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Geographic Distribution Patterns of Soil Microbial Community Assembly Process in Mangrove Constructed Wetlands, Southeast China

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Abstract: Constructed wetlands, as an emerging wastewater treatment system, have been widely used worldwide due to their high purification efficiency and low investment and operating costs. Wetland plants, on the other hand, together with their inter-root microbes, significantly affect the ecological functions of constructed wetlands. The mangrove constructed wetland within Futian District, Shenzhen, China, is a typical wastewater treatment area, but the structure and function of its soil microbial community remain largely unexplored. In this study, the assembly and processes of the soil microbial communities in this constructed wetland were intensively investigated using high-throughput sequencing technology. Our results showed that the three mangrove plants had significant effects on the soil bacterial microbial community α-diversity, insignificant effects on β-diversity, and significant effects on fungal α-diversity and β-diversity. The abundance of genera changed significantly between the treatment groups, such as the genus *Candidatus_Udaeobacter* for bacteria versus *Russula* for fungi, and the random forest model showed that rare genera (e.g., *Acidibacter*, *Dyella*, *Sebacina*, and *Lachnellula*) also play an important role in microbial community construction. Community assembly revealed the deterministic process of soil bacterial and fungal communities under different mangrove species. Overall, this study enhanced our understanding of soil microbial community composition and diversity in constructed wetlands ecosystems, providing insights into their manageability.

Keywords: constructed wetland; mangrove; microbial community; community assembly

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1. Introduction

Compared with traditional wastewater treatment processes such as activated sludge and biofilm [1], constructed wetlands (CWs) have emerged as a widely adopted sustainable technology due to their lower power consumption, operational costs, and carbon emissions [2]. Over the past few decades, CWs have garnered increasing attention, with numerous successful cases demonstrating their efficacy in treating industrial effluent, landfill leachate, and urban runoff [3,4]. Mangrove forests, primarily distributed along the intertidal zones of tropical coastlines and serving as bridges between terrestrial and marine ecosystems, have long been convergence points for urban sewage and industrial wastewater [5]. Leveraging their well-developed root systems and abundant microbial communities, mangrove forests exhibit exceptionally high efficiency in pollutant filtration, nutrient absorption, and carbon sequestration [6]. Although CWs have demonstrated remarkable effectiveness, there is still a need to further enhance their purification efficiency in regions where mangrove forests are densely distributed. Therefore, the strategic integration of

mangrove species into the design of constructed wetlands can help us fully harness the natural water purification functions of these unique ecosystems. Given this background, a thorough exploration of the water purification efficiency and mechanisms of different mangrove species is crucial for the planning and design of constructed wetlands.

Soil microbes are an indispensable component of soil ecosystems, as they decompose organic matter into nutrients absorbable by plants, thereby enhancing soil fertility. Additionally, these microbes improve the soil structure, increase soil permeability and aeration, and reduce soil erosion and water loss, playing a crucial role in environmental protection and the maintenance of ecological balance [7]. CWs, as ecosystems designed by humans to utilize or enhance wetland functions, mimic natural wetland environments and leverage the synergistic interactions among plants, microbes, and water bodies to achieve water purification and ecological restoration [8,9]. Within this intricate and delicate ecological network, soil microbes not only undertake the fundamental tasks of decomposing organic pollutants and transforming nutrients but also actively participate in the self-regulation and recovery processes of the wetland ecosystem [10,11]. By forming symbiotic relationships with the root systems of wetland plants, they promote plant growth and development, thereby enhancing the wetland's capacity to absorb and process pollutants [12,13]. Furthermore, the diversity and activity of soil microbes are crucial factors for the stable operation of constructed wetlands, as they enable resistance to external environmental disturbances and maintain the stability and sustainability of the wetland ecosystem [14,15]. Therefore, in the design and management of constructed wetlands, it is of profound significance to fully consider and protect the ecological functions of soil microbes [16], as this contributes to improving the purification efficiency of the constructed wetlands, preserving ecological balance, and promoting sustainable development [17,18].

Located in Futian District, Shenzhen City, Guangdong Province, the Futian Mangrove Nature Reserve not only acts as a pivotal buffer zone between the protected areas of Shenzhen and Hong Kong but also serves as a crucial ecological corridor facilitating biodiversity exchange between the two regions. However, prior to its designation as a nature reserve, the area suffered severely from wastewater pollution, making the construction of artificial wetlands an indispensable measure to mitigate the environmental impacts of natural wetland degradation, with profound implications for maintaining ecosystem health and promoting sustainable development [19,20]. Furthermore, the construction of artificial wetlands presents a rare opportunity to explore the composition and diversity differences in microbial communities among various mangrove species, a research endeavor that holds significant importance in a global context [9,21]. *Kandelia obovata*, *Sonneratia caseolaris*, and *Aegiceras corniculatum*, these three plant species, are not only critical components of mangrove ecosystems but also play pivotal roles in the functionality of constructed wetlands [22–25]. As the primary producers in these wetlands, they facilitate the growth of diverse microbial communities by providing organic matter and creating favorable microenvironments. The interactions between these plants and their associated microorganisms are crucial for maintaining the ecological balance and enhancing the purification efficiency of artificial wetlands, with the research findings offering valuable insights for the formulation and management of wetland restoration and pollution control strategies worldwide [26,27]. By examining the specific roles and interactions of *Kandelia obovata*, *Sonneratia caseolaris*, and *Aegiceras corniculatum* within constructed wetland systems, this study aimed to offer insights into optimal design and management strategies for sustainable wetland restoration and pollution control.

Given the above background, this study focused on three mangrove species: *Kandelia obovata*, *Sonneratia caseolaris*, and *Aegiceras corniculatum*. We hypothesized that: (1) different mangrove species affect the diversity and composition of soil microbial communities; (2) dif-

ferent mangrove species alter the deterministic and stochastic processes of soil microbial community assembly; and (3) different mangrove species lead to changes in soil physicochemical properties, which in turn influence the soil microbial community. Ultimately, our goal was to deepen our understanding of how soil microorganisms purify wastewater, particularly under the conditions of different mangrove species, and to contribute to the development of more effective and sustainable wastewater treatment strategies utilizing mangrove-associated microbial communities.

2. Material

2.1. Artificial Mangrove Wetland

The study site is located in the Futian Mangrove Nature Reserve (22°32′ N, 114°03′ E) in Shenzhen, which has a southern monsoon climate with an annual mean temperature of 22.6 °C, an extreme high temperature of 38.7 °C (July), and an extreme low temperature of 0.2 °C (January), with small seasonal variations. The average annual precipitation is 1794 mm; the average annual relative humidity is 79%, alternating between wet and dry; the annual cumulative temperature is 2209 h; the average water depth is about 70 cm; and the total area is 400 m^2. The main mangrove species are the native mangrove species autumn eggplant (*Kandelia candel*) and Kiril tree (*Aegiceras corniculatum*) and the alien species sea mulberry (*Sonneratia caseolaris*).

2.2. Experimental Design

The constructed wetland system utilized gravel as the bottom-filling matrix, which was successively overlaid with gravel, fine sand, and mangrove soil, resulting in a seamless transition between the aquifer and the waterproof layer without a clear distinction. The total area is about 400 m^2. It includes two sedimentation tanks and three subsurface flow wetland belts. The bottom of the sedimentation tank is higher than the constructed wetland belt by 5 m, so that the water can flow into the wetland by itself under gravity conditions. Each wetland belt is 5 m. The wetland belt adopts a reinforced concrete structure as a separation area. In the three belts, autumn eggplant, tung flower trees, and sea mulberry are planted, respectively. The sewage used in the wetland is domestic sewage from mangroves in Futian area of Shenzhen. It is pumped to the sedimentation tank and poured into the wetland. The direction of water flow is bottom in and top out. Three annual mangrove seedlings were transplanted into wetlands (two native species, *Kandelia candel* and *Aegiceras corniculatum* and one exotic species, *Sonneratia caseolaris*). Since there were differences in the growth rates of these three mangrove species, adjustments were made to the plant spacing to ensure that each species could grow under optimal light and nutrient conditions. Specifically, for mangrove species with slower growth rates or lower nutrient requirements (such as *Saurophylla sativum*), larger plant spacing (1 m × 1 m) was used to provide more room for growth and nutrient resources. For mangrove species that grow faster or have higher nutrient requirements (such as *Solanum sativum* and *Taurophylla sativum*), smaller plant spacing (0.5 m × 0.5 m) was employed, which allowed more plants to be grown on a limited amount of land while ensuring they had access to adequate light and nutrients, thus promoting their rapid growth. Within three months after the entry of the mangrove species, seawater was pumped to survive, and after three months, sewage was pumped. The hydraulic load of each wetland zone is 333.33 $m^3\ d^{-1}$, and the residence time is about 30 days.

2.3. Sample Collection

In each section of the KC, SC, and AC bands, topsoil collection (0–10 cm) was performed using a five-point sampling method and mixed into a single sample with six repli-

cate groups. Samples were placed in polyethylene bags and transported to the laboratory using a refrigerator filled with ice. Each sample was divided into three parts; one was air-dried and used for physicochemical properties.

2.4. Characterization of Soil Chemical Parameters

Soil pH was measured by mixing 10 g of air-dried soil with 25 mL deionized water (soil–deionized water = 1:2.5) The mixture was shaken for 30 min, allowed to settle, and then measured using a pH meter (pHS-25, China) [28] Total nitrogen (TN) and total potassium (TK) were quantified using an elemental analyzer (Elementar, Langenselbold, Germany) [29]. Total phosphorus (TP) was measured through wet digestion with $HClO_4$-H_2SO_4 followed by spectrophotometric measurement [30]. Cation exchange capacity (CEC) was measured using the ammonium acetate method [31]. Soil electrical conductivity (EC) was assessed with a conductivity meter (DDS-307, Shanghai, China) after preparing a saturated paste extract [32]. Soil organic carbon (SOC) was measured after eliminating inorganic C by fumigating with HCl for 24 h and determined by H_2SO_4-$K_2Cr_2O_7$ oxidation followed by titration with $FeSO_4$ [33].

2.5. DNA Extraction, PCR Amplification, and MiSeq Sequencing

We used the Fast DNA SPIN Extraction Kit (MP Biomedicals; Santa Ana, CA, USA) to extract genomic DNA from 0.5 g of soil according to the instructions [34]. The quantity of DNA was detected using agarose gel electrophoresis (1%) and assayed using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific; Waltham, MA, USA) [35]. We amplified the V3−V4 region of bacterial 16S rRNA using the primer 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), and the ITS rRNA region was amplified using primers ITS1 (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCATCGATGC-3′) [36,37]. This was mainly because the 16S rRNA gene sequence contained two parts: a conserved region and a highly variable region. The V3–V4 region was located in the highly variable region of the 16S rRNA gene, and this specific region not only had enough variability to effectively distinguish different bacterial species but also maintained a certain degree of conservation [38], which made it easy to design universal primers for amplification. In addition, the moderate length of the V3–V4 region (usually about 460 bp) ensured that the region was fully covered [38], thus achieving a high coverage of bacteria. Furthermore, ITS was a non-coding sequence that existed between fungal rRNA genes, consisting of ITS1 and ITS2 [39]. This region is known for its high degree of interspecies variability, which allowed for more sequence differences and thus exhibited superior species differentiation [40]. Although the ITS region varied greatly on the whole, it maintained a certain conservative character in some local regions. By selecting the specific conserved sequences in ITS1 and ITS2 regions as primer binding sites, the fungal ITS sequences could be efficiently amplified, which laid a solid foundation for subsequent fungal diversity analysis and species identification.

A 7 bp sample-specific DNA barcode was added to the primers of each unique sample for multiplex sequencing. PCR amplification was then performed, and the PCR amplification conditions were as follows: The PCR reaction mixture volume was 25 μL and consisted of 12.5 μL PCR Master Mix (TAKARA, Tokyo, Japan), 2.0 μL forward and reverse primers (5 μmol L^{-1}), 2.0 μL DNA, and 6.5 μL H_2O. The PCR amplification procedures included an initial denaturation step at 95 °C for 45 s, followed by 30 cycles at 95 °C for 45 s, 55 °C for 50 s, and 72 °C for 60 s, with a final extension step at 72 °C for 10 min. Each PCR was performed in triplicate [41]. PCR products were purified using the TAKARA DNA Gel Extraction Kit (TAKARA Biosciences) [42]. The purified PCR products were pooled in equal proportions and were quantified using Qubit 3.0 (Life Invitrogen, Waltham, MA,

USA). During DNA extraction, we used detergents and salts for efficient cell lysis and DNA release, followed by purification via phenol–chloroform, high salt precipitation, or column methods to achieve high purity [43]. For sequencing, we conducted rigorous quality checks on DNA samples, employing high-throughput technologies like Illumina and PacBio for precise sequencing [44]. We also repeated sequencing and validated results to promptly identify and rectify any errors, thus ensuring data accuracy.

2.6. Analysis of Sequencing Data

We used the QIIME1 [45] to analyze the raw readings. UPARSE was used for constructing OTUs de novo from next-generation reads [46]. Taxonomic classification was carried out using the Unite 8.0 Fungal Database. The BLAST algorithm was then utilized via QIIME on sequences from the UNITE 8.0 database [47]. The abundance of each operational taxonomic unit (OTU) in each sample and the taxonomies of these OTUs were then tabulated. The alpha diversity indices (e.g., Chao1 index, ACE index, Shannon index, Simpson index, Pielou index, and goods-coverage) were calculated in QIIME1 using the previously mentioned OTU table.

2.7. Statistical Analyses

One-way analysis of variance (ANOVA) was conducted to assess variations in the soil chemical properties and the alpha diversity of soil bacterial and fungal communities, followed by least significant difference (LSD) tests [48]. A Venn diagram was created using R software (Version 4.4.1) with the Vegan package [49]. A redundancy analysis (RDA) was performed using Canoco 4.5 software to evaluate the impact factors of the overall bacterial community structure [50]. Indicator species taxa of soil bacterial and fungal communities were identified using linear discriminant analysis (LDA) effect size (Lefse) analysis [51]. The "microeco" and "ggplot2" packages in R software (Version 4.4.1) were utilized to generate LEfSe graphs, while random forest analyses were conducted using "random Forest", "psych", "reshape2" and "ggplot2" packages in R software (Version 4.4.1) to identify key bacterial and fungal taxa [52]. The random forest model was constructed based on the decision tree classification algorithm [53]. The Kruskal–Wallis test was performed using the "stats" package in the R software (Version 4.4.1) [54]. The correlation heatmaps were visualized using the "corrmorant", "pheatmap", "ggplot2", "ggcorrplot", and "corrgram" packages in the R software (Version 4.4.1) [55]. The Sloan Neutral Community Model was used to reveal the intrinsic mechanisms of soil microbial community assembly and to further define microbial community processes [56].

3. Results

3.1. Soil Physical and Chemical Properties

There were significant differences in soil physicochemical properties among the different plant types (Table 1; $p < 0.05$). The pH value was highest in the SC treatment and lowest in the *Aegiceras corniculatum* treatment. However, the electrical conductivity (EC) was highest in the AC treatment and lowest in the SC treatment. The concentrations of soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), and total potassium (TK) were all highest in the KC treatment.

3.2. Soil Microbial Diversity and Composition

Among the alpha diversity indices of soil bacteria (Table 2; $p < 0.05$), the Shannon, Simpson, and ACE indices in the KC treatment were higher than those in the *Sonneratia caseolaris* and *Aegiceras corniculatum* treatments, although the differences were not significant. In contrast, the alpha diversity indices of soil fungi remained relatively constant. Despite

the minimal changes in soil microbial alpha diversity, significant variations in soil microbial community composition were observed when beta diversity was examined. PCA analysis indicated that the first principal component (PC1) of soil bacteria accounted for 47.99% of the total variance (Figure 1a), suggesting that PC1 plays a crucial role in explaining the variability within the dataset. The second principal component (PC2), accounting for 29.33% of the total variance, although slightly less important than PC1, remains a significant variable for further explaining the variability within the dataset. Similar to soil bacteria, for soil fungi, PC1 accounted for 34.68% of the total variance, and PC2 accounted for 25.67% of the total variance. Both soil bacteria and soil fungi exhibited significant differences among the different plant types. The clear separation of the samples among different treatments in the plot indicates significant differences in their gene expression patterns. Furthermore, the minimal overlap of confidence ellipses among the samples suggests that these differences in the PCA space are statistically significant (Figure 1).

Table 1. Soil physicochemical properties in different treatments.

Sample	pH	TN (mg kg^{-1})	TP (mg kg^{-1})	TK (mg kg^{-1})	EC (μS cm^{-1})	CEC ($cmol^{(+)}$ kg^{-1})	SOC (%)
SC	7.0833 ± 0.03055 a	0.002 ± 0.0001 c	528 ± 37.64306 c	16.6 ± 0.36056 a	68.9667 ± 4.85215 b	30.2667 ± 0.15275 b	19.5667 ± 0.15275 c
AC	5.4467 ± 0.11547 c	0.0028 ± 0.0001 b	734 ± 27.0555 b	16.6667 ± 0.40415 a	96.6333 ± 6.16468 a	18.9 ± 0.26458 c	29.6333 ± 0.28868 b
KC	5.93 ± 0 b	0.0052 ± 0.0001 a	1036.6667 ± 15.27525 a	17.3 ± 0.1 a	87 ± 2.90517 a	32.9333 ± 0.58595 a	59.9333 ± 1.10151 a

Note: SC: *S. caseolaris*, KC: *K. candel*, AC: *A. corniculatum*. Six replications were performed for each treatment. The data are expressed as the mean ± standard deviation; lowercase letters indicate significant differences ($p < 0.05$).

Table 2. Alpha diversity of bacterial and fungal communities in different treatments.

Microbial Community	Sample	Shannon Index	Simpson Index	Pielou Index	Chao1 Index	ACE Index	Goods-Coverage
Bacteria	SC	5.6385 ± 0.3591 b	0.9775 ± 0.0033 b	0.7078 ± 0.024 b	3844.6924 ± 1026.6874 b	3927.0792 ± 1004.675 b	0.9823 ± 0.0045 a
	AC	5.8519 ± 0.1412 ab	0.9879 ± 0.0016 a	0.7305 ± 0.0015 ab	3946.9421 ± 781.6323 b	3999.4834 ± 753.8613 b	0.9866 ± 0.0032 a
	KC	6.297 ± 0.0505 a	0.9931 ± 0.0008 a	0.7501 ± 0.0098 a	5561.2174 ± 319.5831 b	5703.9682 ± 304.6023 b	0.9866 ± 0.0056 a
Fungi	SC	3.106 ± 0.5216 a	0.872 ± 0.0476 a	0.5264 ± 0.0606 a	391.4436 ± 119.745 a	391.2065 ± 115.149 a	0.9996 ± 0.0001 a
	AC	3.6947 ± 0.2397 a	0.9385 ± 0.0106 a	0.5731 ± 0.0193 a	700.3547 ± 134.4325 a	702.6598 ± 130.5442 a	0.999 ± 0.0004 a
	KC	3.0921 ± 0.2662 a	0.834 ± 0.0631 a	0.5011 ± 0.0611 a	535.2602 ± 165.0063 a	534.3394 ± 162.3972 a	0.9992 ± 0.0005 a

Note: SC: *S. caseolaris*, KC: *K. candel*, AC: *A. corniculatum*. Three replications were performed for each treatment. The data are expressed as the mean ± standard deviation; lowercase letters indicate significant differences ($p < 0.05$).

According to the Venn diagram, there are 418 shared soil bacterial OTUs among the three treatments (Figure 2a). The KC group has the highest number of unique OTUs, with 8, while the AC treatment has the fewest, with only 3 unique OTUs. Additionally, among the soil fungal communities, there are 100 shared OTUs among the 3 treatments (Figure 2b). The AC treatment has the highest number of OTUs, totaling 52, whereas the SC treatment

has the fewest, with only 15 OTUs. It is visually observable that the overall number of soil fungal OTUs is slightly lower than that of soil bacterial OTUs. In the bacterial stacked bar chart (Figure 3a), *Candidatus_Udaeobacter* exhibits the highest abundance, significantly outpacing other bacterial genera. Closely following is *Bradyrhizobium*, which also displays a prominent abundance. *Bryobacter* ranks third, and although its abundance is less than the first two, it still comprises a notable proportion. Notably, *Roseiarcus*, *Mucilaginibacter*, and *Granulicella* show significantly higher proportions in the *Aegiceras corniculatum* treatment compared with the other two treatments. In the fungal stacked bar chart (Figure 3b), *Russula* boasts the highest abundance, standing out as the dominant fungus. *Mortierella* trails closely behind; although its abundance is less than that of *Russula*, it still holds a significant position. Moreover, each treatment has its own genus with an extremely high proportion. Among soil bacteria (Figure 4a), the genera *Burkholderia-Caballeronia-Paraburkholderia* and *Granulicella* are most abundant in the *Aegiceras corniculatum* treatment. Conversely, *Haliangium* and *Pseudolabrys* are most numerous in the KC treatment. Among soil fungi (Figure 4b), with the exception of *Cortinarius*, which is most abundant in the *Aegiceras corniculatum* treatment, all other fungal genera are most prevalent in the KC treatment. There are significant differences in soil microbial genera among the various treatments (Figure 4).

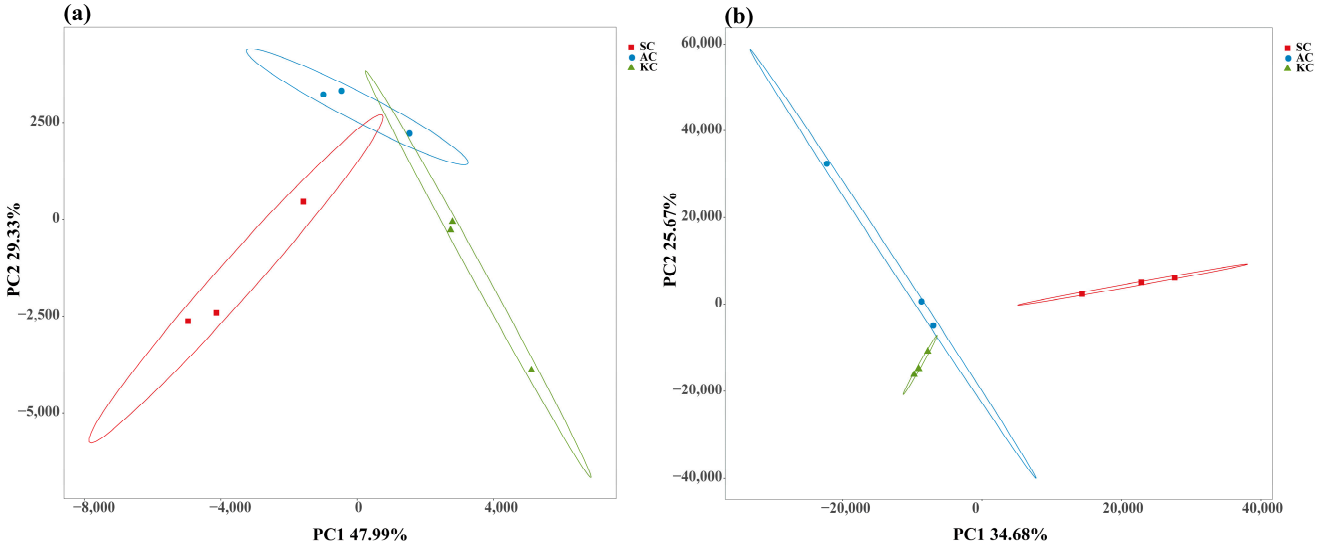


Figure 1. Principal component analysis (PCA) of soil bacterial (**a**) and fungal (**b**) communities across different plant types. Dots of different colors represent different plant types. The *p*-values are used to assess whether the observed differences are merely due to random variations. SC: *Sonneratia caseolaris* treatment; AC: *Aegiceras corniculatum* treatment; KC: *Kandelia candel* treatment.

The random forest classification model showed that *P3OB_51* and *Acidibacter* play an important role in the soil bacterial communities (Figure 5a). However, they are not the most abundant genera (Figure 3a). A similar pattern is observed in soil fungal communities, where genera such as *Sebacina* and *Lachnellula* exert crucial functions despite accounting for < 1% of the total abundance (Figure 5b). In the Circos plot of bacteria, the intricate relationships among various bacterial genera and samples are visually represented (Figure 6a). Herein, *Candidatus_Udaeobacter* occupies a unique position, forming specific associations with multiple samples and exhibiting a relatively high proportion across all treatments. Furthermore, genera such as *Bradyrhizobiu* and *Bryobacter* also exhibit their unique taxonomic positions and inter-genera associations. The Circos plot for fungi unveils a different landscape within the fungal kingdom (Figure 6b). In this plot, *Russula* dominates with the highest proportion in all treatments, occupying a substantial portion.

Although other genera do not have proportions as high as *Russula*, they each play their roles, demonstrating the diversity and complexity within the fungal kingdom. In addition, in the soil bacterial community (Figure 7a), based on the results of LEfSe analysis, we found that *norank_Rokubacteriales* and *norank_bacteriap25* were the key microbial taxa in the SC treatment. In the AC treatment, *Granulicella*, *Acidobacteriaceae_Subgroup_1*, and *Acidobacteriales* emerged as significant microbial taxa. For the KC treatment, *Subgroup_7* and *Gemmatimonadales* were the crucial microbes. In the soil fungal community (Figure 7b), we observed that *Mytilinidion*, *Sebacina*, and *Phialomyces* were the key microbial taxa in the SC treatment. In the AC treatment, *Lipomycetaceae* and *Cordycipitaceae* became important microbial taxa. For the KC treatment, *Geoglossales*, *Geoglossomycetes*, and *Agaricales* were the key microbes.

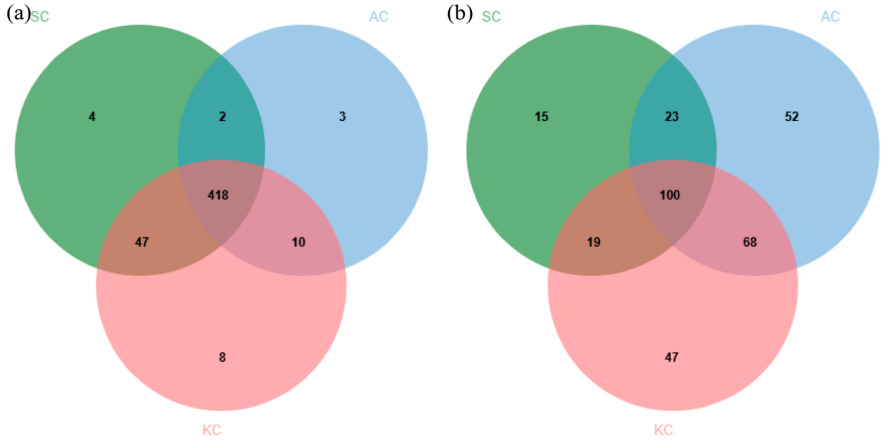


Figure 2. Variance distributions of soil bacterial (**a**) and fungal (**b**) communities under three different treatments (SC, AC, and KC) based on significance levels from Monte Carlo permutation tests. Different colors represent distinct treatment conditions. The overlapping areas depict the common bacterial and fungal OTUs (operational taxonomic units) across different treatments. The non-overlapping areas indicate bacterial and fungal OTUs specific to each treatment condition. The numbers represent the counts of bacterial and fungal OTUs under various treatment conditions. SC: *Sonneratia caseolaris* treatment; AC: *Aegiceras corniculatum* treatment; KC: *Kandelia candel* treatment. The blue part represents the number of bacteria contained in group AC alone, the green part represents the number of bacteria contained in group SC alone, and the pink part represents the number of bacteria contained in group KC alone.

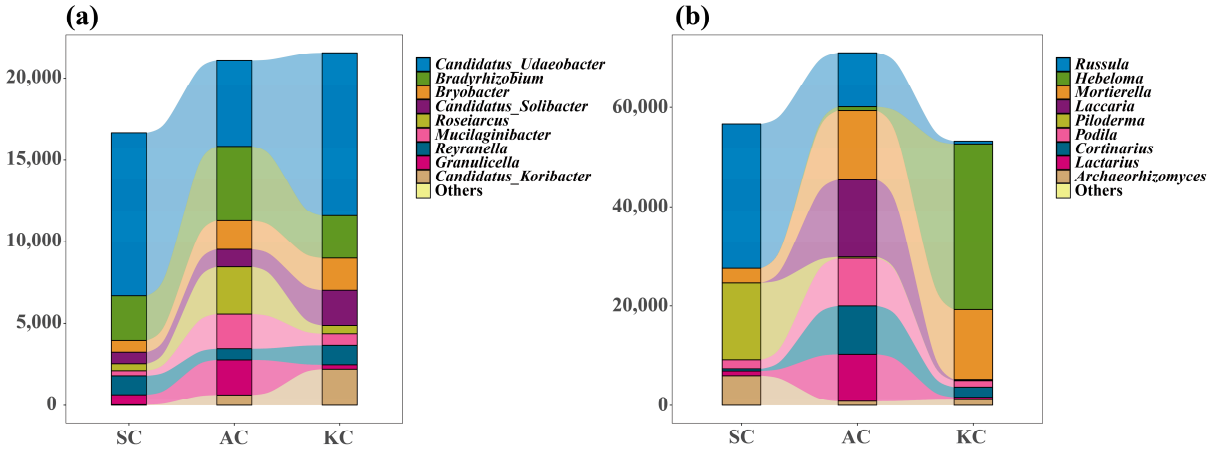


Figure 3. Stacked bar charts representing the top 10 abundant soil bacterial genera (**a**) and soil fungal genera (**b**) under different treatments. SC: *Sonneratia caseolaris* treatment; AC: *Aegiceras corniculatum* treatment; KC: *Kandelia candel* treatment.

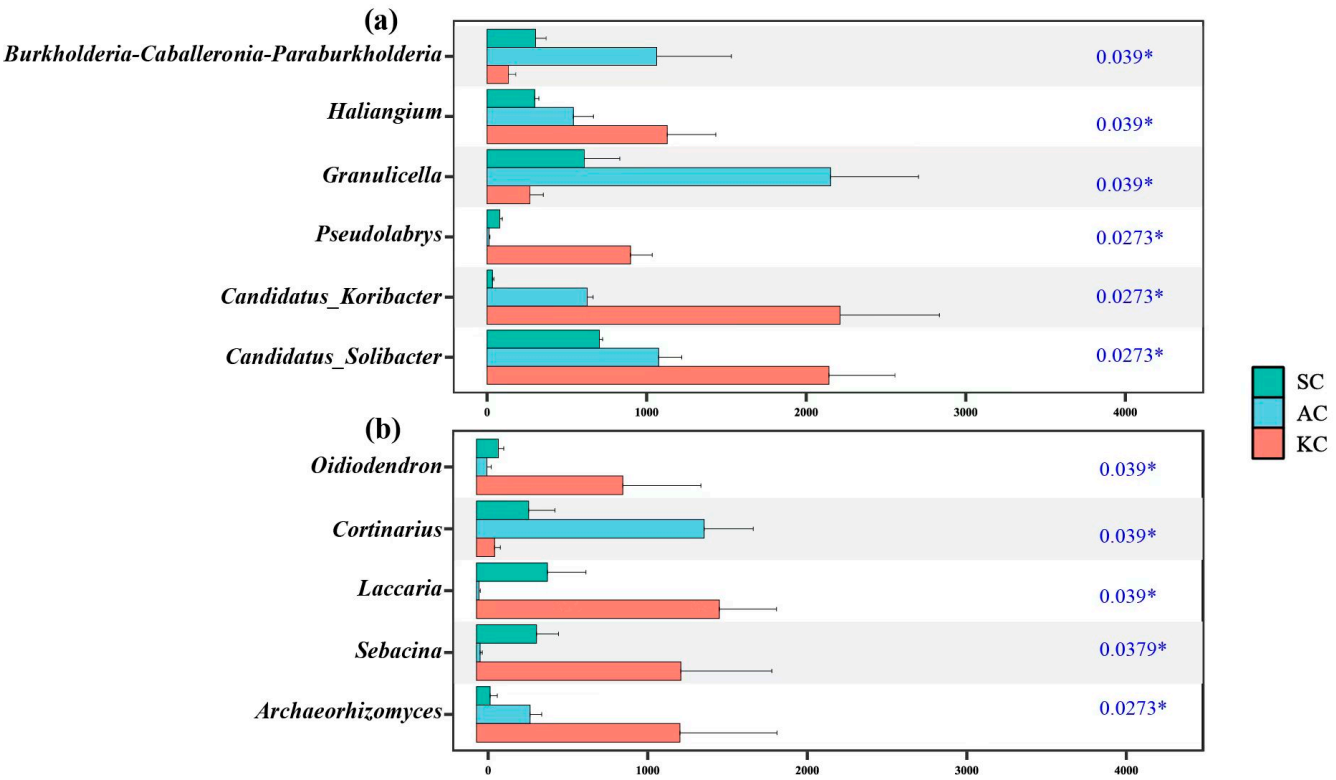


Figure 4. Kruskal–Wallis tests for differences among treatments to examine variations in soil bacterial genera (**a**) and soil fungal genera (**b**) between treatments. Bar graphs of different colors represent distinct treatments. SC: *Sonneratia caseolaris* treatment; AC: *Aegiceras corniculatum* treatment; KC: *Kandelia candel* treatment. * indicates that the genus is significant among different treatments.

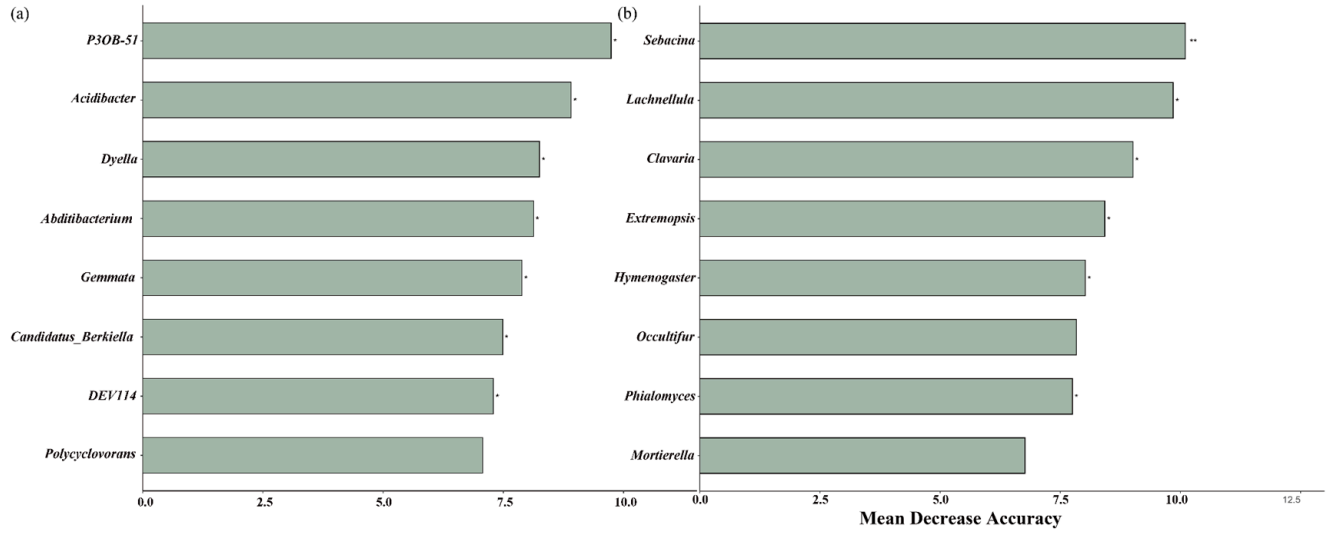


Figure 5. The random forest classification model visually demonstrates the dominant bacterial genera (**a**) and fungal genera (**b**) under different treatments. The random forest variables derived from the classification algorithm highlight the importance of rare genera in predicting different plant types. Green bars represent variables selected using the classification algorithm. Asterisks indicate significant differences. SC: *Sonneratia caseolaris* treatment; AC: *Aegiceras corniculatum* treatment; KC: *Kandelia candel* treatment.

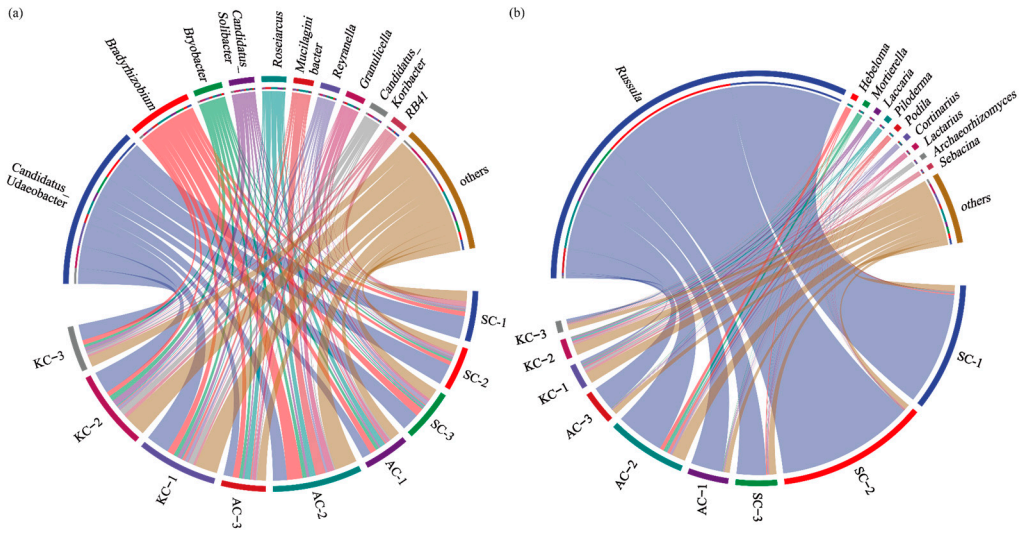


Figure 6. Circos visualization of the top 10 genera of soil bacteria (**a**) and soil fungi (**b**) across different plant types. The width of the lines represents the abundance. There are five circles from outer to inner. The first circle indicates information on different genera and plant type treatments. The second circle displays information on the percentage of relative abundance of each OTU within the total OTUs. The third circle represents the main OTU blocks. The fourth circle shows the OTU sub-blocks, which correspond to the main blocks (third circle), illustrating the abundance of OTUs in each sample and information on the abundance of each OTU included in each treatment. The fifth circle displays the connections between the OTUs and the related information across different treatments corresponding to the fourth circle. SC: *Sonneratia caseolaris* treatment; AC: *Aegiceras corniculatum* treatment; KC: *Kandelia candel* treatment.

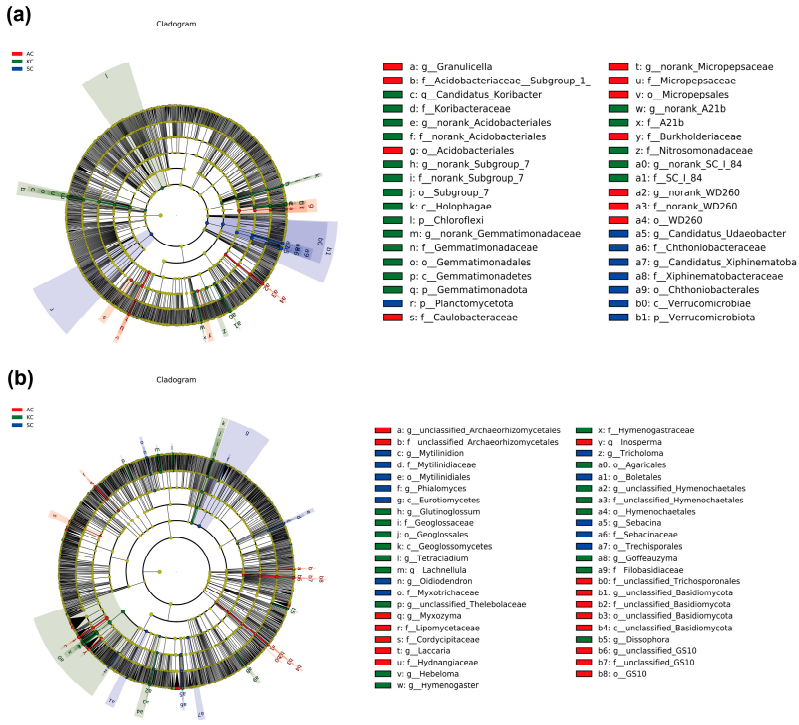


Figure 7. Circular tree diagram of LEfSe (linear discriminant analysis effect size) for discriminating differences between groups in soil bacteria (**a**) and fungi (**b**). Different colors represent different groups. Nodes of different colors in the branches indicate microbial taxa that play significant roles in the corresponding group of that color, while yellow nodes represent microbial taxa that do not play significant roles. The species names denoted by English letters are shown in the legend on the right. If the colors are consistent in the diagram, it indicates that no significant marker was found.

3.3. Drivers Affecting Soil Microbial Communities

There is a close correlation between soil microbial genera and environmental factors. At the bacterial genus level (Figure 8a), *Candidatus_Solibacter*, *Candidatus_Koribacter*, and *Haliangium* exhibit significant positive correlations with TN (total nitrogen), TP (total phosphorus), TK (total potassium), and SOC (soil organic carbon). Both *Granulicella* and *Burkholderia-Caballeronia-Paraburkholderia* show significant negative correlations with CEC (cation exchange capacity). For soil fungal genera (Figure 8b), *Russula* displays significant negative correlations with TN, TP, TK, and SOC, whereas *Archaeorhizomyces* shows the opposite trend, with significant positive correlations with TN, TP, and SOC.

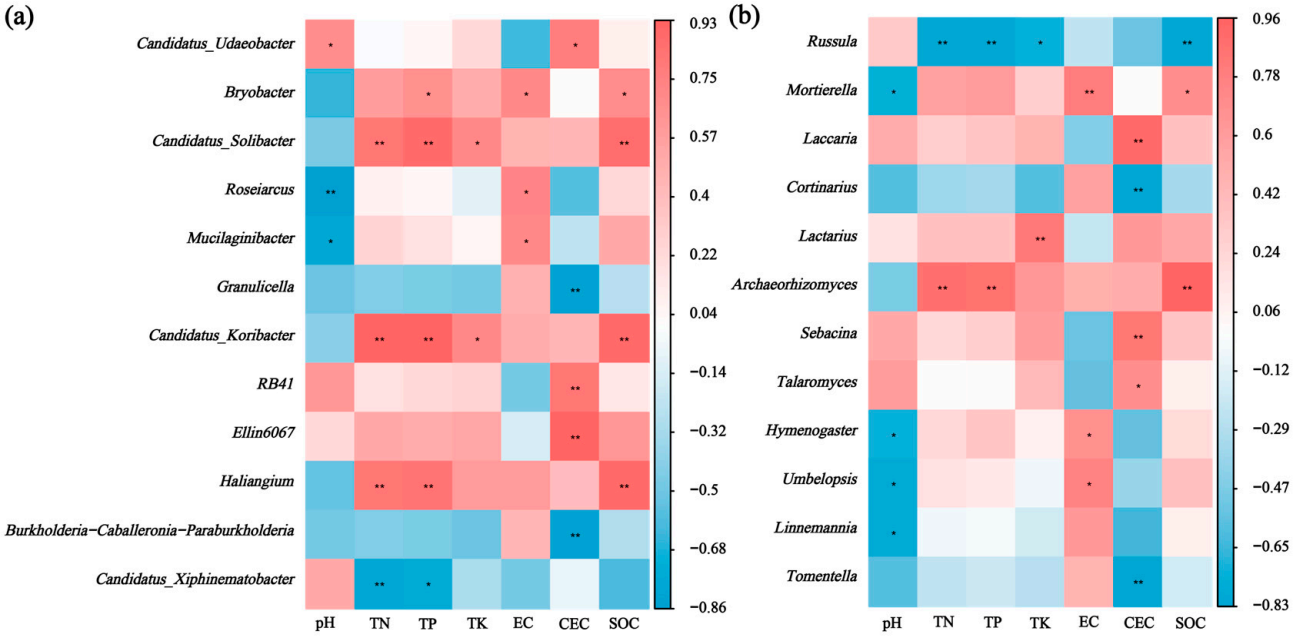


Figure 8. Correlation analysis between soil bacterial (**a**) and soil fungal (**b**) genera and environmental factors across different plant types. * indicates significance at the 0.05 level; ** indicates significance at the 0.01 level; *** indicates significance at the 0.001 level. The color gradient (from red to blue) represents the correlation strength from high to low. Red indicates a positive correlation, while blue indicates a negative correlation.

Furthermore, the results were further confirmed by the RDA plot (Figure 9a), which indicated that among soil bacteria, both pH and CEC in the SC treatment had significant positive correlations and significant impacts on the bacterial community. Additionally, other factors than EC also influenced the SC treatment. The AC treatment, in addition to being significantly and positively influenced by EC, was also affected by all the other factors. The KC treatment was particularly notable, as TN, TP, TK, and SOC all exhibited significant positive correlations. Similar to the soil bacterial community (Figure 9b), soil fungal communities in the KC treatment were significantly and positively influenced by TN, TP, TK, CEC, and SOC.

3.4. The Community Assembly Process of Soil Microbial Communities

We assessed the assembly processes of soil bacterial and fungal communities across different plant types using the Sloan Neutral Community Model (Figure 10). Different plant types exert a significant influence on the assembly processes of soil bacterial and fungal communities, primarily affecting the balance between deterministic and stochastic processes. The establishment of soil bacterial communities is strongly influenced by deterministic processes; the dispersal of bacterial communities diminishes from the KC treatment, through the AC treatment, to the SC treatment (Figure 10a–c). Intriguingly, the

pattern observed in soil fungal communities mirrors that in bacteria, with the assembly processes of fungal communities also being strongly influenced by deterministic processes (Figure 10d–f).

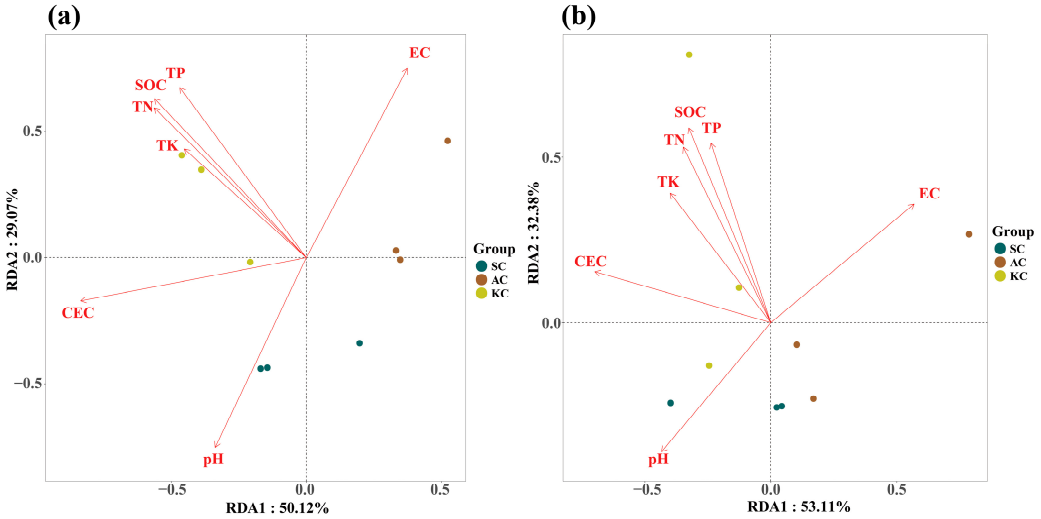


Figure 9. Redundancy analysis between soil bacterial (**a**) and soil fungal (**b**) communities and environmental factors under different plant types. The samples and environmental factors are reflected on the same two-dimensional ordination plot, allowing for an intuitive visualization of the relationships between sample distribution and environmental factors. SC: *Sonneratia caseolaris* treatment; AC: *Aegiceras corniculatum* treatment; KC: *Kandelia candel* treatment.

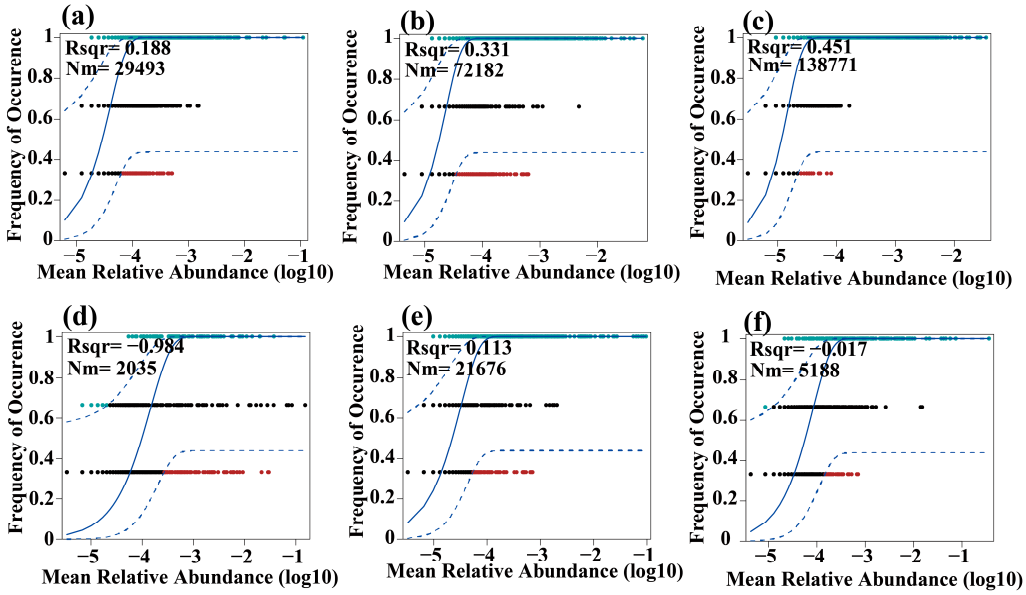


Figure 10. Fitting of soil bacterial ((**a**): SC, (**b**): AC, (**c**): KC) and fungal ((**d**): SC, (**e**): AC, (**f**): KC) communities across different plant types using the Neutral Community Model (NCM). R^2 represents the goodness of fit of the model. Nm: The product of metacommunity size (N) and migration rate (m) (Nm = N × m), which quantitatively estimates the degree of dispersal among communities and determines the correlation between occurrence frequency and relative abundance across regions. The blue solid line best fits the Neutral Community Model (NCM), and the blue dashed lines represent the 95% confidence interval predicted by the NCM. OTUs within this confidence interval (black dots) are considered to be neutrally distributed. SC: *Sonneratia caseolaris* treatment; AC: *Aegiceras corniculatum* treatment; KC: *Kandelia candel* treatment. The solid blue line is the best fit to the neutral community model (NCM), and the dashed blue line indicates 95% confidence intervals around the NCM prediction, and the OTUs within the confidence intervals (black dots) are viewed as neutrally distributed.

4. Discussion

4.1. Soil Microbial Diversity and Composition

In constructed wetlands, different plant species often alter the soil nutrient content, thereby modifying the alpha diversity of soil microbes. In this experimental study, we investigated the impacts of three mangrove species—the native species *Kandelia candel* (KC) and *Aegiceras corniculatum* (AC) as well as the exotic species *Sonneratia caseolaris* (SC)—on the alpha diversity and composition of soil microbial communities. We observed that the Shannon, Simpson, and Pielou indices of soil bacteria in the KC treatment were significantly higher than those in the SC treatment (Table 2). Considering the differences between the native species *Kandelia candel* and the exotic species *Sonneratia caseolaris*, *Kandelia candel*, as a native species [57,58], has interacted with the local soil environment, climatic conditions, and other biotic populations over a long period, forming stable ecological relationships [59,60]. This long-term coevolution may have enabled *Kandelia candel*'s root exudates and leaf litter to provide a more suitable growth environment and nutrient sources for specific soil microorganisms [61,62], thereby promoting their reproduction and increasing diversity. From the perspective of plant physiological and ecological characteristics [14], the root system of *Kandelia candel* is more adept at forming symbiotic relationships with certain specific microorganisms [63], such as attracting beneficial microbial colonization through rhizosphere exudates or providing suitable habitats for microorganisms through the root structure [64,65]. This selective recruitment and the symbiotic relationship contribute to increasing the diversity and richness of soil microorganisms.

As can be seen from Table 1, SOC was the highest in the KC group. It was considered that *K. candel* might have a higher carbon fixation capacity than the other plants, *S. caseolaris* and *A. corniculatum*. This advantage might be due to its unique root structure, faster growth rate, efficient photosynthesis, or a more favorable symbiotic relationship with microorganisms [66]. In addition, the roots of *K. candel* might be able to secrete more substances that promote microbial activity, which in turn contributes to the accumulation and stabilization of organic carbon [67]. At the same time, the constructed wetland environment of the KC group might also have created more favorable conditions for the growth and carbon accumulation of *K. candel*, including suitable water levels, temperatures, light, and soil textures [67]. Given that the highest SOC content was observed in the KC group, future studies could further explore the carbon sequestration mechanism of *K. candel*, including its root structural characteristics, photosynthetic efficiency, and interactions with microorganisms. At the same time, it could also be considered to expand the scale of the experiment and increase the number of samples to more comprehensively evaluate the specific impact of different plants on SOC accumulation in constructed wetlands.

The results of principal component analysis (PCA) indicated that significant changes occurred in the soil bacterial and fungal community structures among the different treatments (Figure 1). The change was closely linked to the profound impact of mangrove species on the soil environment [68]. Specifically, the growth of the different mangrove species significantly alters the physicochemical properties of the soil, including pH value, water content, and oxygen and salinity levels, as well as the distribution and availability of key nutrients such as nitrogen, phosphorus, and potassium [69]. These adjustments in soil properties directly create a novel living environment for soil microorganisms [15,70], subsequently exerting profound influences on their survival and reproduction, and ultimately leading to significant changes in microbial community structure. Furthermore, the root systems of mangroves form an intricate network within the soil, not only providing diverse habitats and microenvironments for microorganisms but also influencing soil oxygen circulation [71,72], water retention, and nutrient distribution through variations in their morphology, density, and arrangement [73]. The diversity of these root structures creates unique conditions for the growth of different microbial species, further promoting the

diversification and structuring of microbial communities. Soil enzymes, as key indicators of microbial activity, participate in vital biochemical processes such as organic matter decomposition and nutrient cycling [74,75]. Mangrove species may indirectly alter the microbial community structure by influencing soil enzyme activity; for instance, they can promote or inhibit the activity of certain enzymes, thereby affecting the rate of organic matter decomposition and the availability of carbon and nutrients in the soil [76,77], ultimately impacting microbial growth [78]. Lastly, soil microorganisms do not exist in isolation; they form a complex network of interactions [79]. The introduction of mangrove species, particularly exotic species like *Sonneratia*, may disrupt this existing balance of microbial interactions. These species may introduce new microbial types or pathogens that compete with, prey on, parasitize, or cooperate with native microorganisms [80,81], further exacerbating changes and dynamic succession in the microbial community structure.

Our study found that the proportions of soil bacterial genera differed among the different treatments (Figure 3a). *Bradyrhizobium* and *Granulicella* had the highest abundance in the AC treatment, while *Candidatus_Udaeobacter* was lowest in the AC treatment (Figure 3a). When the plant species in the constructed wetland is *Aegiceras corniculatum*, the increased abundance of *Bradyrhizobium* and *Granulicella* may be attributed to their better adaptation to specific ecological niches [82,83], which further stimulates specific biochemical pathways or signaling mechanisms that favor the growth and metabolism of these two microorganisms. The symbiosis between *Aegiceras corniculatum* and subsurface soil microorganisms may promote the activity of certain enzymes in the soil that are closely related to the carbon source utilization or energy metabolism of *Bradyrhizobium* and *Granulicella* [84], thereby providing them with a growth advantage. Additionally, *Aegiceras corniculatum* may also create a more favorable living environment for *Bradyrhizobium* and *Granulicella* by influencing the redox state or electron transport chain in the soil [85] while potentially inhibiting the physiological activities of *Candidatus_Udaeobacter*. These mechanisms work together to cause significant changes in the microbial community structure [86], specifically manifested as an increase in the abundance of *Bradyrhizobium* and *Granulicella* and a decrease in the abundance of *Candidatus_Udaeobacter*, an important soil bacterium crucial for stabilizing the structure and function of soil ecosystems as well as for environmental pollution remediation [86]. In the three selected treatments, the relatively high proportion of *Candidatus_Udaeobacter* [86] indicates the significant impact of simulated constructed wetlands on wastewater treatment. In the AC treatment, the increased abundance of *Bradyrhizobium* and *Granulicella* may suggest a competitive relationship with *Candidatus_Udaeobacter* for resources such as carbon and nitrogen sources as well as living space, potentially inhibiting the growth of *Candidatus_Udaeobacter* [87]. Concurrently, the AC treatment might have altered soil environmental conditions (e.g., pH, moisture, and oxygen concentration) that were originally favorable for *Candidatus_Udaeobacter* growth but became less conducive to its reproduction after the change [88].

Soil fungi also exhibited variations, with each treatment having its unique fungal genera. For instance, *Piloderma* was uniquely present in the SC treatment, *Laccaria* was a significant component in the AC treatment, and *Hebeloma* accounted for a high proportion in the KC treatment (Figure 3b). The unique presence of *Piloderma* in the SC treatment likely indicates its high adaptation to the saprophytic lifestyle in the soil environment created by SC treatment [89]. *Piloderma* may have efficiently decomposed organic matter, thereby releasing nutrients crucial for its own growth and potentially enhancing the overall nutrient cycling in the SC treatment [9]. The abundance of *Laccaria* in the AC treatment could be attributed to its ability to form mutualistic relationships with plants, albeit not directly through symbiosis as commonly understood [90], but possibly through mechanisms such as improving nutrient availability or enhancing carbon source utilization. *Laccaria* might

have effectively utilized available carbon sources and contributed to nitrogen fixation, indirectly benefiting plant growth and nutrient uptake in that treatment [91]. The high proportion of *Hebeloma* in the KC treatment may suggest its superior ability to compete for resources and utilize carbon sources in the soil environment created by that treatment [92], effectively mobilizing and making available nutrients that were otherwise inaccessible to plants, thereby enhancing plant nutrient absorption and utilization efficiency in the KC treatment.

In addition to focusing on bacterial genera with significant differences, our study also emphasizes the crucial roles of often-overlooked rare bacterial genera. The random forest classification model indicated that bacterial genera such as *P3OB_51* and *Acidibacter* (Figure 5a), as well as fungal genera like *Sebacina* and *Lachnellula* (Figure 5b), are deemed pivotal in shaping soil microbial communities. Constructed wetlands, due to their unique environmental conditions—including variations in water flow, diverse substrate composition, and rich plant species—serve as habitats for diversified and dynamic microbial communities [8,93]. Such environmental gradients facilitate the establishment of diverse microbial assemblages encompassing both dominant and rare taxa. Among them, some rare bacterial and fungal genera, such as *P3OB_51*, *Acidibacter, Sebacina*, and *Lachnellula*, may play crucial yet previously overlooked roles in the nutrient cycling and pollutant degradation processes. The importance of these rare genera stems from their ability to thrive in specific niches within constructed wetlands, with some excelling at degrading specific pollutants or transforming nutrients under particular conditions (e.g., fluctuations in water levels or oxygen concentrations). The roles of *P3OB_51* in nitrogen cycling and Acidibacter in acidophilic conditions highlight their potential as key factors in nutrient transformation and pollutant degradation in wetland environments. Furthermore, the interactions among microbial genera, plants, and other environmental factors in constructed wetlands further underscore the significance of these rare genera. The mutualistic relationships formed between fungi like *Sebacina* and *Lachnellula* and plant roots can enhance nutrient acquisition and pollutant degradation capabilities, indirectly promoting the growth and activity of associated bacterial genera. These complex interactions and feedback loop networks are crucial for the overall functioning of wetland ecosystems.

The results of LEfSe analysis showed that the Polyangiaceae family, comprising saprotrophic bacteria (Figure 7), played a pivotal role in wastewater purification within the SC treatment. These bacteria are commonly found in soil and decaying organic matter, and they hold immense importance in environmental biocontrol due to their predominantly predatory lifestyle toward microbes [94]. Notably, in terms of antimicrobial activity, they produce secondary metabolites with antibacterial properties that inhibit microbial growth and reproduction through mechanisms such as disrupting cell structure, interfering with nucleic acid and protein synthesis, and inhibiting cell division [95]. Furthermore, these compounds exhibit potential applications in environmental remediation, agriculture, and biotechnology. Additionally, the *Caulobacteriae* family has antibacterial properties [96]. The discovery of these bacterial families reinforces the rationale behind using *Sonneratia caseolaris* in constructed wetlands. Meanwhile, the *Granulicella* bacterial genus, enriched in the AC treatment, plays a significant role and belongs to the *Acidobacteriaceae* family. It exhibits acidophilic characteristics, enabling it to thrive in acidic environments and exert crucial ecological functions [97]. The abundance of bacteria belonging to the *Granulicella* genus increased significantly, potentially linked to improvements in the soil environment, enhanced soil fertility, and the genus's high carbon utilization efficiency. Furthermore, both LEfSe analysis and Circos analysis jointly highlighted the importance of the unique bacterial genera *Candidatus_Koribacter* and *Pseudolabrys* in the KC treatment (Figure 6). *Candidatus_Koribacter* participates in various biogeochemical cycles, including carbon, nitrogen,

and sulfur cycles [98]. These biogeochemical cycles collectively provide a suitable living environment for plants, ensuring they obtain adequate nutrients and water. Additionally, *Candidatus_Koribacter* produces biologically active metabolites that positively affect the normal physiological activities and growth and development of plants. *Pseudolabrys* plays a major role in degrading harmful substances within the KC treatment [99], relying on specific enzyme systems, metabolic pathways, and interactions with other microbes; *Pseudolabrys* also undergoes a series of complex biochemical processes, including adsorption, absorption, transformation, and excretion, to degrade harmful substances.

4.2. Drivers Affecting Soil Microbial Communities

The analysis of the correlation heatmap reveals a significant association between soil pH and specific microorganisms, with *Candidatus_Udacobacter* exhibiting a positive correlation with soil pH (Figure 8), indicating its heightened activity in alkaline or neutral soils, where its abundance or activity increases as soil pH rises. This preference may stem from the more favorable growth conditions provided by alkaline or neutral soils. The acid-tolerant characteristics exhibited by *Roseiarcus* in acidic soils enable it to thrive in extreme environments where other microorganisms struggle to survive [100]. This trait not only demonstrates *Roseiarcus*'s unique adaptability to acidic soil conditions but also potentially promotes plant growth through its metabolic activities, such as releasing nutrients available to plants or producing compounds that stimulate root development [101,102]. On the other hand, *Mucilaginibacter* not only displays special adaptation mechanisms in acidic soils, allowing it to survive and proliferate in such environments [103], but also actively participates in the decomposition of organic matter. This means that *Mucilaginibacter* can utilize the organic matter in acidic soils as a carbon and energy source, breaking it down into smaller molecules like amino acids and monosaccharides through its metabolic processes [104]. These decomposition products are further utilized by other microorganisms or plants, thereby facilitating the cycling and reuse of soil nutrients [105]. Additionally, *Mucilaginibacter*'s special adaptation mechanisms may include tolerance to toxic substances in acidic soils and the maintenance of enzymatic activity at low pH levels, all of which make it an indispensable player in acidic soil ecosystems.

The growth and reproduction of microorganisms depend on essential nutrients such as carbon, nitrogen, phosphorus, and potassium [12], with total nitrogen, phosphorus, and potassium in soil serving as key pathways for their acquisition [106]. *Candidatus_Solibacter* and *Candidatus_Koribacter* exhibit enhanced abilities to utilize soil nitrogen, phosphorus, and potassium, obtaining necessary nutrients through processes like organic matter decomposition and inorganic nutrient mineralization [105,107]. Microorganisms play a crucial role in soil nutrient cycling, breaking down organic matter to release nutrients for plant uptake and utilization, while their metabolic activities also promote nutrient transformation and cycling within the soil [108,109]. By secreting enzymes and altering soil structure, *Candidatus_Solibacter* and *Candidatus_Koribacter* enhance soil nutrient availability [14,109], making nutrients more accessible to plants and other microorganisms. High concentrations of total nitrogen, phosphorus, and potassium in soil favor the growth and reproduction of microorganisms like *Candidatus_Solibacter* and *Candidatus_Koribacter*, enabling them to dominate the microbial community [110,111]. Additionally, balanced levels of nitrogen, phosphorus, and potassium may stimulate microbial metabolic activities, further promoting nutrient transformation and cycling [112]. These two microorganisms likely possess unique ecological characteristics that confer high sensitivity and utilization capacity for soil nutrients such as total nitrogen, phosphorus, and potassium, and they excel at decomposing organic matter or secreting specific enzymes to mineralize inorganic nutrients.

RDA analysis indicates that pH, TK, TN, and TP exert significant influences on soil bacterial and fungal communities in the SC treatment (Figure 9). pH stands out as a pivotal factor shaping soil microbial community structure, directly affecting microbial enzyme activity, cell membrane stability, and nutrient availability, thereby influencing microbial growth and reproduction [113]. Given that different microbial species exhibit varying degrees of adaptability to pH, changes in pH can lead to alterations in the composition and diversity of microbial communities [114,115]. Furthermore, pH indirectly impacts microbial communities by modifying other soil chemical properties such as ion concentrations and the redox potential [15]. In acidic soils, increased aluminum ion solubility may exert toxic effects on certain microorganisms, while in alkaline soils, elevated hydroxide ion concentrations can affect microbial metabolic processes [116,117].

Nitrogen, phosphorus, and potassium are essential nutrients for plant growth and are also indispensable for microbial metabolic activities [118]. The TK content in soil directly influences the microbial acquisition and utilization of potassium, impacting microbial growth and reproduction [119]. In arid or saline soils, potassium ions play a crucial role in osmoregulation, maintaining microbial cell osmotic pressure stability and protecting cells from adverse environmental conditions [120]. Nitrogen is a vital nutrient for microbial growth and reproduction, with soil TN content directly affecting microbial nitrogen acquisition and utilization efficiency [121,122], thereby influencing the microbial community's structure and function. Certain microorganisms, such as nitrogen-fixing bacteria, can convert atmospheric nitrogen into ammonia or nitrate forms utilizable by plants [123]. The activity of these microorganisms is influenced by soil TN content, further affecting the nitrogen cycle of the entire ecosystem [15]. Phosphorus is another essential nutrient for plant growth and is required for microbial metabolic activities [124]. The TP content in soil directly affects microbial phosphorus acquisition and utilization efficiency, impacting microbial community structure and function. The transformation and speciation of phosphorus in soil are influenced by microbial activity, while microbial metabolic processes also depend on the supply of phosphorus [125].

4.3. The Community Assembly Process of Soil Microbial Communities

The neutral community assembly model indicates that the assembly processes of soil bacterial communities under all three treatments are dominated by deterministic processes (Figure 10). As artificially constructed ecosystems, constructed wetlands typically exhibit relatively stable and controllable environmental conditions such as humidity, temperature, and light, which may favor the formation and maintenance of specific soil bacterial community structures through deterministic processes [8,126]. Under these stable environmental conditions, the impact of random disturbances on soil bacterial communities is relatively minor, thus making it more likely that soil bacterial communities are primarily assembled through deterministic processes, including competition and adaptation [127,128]. In constructed wetlands, the plant substrates, namely *Sonneratia caseolaris*, *Aegiceras corniculatum*, and *Kandelia candel*, provide unique ecological niches and nutritional resources for soil bacteria through their root exudates and litter. The specificity of these plant substrates can lead to the adaptation and selection of soil bacterial communities to particular environmental conditions, thereby contributing to the formation of a deterministic assembly pattern [12,129]. Additionally, the complex interactions between different plant species and soil bacteria, which may encompass symbiosis, competition, predation, and other relationships, collectively influence the composition and dynamics of soil bacterial communities [12]. Under the influence of these specific plant substrates, soil bacterial communities may be more inclined to form stable community structures through deterministic processes such as competition and adaptation.

Additionally, the assembly process of the soil fungal community under AC treatment is also dominated by deterministic processes (Figure 10). This may be attributed to the strong influence of niche selection on the soil fungal community under AC treatment. Certain fungal species, due to their superior ability to utilize resources provided by *Aegiceras corniculatum* or to adapt to specific compounds in its root exudates, gain a competitive advantage and develop a unique community structure [130,131]. Competition within the fungal community is a pivotal component of the deterministic process, where, given limited resources, species engage in intense competition for survival and reproduction [127,132]. Those with stronger adaptability and higher resource utilization efficiency are more likely to emerge victorious in this competition [133], subsequently dominating the community structure. Over time, the soil fungal community gradually reaches a dynamic equilibrium under the influence of deterministic processes. Globally, the intricate interaction networks between diverse plant species and soil bacteria, encompassing relationships such as symbiosis, competition, and predation, collectively shape the composition and dynamic changes in soil bacterial communities. Consequently, from a global perspective, the influence of specific plant substrates on soil bacterial communities is widespread and profound. Under the influence of these plant substrates, soil bacterial communities are more inclined to form stable and predictable community structures worldwide through deterministic processes such as competition and adaptation. This finding holds significant importance for understanding the dynamic changes in soil bacterial communities in global artificial wetlands and mangrove ecosystems.

5. Conclusions

In summary, this study provides meaningful insights into the impact of constructed wetlands featuring diverse mangrove species on soil microbial communities. The results indicate that constructed wetlands have a significant effect on the alpha diversity of soil bacterial communities, whereas their influence on the alpha diversity of soil fungal communities is not pronounced. However, beta diversity is notably affected in both soil bacterial and fungal communities. Our findings demonstrate significant changes in the abundance of bacterial genera across different treatment groups, including *Candidatus_Udaeobacter* and *Bradyrhizobium*, as well as notable alterations in the abundance of fungal genera such as *Russula* and *Mortierella*. The random forest classification model reveals that certain rare microbial taxa, such as *Acidibacter*, *Dyella*, *Sebacina*, and *Lachnellula*, play crucial roles in shaping the soil microbial community. Furthermore, a key discovery of our study is that some microbes, such as those belonging to the Polyangiaceae and Caulobacteraceae families, are important in antibiotic resistance and biogeochemical cycling, as they can decompose organic matter and produce secondary metabolites with antibacterial activity. Notably, the assembly of soil microbial communities in all treatment groups is primarily driven by deterministic processes, highlighting the strong influence of environmental factors on their distribution and abundance. Based on these findings, the following management recommendations for constructed wetland ecosystems are proposed: Mangrove species should be selected judiciously to maintain the stability of the soil microbial communities, thereby preserving the natural balance between bacterial and fungal interactions.

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Data Availability Statement: Certain proprietary or sensitive data that were generated during the course of this research are not publicly available due to confidentiality agreements or ethical considerations. However, these data are available from the corresponding author upon reasonable request and with permission from the relevant authorities. Interested researchers are invited to contact Qiong Yang (email: yangking78@126.com) to inquire about accessing these non-public datasets.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Liu, H.; Hu, Z.; Zhang, J.; Ngo, H.h.; Guo, W.; Liang, S.; Fan, J.; Lu, S.; Wu, H. Optimizations on supply and distribution of dissolved oxygen in constructed wetlands: A review. *Bioresour. Technol.* **2016**, *214*, 797–805. [CrossRef] [PubMed]
2. Scholz, M.; Lee, B.H. Constructed wetlands: A review. *Int. J. Environ. Stud.* **2005**, *62*, 421–447. [CrossRef]
3. Wang, W.; Gao, J.; Guo, X.; Li, W.; Tian, X.; Zhang, R. Long-term effects and performance of two-stage baffled surface flow constructed wetland treating polluted river. *Ecol. Eng.* **2012**, *49*, 93–103. [CrossRef]
4. Wu, Y.; Han, R.; Yang, X.; Zhang, Y.; Zhang, R. Long-term performance of an integrated constructed wetland for advanced treatment of mixed wastewater. *Ecol. Eng.* **2017**, *99*, 91–98. [CrossRef]
5. Naskar, M.; Tasneem, S.N.; Mazumder, S.; Mitra, C.; Datta, D. Chapter 17—Mangroves as coastal rainforests: Imminent threats, hazards, and changing status of ecosystem services. In *Biological and Environmental Hazards, Risks, and Disasters*, 2nd ed.; Sivanpillai, R., Shroder, J.F., Eds.; Elsevier: Boston, MA, USA, 2023; pp. 335–357. [CrossRef]
6. Zhuang, W.; Yu, X.; Hu, R.; Luo, Z.; Liu, X.; Zheng, X.; Xiao, F.; Peng, Y.; He, Q.; Tian, Y.; et al. Diversity, function and assembly of mangrove root-associated microbial communities at a continuous fine-scale. *NPJ Biofilm. Microbiomes* **2020**, *6*, 52. [CrossRef]
7. Hartmann, M.; Six, J. Soil structure and microbiome functions in agroecosystems. *Nat. Rev. Earth Environ.* **2023**, *4*, 4–18. [CrossRef]
8. Wu, H.; Wang, R.; Yan, P.; Wu, S.; Chen, Z.; Zhao, Y.; Cheng, C.; Hu, Z.; Zhuang, L.; Guo, Z.; et al. Constructed wetlands for pollution control. *Nat. Rev. Earth Environ.* **2023**, *4*, 218–234. [CrossRef]
9. Chen, G.; Mo, Y.; Gu, X.; Jeppesen, E.; Xie, T.; Ning, Z.; Li, Y.; Li, D.; Chen, C.; Cui, B.; et al. Sustainability of global small-scale constructed wetlands for multiple pollutant control. *NPJ Clean Water* **2024**, *7*, 45. [CrossRef]
10. Rani, A.; Chauhan, M.; Kumar Sharma, P.; Kumari, M.; Mitra, D.; Joshi, S. Microbiological dimensions and functions in constructed wetlands: A review. *Curr. Res. Microb. Sci.* **2024**, *7*, 100311. [CrossRef] [PubMed]
11. De Mandal, S.; Laskar, F.; Panda, A.K.; Mishra, R. Chapter 12—Microbial diversity and functional potential in wetland ecosystems. In *Recent Advancements in Microbial Diversity*; De Mandal, S., Bhatt, P., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 289–314. [CrossRef]
12. Trivedi, P.; Leach, J.E.; Tringe, S.G.; Sa, T.; Singh, B.K. Plant–microbiome interactions: From community assembly to plant health. *Nat. Rev. Microbiol.* **2020**, *18*, 607–621. [CrossRef]
13. Hartman, K.; Tringe, S.G. Interactions between plants and soil shaping the root microbiome under abiotic stress. *Biochem. J.* **2019**, *476*, 2705–2724. [CrossRef] [PubMed]
14. Bastida, F.; Eldridge, D.J.; García, C.; Kenny Png, G.; Bardgett, R.D.; Delgado-Baquerizo, M. Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. *ISME J.* **2021**, *15*, 2081–2091. [CrossRef] [PubMed]
15. Philippot, L.; Chenu, C.; Kappler, A.; Rillig, M.C.; Fierer, N. The interplay between microbial communities and soil properties. *Nat. Rev. Microbiol.* **2024**, *22*, 226–239. [CrossRef]
16. Ferreira, C.S.S.; Kašanin-Grubin, M.; Solomun, M.K.; Sushkova, S.; Minkina, T.; Zhao, W.; Kalantari, Z. Wetlands as nature-based solutions for water management in different environments. *Curr. Opin. Environ. Sci. Health* **2023**, *33*, 100476. [CrossRef]
17. David, G.; Rana, M.S.; Saxena, S.; Sharma, S.; Pant, D.; Prajapati, S.K. A review on design, operation, and maintenance of constructed wetlands for removal of nutrients and emerging contaminants. *Int. J. Environ. Sci. Technol.* **2023**, *20*, 9249–9270. [CrossRef]
18. Shukla, A.; Parde, D.; Gupta, V.; Vijay, R.; Kumar, R. A review on effective design processes of constructed wetlands. *Int. J. Environ. Sci. Technol.* **2022**, *19*, 12749–12774. [CrossRef]
19. Cheng, A.T.; Sims, K.R.E.; Yi, Y. Economic development and conservation impacts of China's nature reserves. *J. Environ. Econ. Manag.* **2023**, *121*, 102848. [CrossRef]
20. Chen, W.; Gu, T.; Xiang, J.; Luo, T.; Zeng, J. Assessing the conservation effectiveness of national nature reserves in China. *Appl. Geogr.* **2023**, *161*, 103125. [CrossRef]
21. Tian, W.; Li, Q.; Luo, Z.; Wu, C.; Sun, B.; Zhao, D.; Chi, S.; Cui, Z.; Xu, A.; Song, Z. Microbial community structure in a constructed wetland based on a recirculating aquaculture system: Exploring spatio-temporal variations and assembly mechanisms. *Mar. Environ. Res.* **2024**, *197*, 106413. [CrossRef] [PubMed]

22. Tian, T.T.; Yang, Q.; Wei, G.L.; Cheung, S.G.; Shin, P.K.S.; Wong, Y.S.; Li, Z.; Chen, Z.H.; Tam, N.F.Y. Changes of substrate microbial biomass and community composition in a constructed mangrove wetland for municipal wastewater treatment during 10-years operation. *Mar. Pollut. Bull.* **2020**, *155*, 111095. [CrossRef]
23. Tian, T.; Tam, N.F.Y.; Zan, Q.; Cheung, S.G.; Shin, P.K.S.; Wong, Y.S.; Zhang, L.; Chen, Z. Performance and bacterial community structure of a 10-years old constructed mangrove wetland. *Mar. Pollut. Bull.* **2017**, *124*, 1096–1105. [CrossRef] [PubMed]
24. Yang, Q.; Tam, N.F.Y.; Wong, Y.S.; Luan, T.G.; Su, W.S.; Lan, C.Y.; Shin, P.K.S.; Cheung, S.G. Potential use of mangroves as constructed wetland for municipal, sewage treatment in Futian, Shenzhen, China. *Mar. Pollut. Bull.* **2008**, *57*, 735–743. [CrossRef]
25. Wu, Y.; Tam, N.F.Y.; Wong, M.H. Effects of salinity on treatment of municipal wastewater by constructed mangrove wetland microcosms. *Mar. Pollut. Bull.* **2008**, *57*, 727–734. [CrossRef]
26. Zhou, L.; Li, X.; Hao, S.; Hong, L.; Chen, L.; Li, Q.Q. Distinct molecular responses of mangrove plants to hypoxia and reoxygenation stresses contribute to their resilience in coastal wetland environment. *Sci. Total Environ.* **2024**, *956*, 177357. [CrossRef] [PubMed]
27. Jing, Y.; Ren, Y.; Yang, D.; Han, H.; Xiao, L.; Chen, G. Resistant physiology of three mangrove species in a constructed wetland sewage treatment system. *Ying Yong Sheng Tai Xue Bao J. Appl. Ecol.* **2006**, *17*, 1544–1546.
28. Kabala, C.; Musztyfaga, E.; Gałka, B.; Łabuńska, D.; Mańczyńska, P. Conversion of Soil pH 1:2.5 KCl and 1:2.5 H_2O to 1:5 H_2O: Conclusions for Soil Management, Environmental Monitoring, and International Soil Databases. *Pol. J. Environ. Stud.* **2016**, *25*, 647–653. [CrossRef] [PubMed]
29. Yang, J.; Masoudi, A.; Li, H.; Gu, Y.; Wang, C.; Wang, M.; Wu, C.; Liu, Y.; Zhao, X.; Yu, Z.; et al. Soil Total Nitrogen Mediated the Impact of Climatic Factors on Urban Soil Organic Matter Under Different Land Uses. *J. Soil Sci. Plant Nutr.* **2024**, *24*, 5487–5504. [CrossRef]
30. Cook, A.M.; Daughton, C.D. [15] Total phosphorus determination by spectrophotometry. In *Methods in Enzymology*; Academic Press: Cambridge, MA, USA, 1981; Volume 72, pp. 292–295.
31. Razzaghi, F.; Arthur, E.; Moosavi, A.A. Evaluating models to estimate cation exchange capacity of calcareous soils. *Geoderma* **2021**, *400*, 115221. [CrossRef]
32. Friedman, S.P. Soil properties influencing apparent electrical conductivity: A review. *Comput. Electron. Agric.* **2005**, *46*, 45–70. [CrossRef]
33. Ma, Y.; Yu, Y.; Nan, S.; Chai, Y.; Xu, W.; Qin, Y.; Li, X.; Bodner, G. Conversion of SIC to SOC enhances soil carbon sequestration and soil structural stability in alpine ecosystems of the Qinghai-Tibet Plateau. *Soil Biol. Biochem.* **2024**, *195*, 109452. [CrossRef]
34. Sagar, K.; Singh, S.P.; Goutam, K.K.; Konwar, B.K. Assessment of five soil DNA extraction methods and a rapid laboratory-developed method for quality soil DNA extraction for 16S rDNA-based amplification and library construction. *J. Microbiol. Methods* **2014**, *97*, 68–73. [CrossRef]
35. Dash, H.R.; Shrivastava, P.; Das, S. Quantification of DNA by Using Agarose Gel Electrophoresis Technique. In *Principles and Practices of DNA Analysis: A Laboratory Manual for Forensic DNA Typing*; Dash, H.R., Shrivastava, P., Das, S., Eds.; Springer: New York, NY, USA, 2020; pp. 119–125.
36. Walters, W.; Hyde, E.R.; Berg-Lyons, D.; Ackermann, G.; Humphrey, G.; Parada, A.; Gilbert, J.A.; Jansson, J.K.; Caporaso, J.G.; Fuhrman, J.A.; et al. Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. *mSystems* **2016**, *1*. [CrossRef] [PubMed]
37. Wang, X.-C.; Liu, C.; Huang, L.; Bengtsson-Palme, J.; Chen, H.; Zhang, J.H.; Cai, D.; Li, J.Q. ITS1: A DNA barcode better than ITS2 in eukaryotes? *Mol. Ecol. Resour.* **2015**, *15*, 573–586. [CrossRef] [PubMed]
38. Zhang, J.; Ding, X.; Guan, R.; Zhu, C.; Xu, C.; Zhu, B.; Zhang, H.; Xiong, Z.; Xue, Y.; Tu, J.; et al. Evaluation of different 16S rRNA gene V regions for exploring bacterial diversity in a eutrophic freshwater lake. *Sci. Total Environ.* **2018**, *618*, 1254–1267. [CrossRef] [PubMed]
39. Liu, J.; Yu, Y.; Cai, Z.; Bartlam, M.; Wang, Y. Comparison of ITS and 18S rDNA for estimating fungal diversity using PCR-DGGE. *World J. Microbiol. Biotechnol.* **2015**, *31*, 1387–1395. [CrossRef] [PubMed]
40. Stadler, M.; Lambert, C.; Wibberg, D.; Kalinowski, J.; Cox, R.J.; Kolařík, M.; Kuhnert, E. Intragenomic polymorphisms in the ITS region of high-quality genomes of the Hypoxylaceae (Xylariales, Ascomycota). *Mycol. Prog.* **2020**, *19*, 235–245. [CrossRef]
41. Li, L.-Y.; Li, Q.; Yu, Y.-H.; Zhong, M.; Yang, L.; Wu, Q.-H.; Qiu, Y.-R.; Luo, S.-Q. A primer design strategy for PCR amplification of GC-rich DNA sequences. *Clin. Biochem.* **2011**, *44*, 692–698. [CrossRef] [PubMed]
42. Brosius, F.C.; Holzman, L.B.; Cao, X. Purification of PCR Products from Agarose Gels for Direct Sequencing. In *PCR Sequencing Protocols*; Rapley, R., Ed.; Springer: Totowa, NJ, USA, 1996; pp. 11–21.
43. Barbosa, C.; Nogueira, S.; Gadanho, M.; Chaves, S. Chapter 7—DNA extraction: Finding the most suitable method. In *Molecular Microbial Diagnostic Methods*; Cook, N., D'Agostino, M., Thompson, K.C., Eds.; Academic Press: San Diego, CA, USA, 2016; pp. 135–154.
44. Guo, Y.; Ye, F.; Sheng, Q.; Clark, T.; Samuels, D.C. Three-stage quality control strategies for DNA re-sequencing data. *Brief Bioinf.* **2014**, *15*, 879–889. [CrossRef]

45. Mohsen, A.; Park, J.; Chen, Y.A.; Kawashima, H.; Mizuguchi, K. Impact of quality trimming on the efficiency of reads joining and diversity analysis of Illumina paired-end reads in the context of QIIME1 and QIIME2 microbiome analysis frameworks. *BMC Bioinform.* **2019**, *20*, 581. [CrossRef]
46. Edgar, R.C. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10*, 996–998. [CrossRef]
47. Bokulich, N.A.; Kaehler, B.D.; Rideout, J.R.; Dillon, M.; Bolyen, E.; Knight, R.; Huttley, G.A.; Gregory Caporaso, J. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **2018**, *6*, 90. [CrossRef]
48. Armstrong, R.; Hilton, A. One—Way Analysis of Variance (Anova). In *Statistical Analysis in Microbiology: Statnotes*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2014; pp. 33–37.
49. Chen, H.; Boutros, P.C. VennDiagram: A package for the generation of highly-customizable Venn and Euler diagrams in R. *BMC Bioinform.* **2011**, *12*, 35. [CrossRef]
50. Takayuki, S. Evaluation of Redundancy Analysis and development of a Generalized Procedure with Applications. *Mol. Ecol. Resour.* **2018**, *18*, 1223–1233.
51. Segata, N.; Izard, J.; Waldron, L.; Gevers, D.; Miropolsky, L.; Garrett, W.S.; Huttenhower, C. Metagenomic biomarker discovery and explanation. *Genome Biol.* **2011**, *12*, R60. [CrossRef] [PubMed]
52. Yu, X.; Zeng, Q. Random forest algorithm-based classification model of pesticide aquatic toxicity to fishes. *Aquat. Toxicol.* **2022**, *251*, 106265. [CrossRef] [PubMed]
53. Sun, Z.; Wang, G.; Li, P.; Wang, H.; Zhang, M.; Liang, X. An improved random forest based on the classification accuracy and correlation measurement of decision trees. *Expert Syst. Appl.* **2024**, *237*, 121549. [CrossRef]
54. Wu, B.L.; Guan, W.H. Reader reaction on the generalized Kruskal-Wallis test for genetic association studies incorporating group uncertainty. *Biometrics* **2015**, *71*, 556–557. [CrossRef] [PubMed]
55. Fey, V.; Jambulingam, D.; Sara, H.; Heron, S.; Sipeky, C.; Schleutker, J. BioCPR-A Tool for Correlation Plots. *Data* **2021**, *6*, 97. [CrossRef]
56. Radic, T.; Likar, M.; Hancevic, K.; Regvar, M.; Carija, M.; Zdunic, G. Dataset on endophytic and rhizoplane fungi on the roots of wild grapevine in Croatia and Bosnia and Herzegovina. *Data Brief* **2021**, *34*, 106692. [CrossRef]
57. Malabrigo, P.; Eduarte, G.; Malabrigo, L.; Coracero, E. *Kandelia candel* (L.) Druce, a True Native Species in the Philippines. *Philipp. J. Sci.* **2021**, *150*, 1121–1129. [CrossRef]
58. Short, A.W.; Chen, R.; Wee, A.K.S. Comparison between parapatric mangrove sister species revealed higher photochemical efficiency in subtropical than tropical coastal vegetation under chilling stress. *Aquat. Bot.* **2021**, *168*, 103323. [CrossRef]
59. Dorey, T.; Frachon, L.; Rieseberg, L.H.; Kreiner, J.M.; Schiestl, F.P. Biotic interactions promote local adaptation to soil in plants. *Nat. Commun.* **2024**, *15*, 5186. [CrossRef] [PubMed]
60. Knight, C.G.; Nicolitch, O.; Griffiths, R.I.; Goodall, T.; Jones, B.; Weser, C.; Langridge, H.; Davison, J.; Dellavalle, A.; Eisenhauer, N.; et al. Soil microbiomes show consistent and predictable responses to extreme events. *Nature* **2024**, *636*, 690–696. [CrossRef] [PubMed]
61. Lang, T.; Ke, X.; Wei, J.; Hussain, M.; Li, M.; Gao, C.; Jiang, M.; Wang, Y.; Fu, Y.; Wu, K.; et al. Dynamics of tannin variations in mangrove leaf litter decomposition and their effects on environmental nitrogen and microbial activity. *Sci. Total Environ.* **2024**, *908*, 168150. [CrossRef]
62. Xing, J.; Pan, D.; Wang, L.; Tan, F.; Chen, W. Proteomic and physiological responses in mangrove Kandelia candel roots under short-term high-salinity stress. *Turk. J. Biol.* **2019**, *43*, 314–325. [CrossRef] [PubMed]
63. Wang, H.-m.; Xiao, X.-r.; Yang, M.-y.; Gao, Z.-l.; Zang, J.; Fu, X.-m.; Chen, Y.-h. Effects of salt stress on antioxidant defense system in the root of *Kandelia candel*. *Bot. Stud.* **2014**, *55*, 57. [CrossRef]
64. Solomon, W.; Janda, T.; Molnár, Z. Unveiling the significance of rhizosphere: Implications for plant growth, stress response, and sustainable agriculture. *Plant Physiol. Biochem.* **2024**, *206*, 108290. [CrossRef]
65. Liu, Y.; Xu, Z.; Chen, L.; Xun, W.; Shu, X.; Chen, Y.; Sun, X.; Wang, Z.; Ren, Y.; Shen, Q.; et al. Root colonization by beneficial rhizobacteria. *FEMS Microbiol. Rev.* **2024**, *48*, fuad066. [CrossRef] [PubMed]
66. Shekhar, V.; Stöckle, D.; Thellmann, M.; Vermeer, J.E.M. Chapter Three—The role of plant root systems in evolutionary adaptation. In *Current Topics in Developmental Biology*; Grossniklaus, U., Ed.; Academic Press: Cambridge, MA, USA, 2019; Volume 131, pp. 55–80.
67. Luzhen, C.; Wenqing, W.; Peng, L. Photosynthetic and physiological responses of *Kandelia candel* L. Druce seedlings to duration of tidal immersion in artificial seawater. *Environ. Exp. Bot.* **2005**, *54*, 256–266. [CrossRef]
68. Carugati, L.; Gatto, B.; Rastelli, E.; Lo Martire, M.; Coral, C.; Greco, S.; Danovaro, R. Impact of mangrove forests degradation on biodiversity and ecosystem functioning. *Sci. Rep.* **2018**, *8*, 13298. [CrossRef] [PubMed]
69. Song, W.; Zhao, Y.; Zhou, J.; Feng, J.; Wang, Z.; Han, G.; Pendall, E.; Lin, G. The effects of climate warming and exogenous nitrogen input on soil N2O emissions from mangroves. *Soil Biol. Biochem.* **2024**, *199*, 109607. [CrossRef]

70. Hu, B.; Guo, P.; Wu, Y.; Deng, J.; Su, H.; Li, Y.; Nan, Y. Study of soil physicochemical properties and heavy metals of a mangrove restoration wetland. *J. Clean. Prod.* **2021**, *291*, 125965. [CrossRef]
71. Srikanth, S.; Lum, S.K.Y.; Chen, Z. Mangrove root: Adaptations and ecological importance. *Trees* **2016**, *30*, 451–465. [CrossRef]
72. Anu, K.; Parveen, K.H.; Sneha, V.K.; Busheera, P.; Jumana, M.; Anu, A.; Muhammed, J.; Augustine, A. Mangroves in environmental engineering: Harnessing the multifunctional potential of nature's coastal architects for sustainable ecosystem management. *Results Eng.* **2024**, *21*, 101765. [CrossRef]
73. Schlüter, S.; Sammartino, S.; Koestel, J. Exploring the relationship between soil structure and soil functions via pore-scale imaging. *Geoderma* **2020**, *370*, 114370. [CrossRef]
74. Bowles, T.M.; Acosta-Martínez, V.; Calderón, F.; Jackson, L.E. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. *Soil Biol. Biochem.* **2014**, *68*, 252–262. [CrossRef]
75. Wang, G.; Gao, Q.; Yang, Y.; Hobbie, S.E.; Reich, P.B.; Zhou, J. Soil enzymes as indicators of soil function: A step toward greater realism in microbial ecological modeling. *Glob. Chang. Biol.* **2022**, *28*, 1935–1950. [CrossRef] [PubMed]
76. Sun, D.; Huang, Y.; Wang, Z.; Tang, X.; Ye, W.; Cao, H.; Shen, H. Soil microbial community structure, function and network along a mangrove forest restoration chronosequence. *Sci. Total Environ.* **2024**, *913*, 169704. [CrossRef] [PubMed]
77. Xing, Y.; Qiu, J.; Chen, J.; Cheng, D.; Yin, Q.; Chen, X.; Xu, L.; Zheng, P. Unveiling hidden interactions: Microorganisms, enzymes, and mangroves at different stages of succession in the Shankou Mangrove Nature Reserve, China. *Sci. Total Environ.* **2024**, *923*, 171340. [CrossRef] [PubMed]
78. Yuan, Z.; Zeng, Z.; Liu, F. Community structures of mangrove endophytic and rhizosphere bacteria in Zhangjiangkou National Mangrove Nature Reserve. *Sci. Rep.* **2023**, *13*, 17127. [CrossRef]
79. Tan, J.; Zuniga, C.; Zengler, K. Unraveling interactions in microbial communities—From co-cultures to microbiomes. *J. Microbiol.* **2015**, *53*, 295–305. [CrossRef] [PubMed]
80. Hibbing, M.E.; Fuqua, C.; Parsek, M.R.; Peterson, S.B. Bacterial competition: Surviving and thriving in the microbial jungle. *Nat. Rev. Microbiol.* **2010**, *8*, 15–25. [CrossRef] [PubMed]
81. Shu, W.-S.; Huang, L.-N. Microbial diversity in extreme environments. *Nat. Rev. Microbiol.* **2022**, *20*, 219–235. [CrossRef]
82. Ma, D.; Guo, Z.; Ding, Q.; Zhao, Z.; Shen, Z.; Wei, M.; Gao, C.; Zhang, L.; Li, H.; Zhang, S.; et al. Chromosome-level assembly of the mangrove plant Aegiceras corniculatum genome generated through Illumina, PacBio and Hi-C sequencing technologies. *Mol. Ecol. Resour.* **2021**, *21*, 1593–1607. [CrossRef]
83. Kulshreshtha, N.M.; Verma, V.; Soti, A.; Brighu, U.; Gupta, A.B. Exploring the contribution of plant species in the performance of constructed wetlands for domestic wastewater treatment. *Bioresour. Technol. Rep.* **2022**, *18*, 101038. [CrossRef]
84. Feng, X.; Li, G.; Xu, S.; Wu, W.; Chen, Q.; Shao, S.; Liu, M.; Wang, N.; Zhong, C.; He, Z.; et al. Genomic insights into molecular adaptation to intertidal environments in the mangrove Aegiceras corniculatum. *New Phytol.* **2021**, *231*, 2346–2358. [CrossRef]
85. Fang, L.; Yang, Y.; Guo, W.; Li, J.; Zhong, C.; Huang, Y.; Zhou, R.; Shi, S. De novo assembly of the transcriptome of Aegiceras corniculatum, a mangrove species in the Indo-West Pacific region. *Mar. Genom.* **2016**, *28*, 49–52. [CrossRef] [PubMed]
86. Willms, I.M.; Rudolph, A.Y.; Göschel, I.; Bolz, S.H.; Schneider, D.; Penone, C.; Poehlein, A.; Schöning, I.; Nacke, H. Globally Abundant "Candidatus Udaeobacter" Benefits from Release of Antibiotics in Soil and Potentially Performs Trace Gas Scavenging. *mSphere* **2020**, *5*, 1110–1128. [CrossRef]
87. Brewer, T.E.; Handley, K.M.; Carini, P.; Gilbert, J.A.; Fierer, N. Genome reduction in an abundant and ubiquitous soil bacterium 'Candidatus Udaeobacter copiosus'. *Nat. Microbiol.* **2016**, *2*, 16198. [CrossRef]
88. Patel, K.F.; Fansler, S.J.; Campbell, T.P.; Bond-Lamberty, B.; Smith, A.P.; RoyChowdhury, T.; McCue, L.A.; Varga, T.; Bailey, V.L. Soil texture and environmental conditions influence the biogeochemical responses of soils to drought and flooding. *Commun. Earth Environ.* **2021**, *2*, 127. [CrossRef]
89. Heinonsalo, J.; Sun, H.; Santalahti, M.; Bäcklund, K.; Hari, P.; Pumpanen, J. Evidences on the Ability of Mycorrhizal Genus Piloderma to Use Organic Nitrogen and Deliver It to Scots Pine. *PLoS ONE* **2015**, *10*, e0131561. [CrossRef] [PubMed]
90. Popa, F.; Rexer, K.-H.; Donges, K.; Yang, Z.L.; Kost, G. Three new Laccaria species from Southwest China (Yunnan). *Mycol. Prog.* **2014**, *13*, 998. [CrossRef]
91. Zhao, Y.; Ji, B.; Liu, R.; Ren, B.; Wei, T. Constructed treatment wetland: Glance of development and future perspectives. *Water Cycle* **2020**, *1*, 104–112. [CrossRef]
92. Bartlett, P.; Eberhardt, U.; Beker, H.J. Demystifying Hebeloma: Introducing hebeloma.org and its database. *IMA Fungus* **2022**, *13*, 18. [CrossRef] [PubMed]
93. Yan, P.; Zhuang, S.; Li, M.; Zhang, J.; Wu, S.; Xie, H.; Wu, H. Combined environmental pressure induces unique assembly patterns of micro-plastisphere biofilm microbial communities in constructed wetlands. *Water Res.* **2024**, *260*, 121958. [CrossRef]
94. Petters, S.; Groß, V.; Söllinger, A.; Pichler, M.; Reinhard, A.; Bengtsson, M.M.; Urich, T. The soil microbial food web revisited: Predatory myxobacteria as keystone taxa? *ISME J.* **2021**, *15*, 2665–2675. [CrossRef] [PubMed]

95. Bill, M.; Chidamba, L.; Gokul, J.K.; Labuschagne, N.; Korsten, L. Bacterial community dynamics and functional profiling of soils from conventional and organic cropping systems. *Appl. Soil Ecol.* **2021**, *157*, 103734. [CrossRef]
96. Soltani, J.; Zaheri-Shoja, M.; Hamzei, J.; Hosseyni-Moghaddam, M.S.; Pakvaz, S. Diversity and bioactivity of bacterial endophyte community of Cupressaceae. *For. Pathol.* **2016**, *46*, 353–361. [CrossRef]
97. Rawat, S.; Männistö, M.; Bromberg, Y.; Häggblom, M. Comparative genomic and physiological analysis provides insights into the role of Acidobacteria in organic carbon utilization in Arctic tundra soils. *FEMS Microbiol. Ecol.* **2012**, *82*, 341–355. [CrossRef]
98. Amoo, A.E.; Enagbonma, B.J.; Babalola, O.O. High-throughput sequencing data of soil bacterial communities from Tweefontein indigenous and commercial forests, South Africa. *Data Brief* **2020**, *28*, 104916. [CrossRef]
99. Zhang, N.; Zhu, C.; Shen, Z.; Tao, C.; Ou, Y.; Li, R.; Deng, X.; Shen, Q.; Dini-Andreote, F. Partitioning the Effects of Soil Legacy and Pathogen Exposure Determining Soil Suppressiveness via Induced Systemic Resistance. *Plants* **2022**, *11*, 2816. [CrossRef]
100. Zhou, X.; Tahvanainen, T.; Malard, L.; Chen, L.; Pérez-Pérez, J.; Berninger, F. Global analysis of soil bacterial genera and diversity in response to pH. *Soil Biol. Biochem.* **2024**, *198*, 109552. [CrossRef]
101. Centenaro, G.; Hudek, C.; Zanella, A.; Crivellaro, A. Root-soil physical and biotic interactions with a focus on tree root systems: A review. *Appl. Soil Ecol.* **2018**, *123*, 318–327. [CrossRef]
102. Li, H.; La, S.; Zhang, X.; Gao, L.; Tian, Y. Salt-induced recruitment of specific root-associated bacterial consortium capable of enhancing plant adaptability to salt stress. *ISME J.* **2021**, *15*, 2865–2882. [CrossRef] [PubMed]
103. Li, Y.; Yu, Y.; Yang, X.; Pat-Espadas, A.M.; Vinuesa, P.; Herzberg, M.; Chen, J.; Rosen, B.P.; Feng, R.; Rensing, C. Adaptation to metal(loid)s in strain Mucilaginibacter rubeus P2 involves novel arsenic resistance genes and mechanisms. *J. Hazard. Mater.* **2024**, *462*, 132796. [CrossRef] [PubMed]
104. López-Mondéjar, R.; Zühlke, D.; Becher, D.; Riedel, K.; Baldrian, P. Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. *Sci. Rep.* **2016**, *6*, 25279. [CrossRef] [PubMed]
105. Séneca, J.; Söllinger, A.; Herbold, C.W.; Pjevac, P.; Prommer, J.; Verbruggen, E.; Sigurdsson, B.D.; Peñuelas, J.; Janssens, I.A.; Urich, T.; et al. Increased microbial expression of organic nitrogen cycling genes in long-term warmed grassland soils. *ISME Commun.* **2021**, *1*, 69. [CrossRef]
106. Pathak, H.; Fagodiya, R.K.; Singh, A. Nitrogen, phosphorus and potassium budget in crop production in South-Asia: Regional and country trends during the last five decades. *Sci. Rep.* **2024**, *14*, 29136. [CrossRef]
107. Lacerda-Júnior, G.V.; Noronha, M.F.; Cabral, L.; Delforno, T.P.; de Sousa, S.T.P.; Fernandes-Júnior, P.I.; Melo, I.S.; Oliveira, V.M. Land Use and Seasonal Effects on the Soil Microbiome of a Brazilian Dry Forest. *Front. Microbiol.* **2019**, *10*, 648. [CrossRef] [PubMed]
108. Wu, H.; Cui, H.; Fu, C.; Li, R.; Qi, F.; Liu, Z.; Yang, G.; Xiao, K.; Qiao, M. Unveiling the crucial role of soil microorganisms in carbon cycling: A review. *Sci. Total Environ.* **2024**, *909*, 168627. [CrossRef]
109. Nabi, M. Chapter eleven—Role of microorganisms in plant nutrition and soil health. In *Sustainable Plant Nutrition*; Aftab, T., Hakeem, K.R., Eds.; Academic Press: Cambridge, MA, USA, 2023; pp. 263–282. [CrossRef]
110. Zhang, G.; Fu, G. Changes in soil organic carbon, total nitrogen and total phosphorus in 2000–2020 and their driving mechanisms in Tibetan alpine grasslands. *Glob. Planet. Change* **2024**, *239*, 104484. [CrossRef]
111. Tian, H.; Qiao, J.; Zhu, Y.; Jia, X.; Shao, M. Vertical distribution of soil available phosphorus and soil available potassium in the critical zone on the Loess Plateau, China. *Sci. Rep.* **2021**, *11*, 3159. [CrossRef]
112. Dai, Z.; Liu, G.; Chen, H.; Chen, C.; Wang, J.; Ai, S.; Wei, D.; Li, D.; Ma, B.; Tang, C.; et al. Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems. *ISME J.* **2020**, *14*, 757–770. [CrossRef]
113. Shi, Y.; Li, Y.; Yang, T.; Chu, H. Threshold effects of soil pH on microbial co-occurrence structure in acidic and alkaline arable lands. *Sci. Total Environ.* **2021**, *800*, 149592. [CrossRef] [PubMed]
114. Kerfahi, D.; Guo, Y.; Dong, K.; Wang, Q.; Adams, J.M. pH is the major predictor of soil microbial network complexity in Chinese forests along a latitudinal gradient. *CATENA* **2024**, *234*, 107595. [CrossRef]
115. Xiang, J.; Gu, J.; Wang, G.; Bol, R.; Yao, L.; Fang, Y.; Zhang, H. Soil pH controls the structure and diversity of bacterial communities along elevational gradients on Huangshan, China. *Eur. J. Soil Biol.* **2024**, *120*, 103586. [CrossRef]
116. Bojórquez-Quintal, E.; Escalante-Magaña, C.; Echevarría-Machado, I.; Martínez-Estévez, M. Aluminum, a Friend or Foe of Higher Plants in Acid Soils. *Front. Plant. Sci.* **2017**, *8*, 1767. [CrossRef]
117. Ur Rahman, S.; Han, J.-C.; Ahmad, M.; Ashraf, M.N.; Khaliq, M.A.; Yousaf, M.; Wang, Y.; Yasin, G.; Nawaz, M.F.; Khan, K.A.; et al. Aluminum phytotoxicity in acidic environments: A comprehensive review of plant tolerance and adaptation strategies. *Ecotoxicol. Environ. Saf.* **2024**, *269*, 115791. [CrossRef]
118. Shrivastav, P.; Prasad, M.; Singh, T.B.; Yadav, A.; Goyal, D.; Ali, A.; Dantu, P.K. Role of Nutrients in Plant Growth and Development. In *Contaminants in Agriculture: Sources, Impacts and Management*; Naeem, M., Ansari, A.A., Gill, S.S., Eds.; Springer International Publishing: Cham, Switzerland, 2020; pp. 43–59. [CrossRef]
119. Cao, T.; Luo, Y.; Shi, M.; Tian, X.; Kuzyakov, Y. Microbial interactions for nutrient acquisition in soil: Miners, scavengers, and carriers. *Soil Biol. Biochem.* **2024**, *188*, 109215. [CrossRef]

120. Mostofa, M.G.; Rahman, M.M.; Ghosh, T.K.; Kabir, A.H.; Abdelrahman, M.; Rahman Khan, M.A.; Mochida, K.; Tran, L.-S.P. Potassium in plant physiological adaptation to abiotic stresses. *Plant Physiol. Biochem.* **2022**, *186*, 279–289. [CrossRef] [PubMed]
121. Kuypers, M.M.M.; Marchant, H.K.; Kartal, B. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* **2018**, *16*, 263–276. [CrossRef]
122. Kishorekumar, R.; Bulle, M.; Wany, A.; Gupta, K.J. An Overview of Important Enzymes Involved in Nitrogen Assimilation of Plants. *Methods Mol. Biol.* **2020**, *2057*, 1–13. [CrossRef]
123. Bhardwaj, A.; Singh, P.; Gupta, N.; Bhattacharjee, S.; Srivastava, A.; Parida, A.; Mishra, A.K. Chapter 20—Cyanobacteria: A key player in nutrient cycling. In *Cyanobacteria*; Mishra, A.K., Singh, S.S., Eds.; Academic Press: Cambridge, MA, USA, 2024; pp. 579–596. [CrossRef]
124. Langhans, C.; Beusen, A.H.W.; Mogollón, J.M.; Bouwman, A.F. Phosphorus for Sustainable Development Goal target of doubling smallholder productivity. *Nat. Sustain.* **2022**, *5*, 57–63. [CrossRef]
125. Luo, L.; Ma, Y.; Sanders, R.L.; Xu, C.; Li, J.; Myneni, S.C.B. Phosphorus speciation and transformation in long-term fertilized soil: Evidence from chemical fractionation and P K-edge XANES spectroscopy. *Nutr. Cycl. Agroecosyst.* **2017**, *107*, 215–226. [CrossRef]
126. Nuamah, L.A.; Li, Y.; Pu, Y.; Nwankwegu, A.S.; Haikuo, Z.; Norgbey, E.; Banahene, P.; Bofah-Buoh, R. Constructed wetlands, status, progress, and challenges. The need for critical operational reassessment for a cleaner productive ecosystem. *J. Clean. Prod.* **2020**, *269*, 122340. [CrossRef]
127. Jiao, S.; Yang, Y.; Xu, Y.; Zhang, J.; Lu, Y. Balance between community assembly processes mediates species coexistence in agricultural soil microbiomes across eastern China. *ISME J.* **2020**, *14*, 202–216. [CrossRef]
128. Wang, Y.; Zhu, K.; Chen, X.; Wei, K.; Wu, R.; Wang, G. Stochastic and deterministic assembly processes of bacterial communities in different soil aggregates. *Appl. Soil Ecol.* **2024**, *193*, 105153. [CrossRef]
129. Lidbury, I.D.E.A.; Borsetto, C.; Murphy, A.R.J.; Bottrill, A.; Jones, A.M.E.; Bending, G.D.; Hammond, J.P.; Chen, Y.; Wellington, E.M.H.; Scanlan, D.J. Niche-adaptation in plant-associated Bacteroidetes favours specialisation in organic phosphorus mineralisation. *ISME J.* **2021**, *15*, 1040–1055. [CrossRef] [PubMed]
130. Yarzábal Rodríguez, L.A.; Álvarez Gutiérrez, P.E.; Gunde-Cimerman, N.; Ciancas Jiménez, J.C.; Gutiérrez-Cepeda, A.; Ocaña, A.M.F.; Batista-García, R.A. Exploring extremophilic fungi in soil mycobiome for sustainable agriculture amid global change. *Nat. Commun.* **2024**, *15*, 6951. [CrossRef] [PubMed]
131. Pérez-Izquierdo, L.; Rincón, A.; Lindahl, B.D.; Buée, M. Chapter 13—Fungal community of forest soil: Diversity, functions, and services. In *Forest Microbiology*; Asiegbu, F.O., Kovalchuk, A., Eds.; Academic Press: Cambridge, MA, USA, 2021; pp. 231–255. [CrossRef]
132. Wang, C.; Kuzyakov, Y. Mechanisms and implications of bacterial-fungal competition for soil resources. *ISME J.* **2024**, *18*, wrae073. [CrossRef] [PubMed]
133. Zhang, X.; Li, B.; Peñuelas, J.; Sardans, J.; Cheng, D.; Yu, H.; Zhong, Q. Resource-acquisitive species have greater plasticity in leaf functional traits than resource-conservative species in response to nitrogen addition in subtropical China. *Sci. Total Environ.* **2023**, *903*, 166177. [CrossRef] [PubMed]

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