%

To obtain the species classification information corresponding to each OTU, the UCLUST algorithm was used to taxonomically analyze the representative sequences with 97% OTU similarity. During that procedure, the community composition of each sample was counted at each classification level: domain, kingdom, phylum, class, order, family, genus, and species. We detected a total of 43 bacterial phyla in all the coastal and nursery rhizospheric samples, which were further classified into 105 classes, 235 orders, 285 families, 425 genera, and 429 species (Table 2).

Table 2. Taxonomic information of the total rhizosphere bacterial species.

Domain	Phylum	Class	Order	Family	Genus	Species	OTU
1	43	105	235	285	425	429	5417

3.2. Alpha Rarefaction Curves and Alpha Diversity

Rarefaction curves were constructed for each individual sample to compare the richness of bacteria, using a 97% sequence similarity cut-off value in Mothur. As can be seen in Figure 1, the rhizosphere bacterial microbiome of the coastal field-grown *S. sebiferum* trees had much higher abundance than that of the nursery-grown trees. The rarefaction curves for evaluating the OTU richness per sample generally approached saturation. Further, to estimate the species abundance and diversity of the studied environmental communities, their alpha diversity was analyzed based on the assessment of statistical indexes. The estimator for community richness had an average value of 2279.75 in the tree rhizosphere soils of the nursery-grown *S. sebiferum*. However, the rhizosphere in the coastal fields showed relatively higher bacterial richness, ranging from 2991 to 3101, which was 34.02% higher than that in the rhizosphere of nursery-grown trees (Table 3, Figure 2). Notably, distinct differences between the soil samples from the coastal and the nursery rhizospheres were established at $p < 0.05$. The Chao1 estimator for the coastal rhizosphere soil ranged from 3323.00 to 3438.53, whereas its average value in the nursery rhizosphere soil was 2629.14. This result indicated a relatively higher richness of rhizosphere bacterial species in the coastal rhizosphere soil. A higher Shannon index was found in the coastal rhizosphere soils, suggesting a role of increased community diversity by recruiting certain bacterial species. Furthermore, the rank-abundance distribution curve confirmed our conclusions in this respect. The coastal rhizosphere communities had a larger range of the curve on the horizontal axis, suggesting higher abundance of bacterial species (Figure 3).

Table 3. Alpha-diversity estimators of the rhizosphere bacterial communities.

Sample	Reads	Richness	Chao	Shannon	Coverage
S-1	32,264	3096	3438.53	7.10	0.98
S-2	44,622	3101	3323.00	7.01	0.99
S-3	28,377	2991	3340.12	7.06	0.98
S-4	35,996	3033	3401.81	7.02	0.98
C-1	28,962	2393	2780.65	6.63	0.98
C-2	39,562	2262	2644.58	6.30	0.99
C-3	35,259	2189	2510.12	6.27	0.99
C-4	50,899	2275	2581.20	6.19	0.99

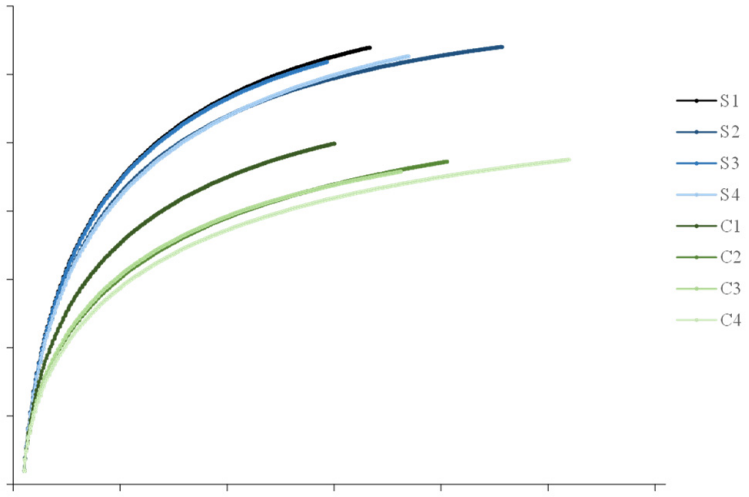

Figure 1. Rarefaction curves of the examined samples. The sequence similarity cut-off value in Mothur was 97%.

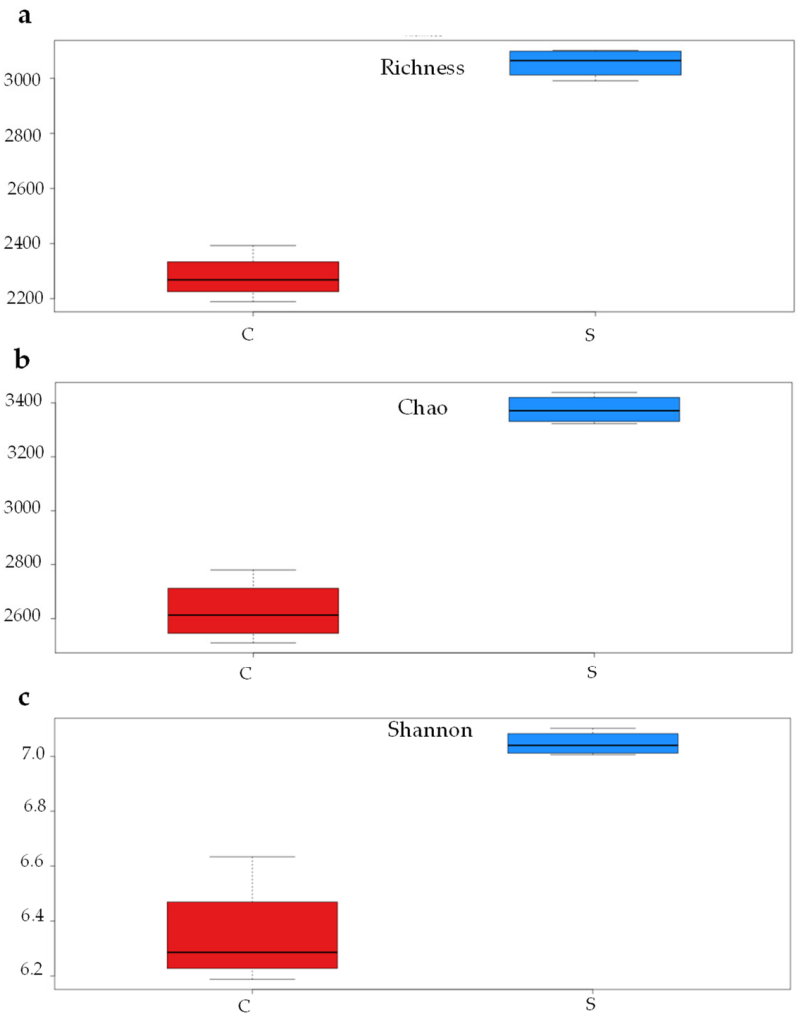

Figure 2. Boxplot analysis of the alpha-diversity estimates of different bacterial communities. (**a**). Richness of OTUs; (**b**). Chao - the Chao1 estimator; (**c**). Shannon - the Shannon index.

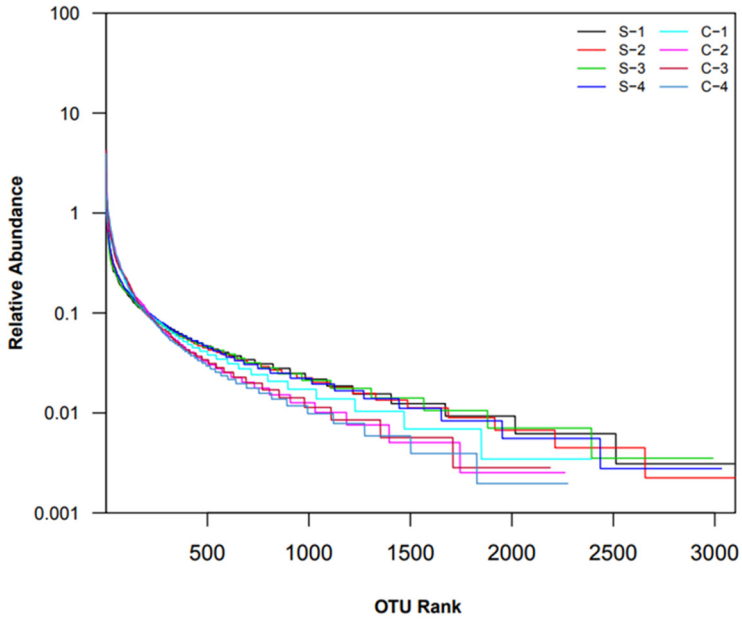

Figure 3. Rank-abundance distribution curve of different bacterial communities.

3.3. Beta Diversity

In our beta-diversity analysis, the Bray–Curtis ordination was employed to calculate the distance between the two samples and compare the community composition. The boxplot showed a significant difference in the inter-group distance coefficient between the nursery and coastal samples, determined multiple comparison Student's *t*-test (Figure 4).

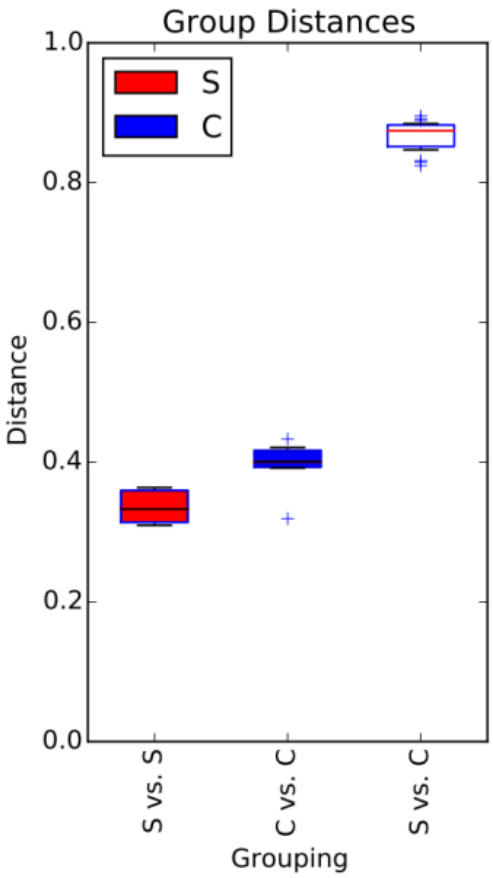

Figure 4. Box-whisker plot for the group distances.

The community structures in the samples were established using PCA, which revealed strong clustering of rhizosphere bacterial communities from different samples. Based on the OTU level, PC1 explained 65.32% and PC2 14.7% of the total variation. These results indicated that the different saline-alkaline degrees of the soil samples collected from the two study sites might be the reason for clustering of rhizosphere bacterial communities. Principal co-ordinates analysis (PCoA) and nonmetric multidimensional scaling (NMDS) analysis confirmed our present conclusions (Figure 5).

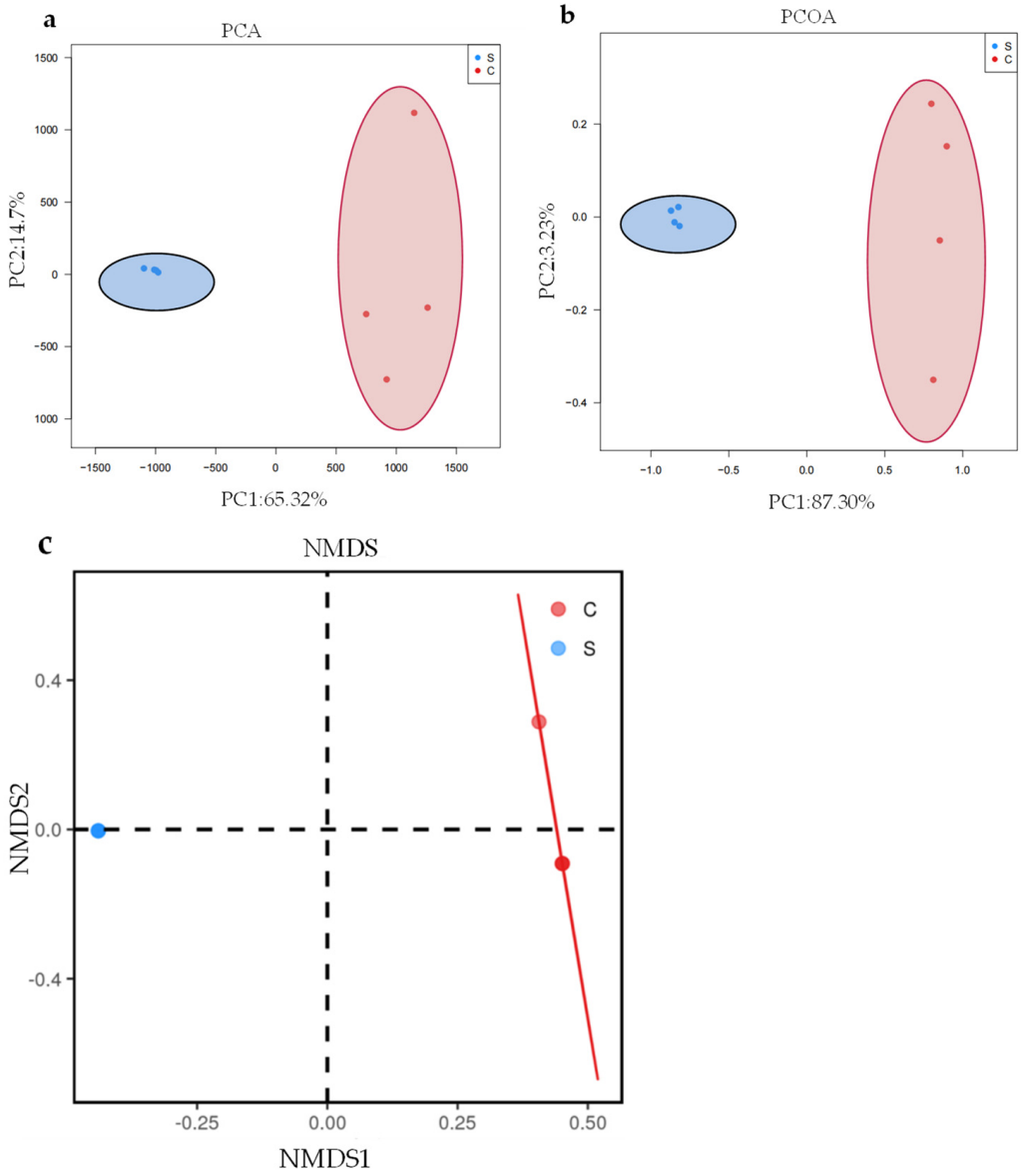

Figure 5. Graphic results of the principal component, principal co-ordinates, and nonmetric multidimensional scaling analyses between nursery and coastal saline-alkaline soil-grown trees at each taxonomic level. (**a**) Principal component analysis; (**b**) Principal co-ordinates analysis; (**c**) Nonmetric multidimensional scaling.

3.4. Taxonomic Distribution

As can be seen in the Venn diagram, 1500 OTUs overlapped between the environmental samples, whereas 2256 OTUs were found only in the coastal field-grown *S. sebiferum* rhizosphere soils; 1461 OTUs were detected only the in nursery rhizosphere soils (Figure 6a). To investigate the taxonomic community structure of the rhizosphere bacterial communities, the OTUs were clustered for the microbial community bar plot and heatmap analyses. In

all eight samples from the coastal and nursery rhizosphere soils, we detected a total of 45 bacterial phyla. Microbial community heatmap analysis showed a differential abundance pattern of the bacterial community between the coastal and nursery rhizosphere soils (Figure 7). The following bacterial phyla were predominant in the rhizosphere communities in the coastal soil: Proteobacteria (35.66% ± 4.00%), Acidobacteriota (16.29% ± 4.00%), Bacteroidota (9.93% ± 3.28%), Chloroflexi (8.78% ± 1.79%), and Gemmatimonadota (5.10% ± 1.05%). Similar results were obtained for rhizosphere communities found in the nursery soil samples. Proteobacteria (36.23% ± 2.93%) was the most abundant phylum, followed by Acidobacteriota (18.00% ± 3.87%). However, the remaining phyla showed high variability in their abundance in different samples. Gemmatimonadota (8.85% ± 3.13%) and Bacteroidota (7.30% ± 1.69%) had relatively higher abundance in the nursery rhizosphere soils than in the coastal soils. These results indicated that the bacterial communities in the rhizosphere of *S. sebiferum* had a comparatively similar phylogenetic composition to that of the coastal and nursery soils. Proteobacteria and Acidobacteriota were the dominant rhizosphere bacterial representatives in *S. sebiferum* regardless of soil salinity. Most notably, over 60% of the bacterial phyla had average abundance lower than 1%. In the coastal environments, rare groups (relative abundance < 1%) had a proportion of approximately 2.47%, whereas the average proportion in the nursery rhizosphere soils was 0.36%. Notably, the phyla Latescibacterota, Methylomirabilota, NB1-j, and Nitrospirota were largely absent (<1%) in the nursery rhizosphere soil (Figures 6b and 7). We speculated that the rare groups were more sensitive to the *S. sebiferum* tree growth environments.

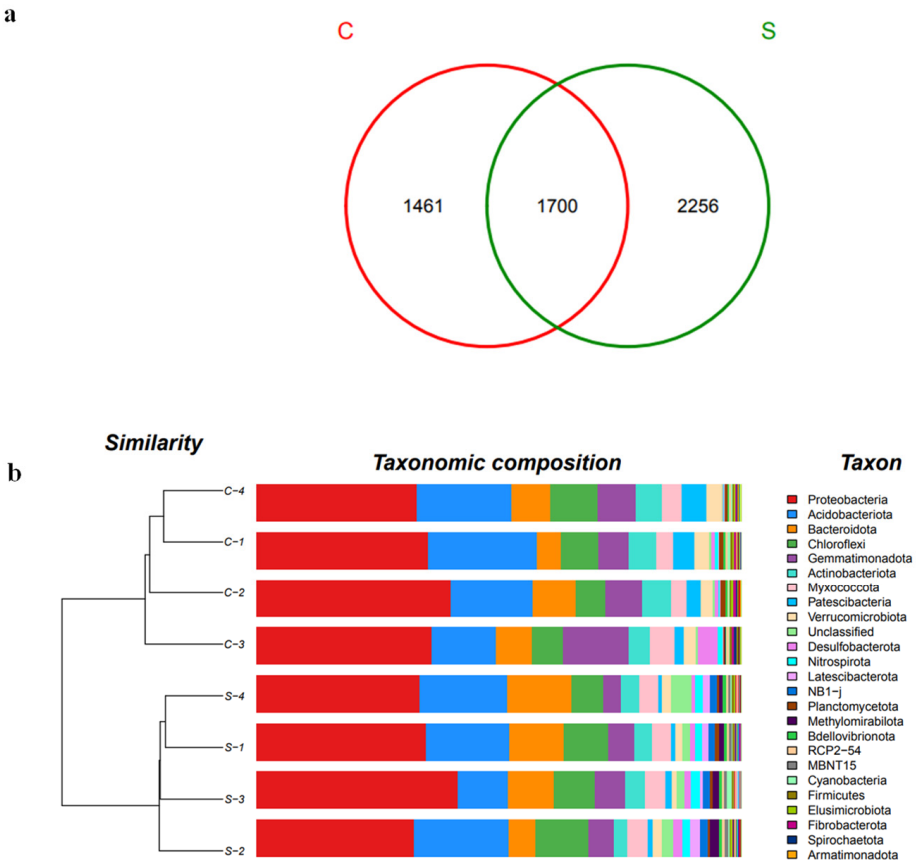

Figure 6. Venn diagram and taxonomic distribution of microbial community. (**a**) Venn diagram; (**b**) Taxonomic distribution.

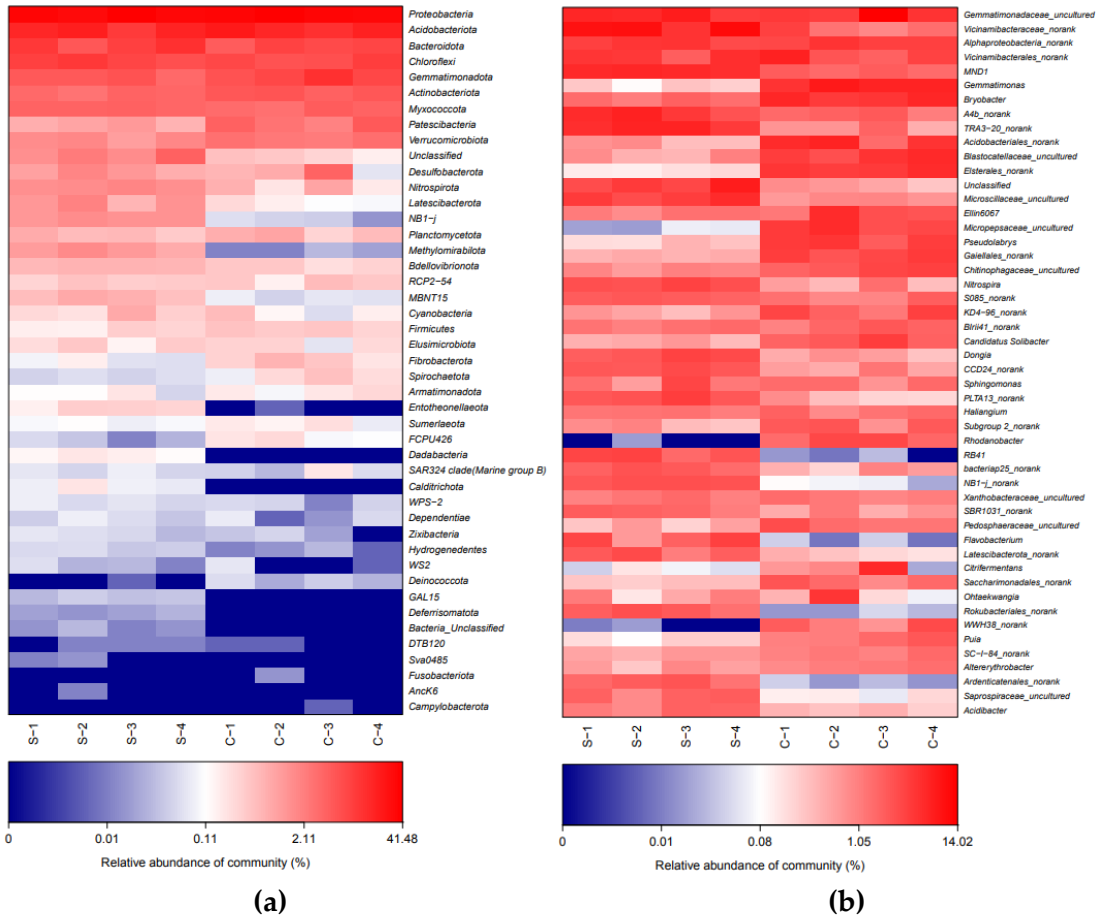

Figure 7. Microbial community heatmap analysis at the phylum (**a**) and genus (**b**) levels based on Bray–Curtis similarity.

3.5. Biomarkers of the Bacterial Microbiome in Rhizosphere Soils

We further compared taxa in the coastal soil vs. nursery soil groups by LEfSe. The analysis was conducted to discover high-dimensional biomarkers. Briefly, the non-parametric factorial Kruskal–Wallis sum-rank test was used to find biomarkers with statistically significant abundance differences. These differentially abundant taxa with a LDA score > 2 and $p < 0.05$ were defined as potential biomarkers. Then, LDA was applied to estimate the effect of each significantly different taxon.

As can be observed in Table 4, for tallow trees, a total of 43 rhizosphere bacterials within the phylum of Proteobacteria, Nitrospirota, NB1-j, Methylomirabilota, Latescibacterota, Chloroflexi, Bacteroidota, and Acidobacteriota were identified as significant abundance bacterials in coastal environments. Further, we used a cladogram to represent the connection between the significantly different taxa at the taxonomic levels. As displayed in the figure, the phyla Actinobacteriota, Verrucomicrobiota, and Patescibacteria, and the classes Alphaproteobacteria and Acidobacteriae were biomarkers in the nursery rhizosphere soil. The SA microbiome at the phylum taxonomic level was characterized by a preponderance of Nitrospirota, Methylomirabilota, NB1-j, and Latescibacterota (Figure 8).

Table 4. Rhizosphere bacterial groups with significant abundance in coastal environments.

Bacterial Communities	Abundance	LDA	*p*-Value
Myxococcota, bacteriap25	4.06	3.64	2.09×10^{-2}
Proteobacteria, Gammaproteobacteria, Steroidobacterales	4.14	3.79	2.09×10^{-2}
Chloroflexi, Anaerolineae, SBR1031, A4b	4.43	3.95	2.09×10^{-2}
Latescibacterota	4.13	3.81	2.09×10^{-2}
Proteobacteria, Gammaproteobacteria, CCD24	4.14	3.67	2.09×10^{-2}
Proteobacteria, Gammaproteobacteria, Burkholderiales, Nitrosomonadaceae, MND1	4.50	3.98	2.09×10^{-2}
Acidobacteriota, Vicinamibacteria, Vicinamibacterales, Vicinamibacteraceae	4.70	4.29	2.09×10^{-2}
Proteobacteria, Alphaproteobacteria, Dongiales, Dongiaceae	4.18	3.76	2.09×10^{-2}
Acidobacteriota, Vicinamibacteria	4.89	4.29	4.33×10^{-2}
Acidobacteriota, Vicinamibacteria, Vicinamibacterales	4.86	4.23	4.33×10^{-2}
Proteobacteria, Gammaproteobacteria, Burkholderiales	5.08	4.27	2.09×10^{-2}
Proteobacteria, Gammaproteobacteria, Pseudomonadales	4.21	3.77	2.09×10^{-2}
Bacteroidota, Bacteroidia, Chitinophagales, Saprospiraceae	4.02	3.64	2.09×10^{-2}
Chloroflexi, Anaerolineae, Ardenticatenales	4.04	3.76	2.02×10^{-2}
Acidobacteriota, Blastocatellia, 11_24	3.97	3.67	2.09×10^{-2}
Nitrospirota, Nitrospiria	4.21	3.78	2.09×10^{-2}
NB1-j	4.16	3.86	2.09×10^{-2}
Proteobacteria, Gammaproteobacteria	5.34	4.67	2.09×10^{-2}
Proteobacteria, Gammaproteobacteria, Burkholderiales, Sutterellaceae	3.88	3.58	1.39×10^{-2}
Acidobacteriota, Blastocatellia, Pyrinomonadales, Pyrinomonadaceae	4.18	3.89	2.09×10^{-2}
Bacteroidota, Bacteroidia, Flavobacteriales	4.18	3.80	2.09×10^{-2}
Bacteroidota, Bacteroidia, Flavobacteriales, Flavobacteriaceae, Flavobacterium	4.13	3.78	1.94×10^{-2}
Nitrospirota, Nitrospiria, Nitrospirales	4.21	3.77	2.09×10^{-2}
Proteobacteria, Gammaproteobacteria, Burkholderiales, TRA3_20	4.49	4.12	2.09×10^{-2}
Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae	3.91	3.59	2.09×10^{-2}
Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	3.91	3.55	2.09×10^{-2}
Nitrospirota, Nitrospiria, Nitrospirales, Nitrospiraceae, Nitrospira	4.21	3.80	2.09×10^{-2}
Acidobacteriota, Subgroup_5	3.84	3.54	2.09×10^{-2}
Proteobacteria, Gammaproteobacteria, Steroidobacterales, Steroidobacteraceae	4.07	3.72	2.09×10^{-2}
Methylomirabilota, Methylomirabilia	4.09	3.81	2.02×10^{-2}
Methylomirabilota, Methylomirabilia, Rokubacteriales	4.08	3.77	2.02×10^{-2}
Acidobacteriota, Blastocatellia, Pyrinomonadales	4.18	3.89	2.09×10^{-2}
Nitrospirota	4.21	3.77	2.09×10^{-2}
Acidobacteriota, Blastocatellia, Pyrinomonadales, Pyrinomonadaceae, RB41	4.18	3.90	2.09×10^{-2}
Chloroflexi, Anaerolineae	4.73	4.26	2.09×10^{-2}
Proteobacteria, Alphaproteobacteria, Dongiales	4.18	3.79	2.09×10^{-2}
Chloroflexi, Anaerolineae, SBR1031	4.56	4.05	2.09×10^{-2}
Proteobacteria, Gammaproteobacteria, PLTA13	4.19	3.81	2.09×10^{-2}
Nitrospirota, Nitrospiria, Nitrospirales, Nitrospiraceae	4.21	3.77	2.09×10^{-2}
Bacteroidota, Bacteroidia, Flavobacteriales, Flavobacteriaceae	4.13	3.80	1.94×10^{-2}
Proteobacteria, Alphaproteobacteria, Azospirillales	4.02	3.71	2.02×10^{-2}
Proteobacteria, Alphaproteobacteria, Dongiales, Dongiaceae, Dongia	4.18	3.75	2.09×10^{-2}
Methylomirabilota	4.09	3.80	2.02×10^{-2}

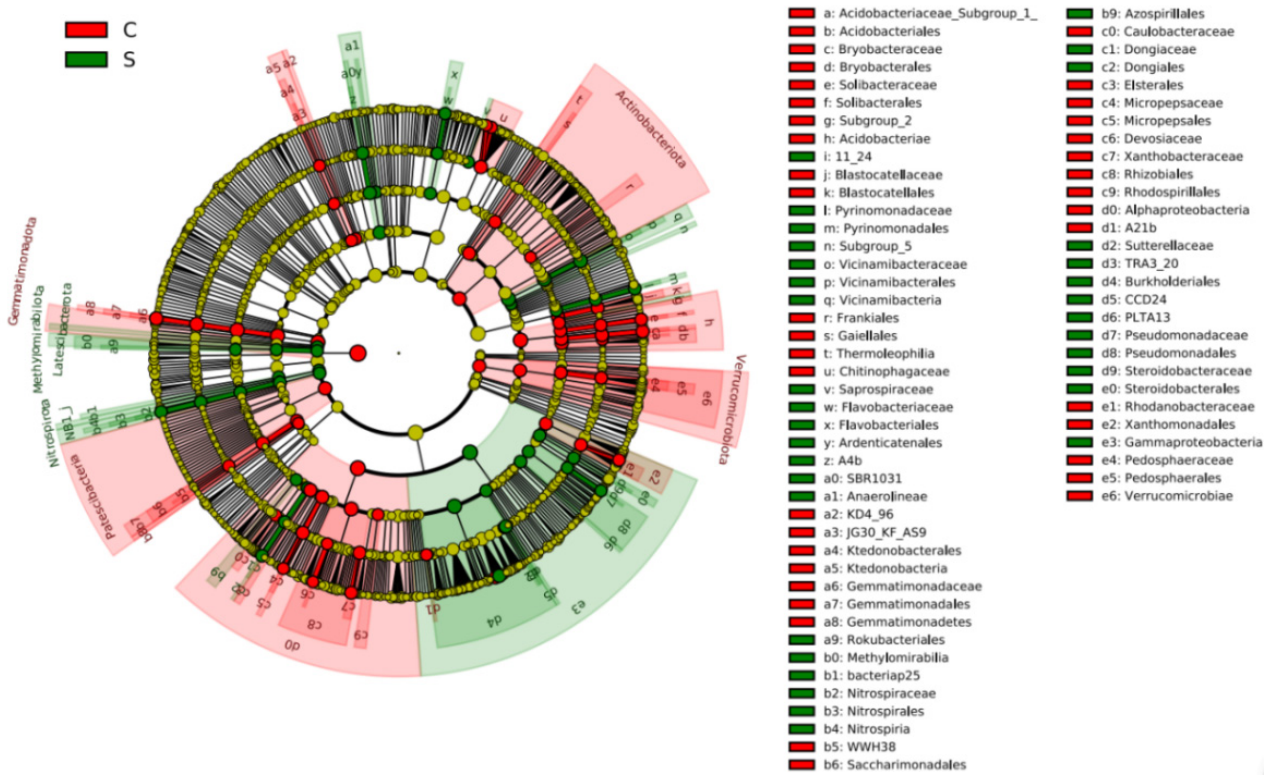

Figure 8. Cladogram depicting the taxonomic levels, from the phylum to the species. Each small circle at a different taxonomic level represents a taxonomic unit at that level. Red nodes denote the rhizosphere microbial groups that play an important role in nursery cultivated tallow trees. The green nodes indicate the microbial groups that have important associations with saline-alkaline environments. Yellow nodes represent the microbial groups that do not play an important role in both groups.

4. Discussion

Coastal areas are characterized by limited faunal and floral diversity. Coastal areas are typically saltwater–freshwater mixing zones, and soil salinity levels may change tremendously along with soil moisture level variations, such as those during drought and flooding. Therefore, the harsh natural conditions hamper the establishment of a critical salinity value for trees grown in coastal regions [13]. As previously reported, global soil microbial community composition is influenced more by salinity than by extremes of temperature, pH, or other chemical factors [14]. Several studies found associations between the shifts in the compositions of bacterial communities and soil salinity [14,15]. In the present study, we found that the community of the rhizosphere bacterial of *S. sebiferum* was considerably affected by coastal soil environment. Moreover, the results suggested that certain species of bacteria phyla were only found in coastal rhizosphere soils, probably depending on saline environmental forces, and also on positive or negative interactions between the root of *S. sebiferum* trees and the microbial groups.

In this study, we identified the bacterial community present in the rhizosphere of *S. sebiferum* trees as a bioindicator for a coastal environment. This community included the groups Latescibacterota, Methylomirabilota, NB1-j, and Nitrospirota, which were largely absent (<1%) in the analyzed nursery rhizosphere soil. These results highlighted the most significant involvement of these bacterial groups in plant–microorganism interactions. The distribution of Latescibacterota (previously known as WS3) and Nitrospirae was reported to be pH-related as they were more abundant in the alkaline soil [16]. In earlier research, Methylomirabilota- and Nitrospirota- associated species were found to be involved in nitrogen cycling [3,17]. The phylum Nitrospirota, known also as Nitrospirae or Nitrospira,

encompasses a limited number of bacterial species [18]. The members of Nitrospirota perform nitrite oxidation for energy acquisition [19]. A recent study showed a novel function of the phylum Nitrospirota, disproportionation of thiosulfate and elemental sulfur [20]. NB1-j is typically found in deep-sea sediments; it is still poorly characterized and uncultivated [21]. Latescibacterota, Methylomirabilota, NB1-j, and Nitrospirota were enriched around the root system of the experimental *S. sebiferum* trees. The interaction between the tree and these bacteria might facilitate the adaptation of the trees to coastal environments, especially through affecting their inorganic nitrogen or sulfide cycling in the rhizosphere.

Proteobacteria, which was the most abundant representative of halophilic microorganisms in coastal soils, was reported to be greatly important to global carbon, nitrogen, and sulfur cycling [22,23]. In this study, the rhizosphere bacteria of the phylum Proteobacteria seemed to have similar proportions in the rhizosphere of *S. sebiferum* trees cultivated in coastal and nursery soils. However, the Gammaproteobacteria and Alphaproteobacteria classes, which belong to the phylum Proteobacteria, appeared to have the highest abundance in the coastal saline-alkali rhizosphere soils. The typical class Gammaproteobacteria was the most abundant taxon among bacterial groups, with a significant difference, although it was not identified as an indicator by our cladogram (Table 3). Previous studies showed that Gammaproteobacteria was predominant in the saline and hypersaline lakes and was critically involved in the ecosystem carbon, nitrogen, and sulfur cycles [24]. In addition, Gammaproteobacteria representatives perform 70–86% of the dark carbon and sulfur fixation in coastal sediments. Given their high abundance in coastal field-grown *S. sebiferum* rhizosphere soils, we speculated that Gammaproteobacteria interacts with tree roots; these bacteria accumulate around the root system, where they convert organic matter into inorganic, effectively providing trees with readily available nutrients. In addition, Gammaproteobacteria might effectively regulate the ecological adaptability of *S. sebiferum* trees, facilitating their resistance to adverse environmental changes, especially saline-alkali stress.

5. Conclusions

The rhizospheric soil of *S. sebiferum* trees in coastal environments has relatively higher community diversity than that in forestry nursery environments. The phyla Proteobacteria, Acidobacteriota, Bacteroidota, Chloroflex, and Gemmatimonadota are the dominant rhizospheric bacteria in the rhizosphere soil of *S. sebiferum* trees. However, Latescibacterota, Methylomirabilota, NB1-j, and Nitrospirota were distinct for the rhizosphere soils of trees grown in coastal fields. Hence, these bacterial groups were identified as biomarkers for the coastal environment at the phylum taxonomic level. *S. sebiferum* trees might promote its adaptability through interactions between the tree roots and the bacteria in the rhizosphere, which increases their adaptability to coastal environment, at least partially by recruiting certain species of the bacterial microbiome.

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References

1. Chen, X.; Mao, Y.; Huang, S.; Ni, J.; Lu, W.; Hou, J.; Wang, Y.; Zhao, W.; Li, M.; Wang, Q.; et al. Selection of Suitable Reference Genes for Quantitative Real-time PCR in *Sapium sebiferum*. *Front. Plant. Sci.* **2017**, *8*, 637. [CrossRef] [PubMed]
2. Boldor, D.; Kanitkar, A.; Terigar, B.G.; Leonardi, C.; Lima, M.; Breitenbeck, G.A. Microwave Assisted Extraction of Biodiesel Feedstock from the Seeds of Invasive Chinese Tallow Tree. *Environ. Sci. Technol.* **2010**, *44*, 4019–4025. [CrossRef] [PubMed]
3. Barrilleaux, T.C.; Grace, J.B. Growth and invasive potential of Sapium sebiferum (Euphorbiaceae) within the coastal prairie region: The effects of soil and moisture regime. *Am. J. Bot.* **2000**, *87*, 1099–1106. [CrossRef] [PubMed]
4. Zhao, D.; Tao, J. Recent advances on the development and regulation of flower color in ornamental plants. *Front. Plant. Sci.* **2015**, *6*, 261. [CrossRef] [PubMed]
5. Jones, R.H.; McLeod, K.W. Growth and photosynthetic responses to a range of light environments in Chinese tallowtree and Carolina ash seedlings. *For. Sci.* **1990**, *36*, 851–862.
6. Shah, F.A.; Wei, X.; Wang, Q.; Liu, W.; Wang, D.; Yao, Y.; Hu, H.; Chen, X.; Huang, S.; Hou, J.; et al. Karrikin improves osmotic and salt stress tolerance via the regulation of the redox homeostasis in the oil plant Sapium sebiferum. *Front. Plant. Sci.* **2020**, *11*, 216. [CrossRef]
7. Conner, W.H. The Effect of Salinity and Waterlogging on Growth and Survival of Baldcypress and Chinese Tallow Seedlings. *J. Coast. Res.* **1994**, *10*, 1045–1049.
8. Conner, W.H.; McLeod, K.W.; McCarron, J.K. Flooding and salinity effects on growth and survival of four common forested wetland species. *Wetl. Ecol. Manag.* **1997**, *5*, 99–109. [CrossRef]
9. Conner, W.H.; George, R.A. Impact of Saltwater Flooding on Red Maple, Redbay, and Chinese Tallow Seedlings. *Castanea* **1993**, *58*, 214–219.
10. Edgar, R.C.; Haas, B.J.; Clemente, J.C.; Quince, C.; Knight, R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **2011**, *27*, 2194–2200. [CrossRef]
11. Edgar, R. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10*, 996–998. [CrossRef] [PubMed]
12. DeSantis, T.Z.; Hugenholtz, P.; Larsen, N.; Rojas, M.; Brodie, E.L.; Keller, K.; Huber, T.; Dalevi, D.; Hu, P.; Andersen, G.L. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* **2006**, *72*, 5069–5072. [CrossRef] [PubMed]
13. Lozupone, C.A.; Knight, R. Global patterns in bacterial diversity. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 11436–11440. [CrossRef] [PubMed]
14. Ma, B.; Gong, J. Biotechnology. A meta-analysis of the publicly available bacterial and archaeal sequence diversity in saline soils. *World J. Microbiol. Biotechnol.* **2013**, *29*, 2325–2334. [CrossRef] [PubMed]
15. Zhang, Y.; Shen, H.; He, X.; Thomas, B.W.; Lupwayi, N.Z.; Hao, X.; Thomas, M.C.; Shi, X. Fertilization shapes bacterial community structure by alteration of soil pH. *Front. Microbiol.* **2017**, *8*, 1325. [CrossRef] [PubMed]
16. Zhang, X.; Liao, X.; Huang, L.; Shan, Q.; Hu, A.; Yan, D.; Zhang, J.; Long, X.-E. Sediments. Soil profile rather than reclamation time drives the mudflat soil microbial community in the wheat-maize rotation system of Nantong, China. *J. Soils Sediments* **2021**, *21*, 1672–1687. [CrossRef]
17. Coutinho, F.H.; Cabello-Yeves, P.J.; Gonzalez-Serrano, R.; Rosselli, R.; López-Pérez, M.; Zemskaya, T.I.; Zakharenko, A.S.; Ivanov, V.G.; Rodriguez-Valera, F. New viral biogeochemical roles revealed through metagenomic analysis of Lake Baikal. *Microbiome* **2020**, *8*, 163. [CrossRef]
18. Umezawa, K.; Kojima, H.; Kato, Y.; Fukui, M. Disproportionation of inorganic sulfur compounds by a novel autotrophic bacterium belonging to Nitrospirota. *Syst. Appl. Microbiol.* **2020**, *43*, 126110. [CrossRef]
19. Umezawa, K.; Kojima, H.; Kato, Y.; Fukui, M. Dissulfurispira thermophila gen. nov., sp. nov., a thermophilic chemolithoautotroph growing by sulfur disproportionation, and proposal of novel taxa in the phylum Nitrospirota to reclassify the genus Thermodesulfovibrio. *Syst. Appl. Microbiol.* **2021**, *44*, 126184. [CrossRef]
20. Agostini, L.; Moreira, J.C.F.; Bendia, A.G.; Kmit, M.C.P.; Waters, L.G.; Santana, M.F.M.; Sumida, P.Y.G.; Turra, A.; Pellizari, V.H. Deep-sea plastisphere: Long-term colonization by plastic-associated bacterial and archaeal communities in the Southwest Atlantic Ocean. *Sci. Total Environ.* **2021**, *793*, 148335. [CrossRef]
21. Kersters, K.; De Vos, P.; Gillis, M.; Swings, J.; Vandamme, P.; Stackebrandt, E. Introduction to the Proteobacteria. In *The Prokaryotes: A Handbook on the Biology of Bacteria*; Springer: Berlin, Germany, 2006; Volume 5, pp. 3–37.
22. Spain, A.M.; Krumholz, L.R.; Elshahed, M.S. Abundance, composition, diversity and novelty of soil Proteobacteria. *ISME J.* **2009**, *3*, 992–1000. [CrossRef] [PubMed]
23. Liu, W.; Jiang, H.; Yang, J.; Wu, G. Gammaproteobacterial diversity and carbon utilization in response to salinity in the Lakes on the Qinghai–Tibetan Plateau. *Geomicrobiol. J.* **2018**, *35*, 392–403. [CrossRef]
24. Dyksma, S.; Bischof, K.; Fuchs, B.M.; Hoffmann, K.; Meier, D.; Meyerdierks, A.; Pjevac, P.; Probandt, D.; Richter, M.; Stepanauskas, R.; et al. Ubiquitous Gammaproteobacteria dominate dark carbon fixation in coastal sediments. *ISME J.* **2016**, *10*, 1939–1953. [CrossRef] [PubMed]