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PGPR Promotes the Recovery of Submerged Macrophytes via Indigenous Microbiome Modulations under Combined Abiotic Stress

Yahua Li [1], Xiangfen Liu [2], Qianzheng Li [2], Yao Guo [2], Yi Zhang [2], Chuan Wang [2,3,4,*], Qiaohong Zhou [2] and Zhenbin Wu [1,2]

[1] School of Environmental Studies, China University of Geosciences, Wuhan 430074, China
[2] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
[3] Faculty of Resources and Environmental Science, Hubei University, Wuhan 430062, China
[4] Hubei Key Laboratory of Regional Development and Environmental Response, Hubei University, Wuhan 430062, China
* Correspondence: wangchuan@hubu.edu.cn; Tel./Fax: +86-27-8866-8078

Abstract: The restoration of submerged macrophytes is commonly limited by abiotic stress. Here, we isolated PGPR strains from the rhizosphere of submerged macrophytes using ACC deaminase selective medium, and evaluated their growth promoting effects on *Vallisneria natans* (*V. natans*) under low light intensity and (or) high sediment organic matter load, and also explored the indigenous microbiome response of *V. natans* seedlings to PGPR inoculants. Ten isolates were chosen from the 252 isolated strains based on the ACC deaminase activity and the production of IAA and siderophore. M1 (*Pseudomonas vancouverensis*) and E15 (*Enterobacter ludwigii*) had the best growth promoting effects under low light stress and under double stress of low light and high sediment organic matter load, and the shoot height increased by 36% and 46%, respectively. The results of indigenous microbiome analysis showed that PGPR inoculants could regulate the relative abundance of *unclassified_f_Enterobacteriaceae* and improve the α-diversity of the rhizosphere bacterial community. Under high sediment organic matter load, inoculation of PGPR obviously shifted the β-diversity of rhizosphere bacterial communities to promote the early growth of *V. natans*. This study expands the application of plant–microbe interaction in the field of freshwater ecological restoration.

Keywords: PGPR; submerged macrophytes; light intensity; sediment organic matter load; indigenous microbiome

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1. Introduction

The deterioration of aquatic ecosystems is a global issue [1]. The dominance and community stability of submerged macrophytes have a significant impact on the health of an aquatic ecosystem [2]. It usually takes decades for the restoration of submerged macrophytes in eutrophic water bodies to occur, even after the water quality has improved [3]. Consequently, the artificial restoration of submerged macrophytes is a crucial part of many aquatic ecology restoration projects. However, low light intensity and high sediment organic matter load are prominent factors that restrict the germination and early growth of submerged macrophytes. The light compensation point is the light intensity at which the rate of photosynthesis is equal to the rate of respiration. The submerged macrophytes can only accumulate dry matter and maintain continuous growth when the light intensity is greater than the photosynthetic compensation point [4]. Most submerged macrophytes disappeared due to the deterioration of the underwater light environment [5]. The high sediment organic matter load may produce an anaerobic sediment environment and inhibit the growth of submerged macrophytes [6,7]. In our previous study on the long-term monitoring of the West Lake, Hangzhou, China, it was also found that the sediment organic

matter load and the biomass of submerged macrophytes are significantly negatively correlated [8]. Therefore, it is important to find a sustainable approach to enhance the growth of submerged macrophytes under combined abiotic stress, such as limited light and high sediment organic matter load.

Plant-growth-promoting rhizobacteria (PGPR) can promote plant growth and reduce the susceptibility of plants to disease. The growth-promoting effects of bacteria such as *Azotobacter*, *Bacillus* and *Pseudomonas* have been widely reported [9–11]. Many studies have demonstrated that PGPR, which has growth-promoting properties such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase and indolyl-3-acetic acid (IAA) production, can alleviate the inhibition of abiotic stress such as salt [12,13], drought [14], heavy metal [15,16] and waterlogging [17] on plant growth. The direct mechanisms include the ability to produce plant hormones [18], N_2 fixation [19], phosphate solubilization [9], etc. The indirect mechanisms (antagonism against phytopathogens) can be achieved through siderophore production [9], synthesis of antibiotics, and competition for binding sites [20]. Additionally, previous studies have demonstrated that PGPR can promote plant growth by modulating the structure of the rhizosphere's indigenous microbiome [21]. The forms of plant microbiome modulations include the colonization of microbial inoculants, targeted changes toward plant-beneficial local microbiota members [22,23], and stabilizing or increasing microbial diversity [24].

At present, it is unclear whether PGPR can enhance the growth and recovery of submerged macrophytes in combined abiotic stress environments. Ravanbakhsh et al. showed that ACC deaminase-producing bacteria inhibited the growth of the aquatic plant *Rumex palustris* [25]. Our group has screened several bacterial strains from submerged plant rhizospheres, and these strains effectively promoted the growth of *V. natans* seedlings under high sediment organic matter load [26]. However, low light intensity, as a more common stress factor, often coexists with high sediment organic matter load, which seriously restricts the growth of submerged macrophytes, and increases the randomness and uncertainty of their recovery [7]. Therefore, it is necessary to explore whether PGPR is helpful to the recovery of submerged macrophytes under mixed abiotic stress and to understand the modulations of PGPR inoculants on submerged macrophytes' indigenous microbiome.

This work hypothesized that PGPR could alleviate the inhibition of mixed abiotic stress on submerged macrophytes through indigenous microbiome modulations. The present study aimed at (1) obtaining PGPR with multiple plant-growth-promoting (PGP) properties from submerged macrophytes rhizospheres; (2) evaluating the growth promotion potential of PGPR under different light intensities and sediment organic matter load using *V. natans* as the test submerged macrophytes; and (3) exploring PGPR-promoting mechanisms from the perspective of rhizosphere microbial community regulation. Our findings will establish a theoretical foundation for the application of PGPR in freshwater ecological restoration.

2. Materials and Methods

2.1. Rhizosphere Samples Collection

Two regions of the West Lake in Hangzhou, China (Maojiabu and Xilihu), have significantly different sediment organic matter loads and submerged macrophytes coverage according to years of ecological survey [8,27]. These variations provided suitable sites for collecting rhizosphere samples at various sediment organic matter loads. For PGPR isolation, we obtained rhizosphere samples from submerged macrophytes including *Hydrilla verticillata*, *Vallisneria natans*, *Potamogeton maackianus*, *Potamogeton wrightii*, which are located in different lake habitats with different light intensities and sediment organic matter loads at West Lake. In addition, in order to expand the sampling area, we collected some rhizosphere samples from Donghu in Wuhan and Shahu in Yinchuan, China. Temperatures in the sampling areas were 19–37 °C (Hangzhou), 21–34 °C (Wuhan), and 11–36 °C (Yinchuan) during the sampling period (June 2020). We also obtained *V. natans* rhizosphere samples from a microecosystem in which the *V. natans* growth was obviously inhibited by

the sediment with high organic matter. Details of the collected rhizosphere samples are shown in Table S1.

The roots and closely attached sediment of submerged macrophytes were cut with sterilized scissors, placed in sterile bags, stored at 4 °C, and sent to the laboratory for rhizosphere bacteria separation as soon as possible. The rhizosphere samples of *Chara vulgaris* were taken from the rhizoid, and the other procedures were the same. Three replicates of rhizospheric samples were collected.

2.2. Isolation of Rhizosphere Bacteria That Produce ACC Deaminase

The collected rhizosphere samples (3–5 g) were transferred into sterile water. Shaking (200 rpm, 28 °C, 30 min) led the bacteria to be released into the sterile water. Bacteria capable of producing ACC deaminase were isolated according to the method described by Penrose and Glick [28]. Following the serial dilution method, the enriched bacteria liquid was spread on Dworkin and Foster (DF) minimal medium [29] amended with 3 mM ACC in place of $(NH_4)_2SO_4$, as the sole nitrogen source, and incubated at 28 °C for 24–72 h. Following incubation, single bacterial colonies were selected based on the differences in their shapes and colors, and they were then grown individually to generate new individual cultures. After purification, single individual bacterial colonies were stored in glycerol stock (20%) at −80 °C for subsequent use.

2.3. Determining the Selected Strain's Plant-Growth-Promoting (PGP) Properties

ACC deaminase activity was quantified using the Penrose and Glick method [28] based on the strain's capacity to utilize ACC as a nitrogen source. The unit enzyme activity is the amount that ACC deaminase catalyzes ACC to produce a-ketobutyric acid per minute. The unit enzyme activity was divided by the total protein content to obtain the ACC deaminase activity of the bacteria. The ACC deaminase activity was expressed as nmol α-ketobutyrate mg^{-1} h^{-1}. IAA production was measured based on Gordon and Weber [30]. Chrome Azurol S agar plates were used to evaluate the siderophores' production [31], and the diameter of orange halos surrounding bacterial colonies was measured to indicate iron chelation.

2.4. Identification of the Selected Strains and Construction of Phylogenetic Tree

For the selected strains with better comprehensive PGP properties, 16S rRNA gene sequencing was carried out, and the sequence was determined using Tsingke Biotechnology Co., Ltd. (Beijing, China). The details are provided in the supplementary materials. Using the BLAST tools on the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST (accessed on 30 January 2021)), the sequences were compared. The neighbor-joining algorithm in MEGA 11 was used to create the phylogenetic tree. The 16S rRNA gene sequences were uploaded to NCBI GenBank database, and the accession number is listed in Table 1.

2.5. Effects of PGPR on V. natans Seed Germination and Early Growth under Different Abiotic Stress Environments

2.5.1. Setting of Environmental Stress Conditions

Through preliminary experiments, the growth conditions of plants under different gradients of light intensity and sediment organic matter load were compared. The light gradient was 0.08%, 4%, 11%, 21%, 34%, 51% and 100% (5000 Lux); 1000 Lux and 5000 Lux were set as low light conditions (LL) and suitable light conditions (SL). Sediments from Maojiabu and Xilihu, two different regions of West Lake in Hangzhou, China, were chosen as suitable (SO) and high sediment organic matter loads (HO). Chemical properties of the two type sediments are shown in Table 2. For the activity of sediment organic matter, according to the concentration of $KMnO_4$ oxidant, sediment organic matter was divided into four parts, including high active organic matter (can be oxidized by 33 mmol /L $KMnO_4$), middle active organic matter (can be oxidized by 167 mmol /L $KMnO_4$), low

active organic matter (can be oxidized by 333 mmol /L $KMnO_4$), and inactive organic matter (cannot be oxidized by 333 mmol /L $KMnO_4$) [32].

Table 1. Phylogenetic affiliation and the traits of each chosen PGPR strain, including 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (nmol ketobutyrate mg^{-1} protein h^{-1}), indole-3-acetic acid (IAA) production (μg mL^{-1} ± SD), and siderophore production (colony diameter in mm).

Selected Strains	Family Name	Strain Species	GenBank Accession Number	ACC Deaminase Activity	IAA Production	Siderophore Production	P.S.
C2	*Pseudomonadaceae*	*Pseudomonas plecoglossicida*	ON955842	5223 ± 651	2.22 ± 0.21	1.1 ± 0	ACC deaminase activity group
C17	*Enterobacteriaceae*	*Citrobacter farmeri*	ON936096	ND	41.30 ± 1.23	0.7 ± 0	Higher IAA production group
D2	*Achromobacteriaceae*	*Achromobacter insuavis*	ON936100	3608 ± 2050	2.75 ± 0.37	0.98 ± 0.10	ACC deaminase activity group
E15	*Enterobacteriaceae*	*Enterobacter ludwigii*	ON936101	ND	30.45 ± 0.56	0.88 ± 0.03	Higher IAA production group
F19	*Enterobacteriaceae*	*Klebsiella grimontii*	ON936099	ND	42.42 ± 1.09	0.85 ± 0.06	Higher IAA production group
F25	*Enterobacteriaceae*	*Klebsiella michiganensis*	ON936102	ND	42.72 ± 2.15	0.88 ± 0.05	Higher IAA production group
H13	*Enterobacteriaceae*	*Klebsiella oxytoca*	ON936097	ND	29.22 ± 0.84	1.10 ± 0.14	Higher IAA production group
H22	*Pseudomonadaceae*	*Pseudomonas migulae*	ON936103	6357 ± 1927	1.62 ± 0.25	1.9 ± 0.08	ACC deaminase activity group
L4	*Xanthomonadaceae*	*Stenotrophomonas pavanii*	ON936098	376 ± 20.32	1.98 ± 0.31	0.42 ± 0.05	ACC deaminase activity group
M1	*Pseudomonadaceae*	*Pseudomonas vancouverensis*	ON955843	6541 ± 326	3.16 ± 0.21	1.53 ± 0.10	ACC deaminase activity group

The test of the PGP traits was performed in three replicates for all strains.

Table 2. Chemical properties of the sediment obtained from Maojiabu (sediments with low organic matter levels) and Xilihu (sediments with high organic matter levels), the two different areas of West Lake, Hangzhou, China. Organic matter (OM); Total nitrogen (TN); Nitrite nitrogen (TN); Ammonium nitrogen (NH_3-N); Total phosphorus (TP); Inorganic phosphorus (IP); Organic phosphorus (OP).

Sediment Chemical Properties	Sediments with Low OM Levels	Sediments With High OM Levels
OM (mg g^{-1})	7.555 ± 0.219	26.390 ± 0.011
High active OM (mg g^{-1})	0.908 ± 0.071	3.464 ± 0.058
Middle active OM (mg g^{-1})	1.145 ± 0.167	5.925 ± 0.141
Low active OM (mg g^{-1})	0.921 ± 0.132	5.987 ± 0.109
Inactive OM (mg g^{-1})	4.581 ± 0.009	11.014 ± 0.012
TN (mg g^{-1})	0.660 ± 0.021	2.388 ± 0.065
NO_2-N (mg kg^{-1})	0.003 ± 0.006	0.025 ± 0.020
NH_3-N (mg g^{-1})	0.056 ± 0.002	0.067 ± 0.000
TP (mg g^{-1})	0.780 ± 0.016	1.173 ± 0.011
IP (mg g^{-1})	0.492 ± 0.133	0.554 ± 0.021
OP (mg g^{-1})	0.288 ± 0.016	0.619 ± 0.011

Each test was performed in three replicates for the two sediments.

In order to evaluate the effects of PGPR on the germination and early growth of *V. natans* under different environmental stress conditions, four environmental conditions were set in this experiment: (a) Suitable environment: suitable light intensity and suitable sediment organic matter load (SL + SO); (b) Single high sediment organic matter load stress: suitable light intensity and high sediment organic matter load (SL + HO); (c) Single low light stress: low light intensity and suitable sediment organic matter load (LL + SO); (d) Double stress: low light intensity and high sediment organic matter load (LL + HO).

This experiment was carried out in a standard laboratory located in Wuhan, China. Plants were grown at 25 ± 2 °C, 12 h light. The sediment was laid in plastic pot (10 cm bottom diameter, 14 cm height, 11 cm top diameter) at a height of 3 cm; then, slowly, we added 9 cm ultrapure water to the upper part of sediment. The seeds of *V. natans* were sterilized with 70% ethanol for 3 min, washed with sterile water three times, and dried in the shade. A total of 0.02 g sterilized *V. natans* seeds were sown equally on the surface of sediment.

2.5.2. Bacterial Inoculation and Plant Treatments

The strains with better comprehensive PGP properties were screened from the isolated and purified strains to test their capacity to promote *V. natans* seed germination and early growth under different environmental conditions. A bacterial solution was prepared according to Zhang et al. [33]. To the inoculated group, 5 mL (2×10^8 CFU/mL) of bacterial suspension was added every 5 days for a total of eight additions, and to the control group we added sterile water. Ultrapure water was added every 3 days to replenish evaporated water. For each treatment, three pot replicates were set.

The experiment lasted for 41 days, which was adequate for the development of treatment-related differences in growth. At the end, the number of seedlings and shoot height were recorded. The roots and attached sediment were collected in sterile water and shaken at 28 °C 200 rpm for 30 min. Then, the root tissues were taken out and the suspension was centrifuged at 12,000× *g* 4 °C for 10 min. The precipitation was collected and stored at −80 °C for rhizosphere indigenous microbiome analysis [34].

2.5.3. Rhizosphere Indigenous Microbiome Analysis

On the rhizosphere sediment samples of the controls and the treatments in which the strains showed a significant growth-promoting effect in various environments, we performed a bacterial 16S rRNA gene profiling using Illumina sequencing (Illumina, San Diego, CA, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The details are provided in the supplementary materials. The raw rRNA gene sequence data were uploaded to the NCBI Sequence Read Archive (SRA) database under accession number PRJNA867510.

2.6. Data Analysis

The alpha diversity (α-diversity) of microbial communities was estimated using the Simpson diversity index [35], which was calculated as follows:

$$\text{Simpson diversity index} = 1 - \frac{\sum_{i=1}^{S_{obs}} n_i(n_i - 1)}{N(N - 1)}$$

where S_{obs} is the number of OTUs actually observed, n_i is the number of sequences contained in the *i*th OUT, and *N* is the number of all sequences. The beta diversity (β-diversity) of microbial communities was analyzed with principal component analysis (PCA), and adonis was used to analyze the differences between the two groups: the non-inoculated control and the PGPR-inoculated group.

Statistical analysis of data was performed using R (version 4.1.3). Univariate ANOVA, with LSD post hoc test, was performed to assess the significant differences in relative shoot height between different experimental groups. Significant differences of bacterial relative abundance and Simpson diversity index between the inoculated and the control groups were analyzed via Student's t test. Linear regression was used to analyze the correlation between bacterial community characteristics and shoot height. Before all tests, data were checked for normality and homogeneity. When the residuals did not meet normality or homogeneity in linear regression, p values were obtained using the lmorigin function and permutation tests [36,37]. Significance levels for all tests were set at p-value < 0.05.

3. Results and Discussion

3.1. Screening of PGPR from Submerged Macrophytes' Rhizosphere

3.1.1. PGPR Isolation and Their PGP Properties

Through the screening of ACC deaminase selective medium, 252 isolates with ACC deaminase activity were obtained (Table S1). All strains were examined for ACC deaminase activity, IAA and siderophore production. In plants, ACC is the immediate precursor of ethylene. ACC exudated from plant roots under stress conditions may be transferred into ACC deaminase-producing bacteria cells based on the concentration gradient or candidate attractants such as chelators, exopolysaccharides, peptides/chaperones, or hormones [38]. Bacteria with ACC deaminase activity can convert ACC into 2-oxybutyrate and NH_3, which can inhibit the formation of excessive ethylene in a host under stress and increase the host's stress tolerance [39]. The amount of ACC deaminase activity required for a bacterium to grow on ACC is approximately 20 nmol α-ketobutyrate mg^{-1} h^{-1} [28]. In this study, all strains could successfully grow in the ACC deaminase selective medium; however, their ACC deaminase activity varied greatly. There are only 11 isolates recorded with ACC deaminase activity, ranging from 376 to 6540 nmol α-ketobutyrate mg^{-1} h^{-1} (Figure S1). Auxins are powerful molecules and control plant cells' division, expansion, and differentiation [40]. IAA is a common endogenous auxin in plants. Matsuda et al. discovered that, in addition to plants, a significant number of microorganisms also contain IAA synthesis-related genes [41]. Numerous studies have demonstrated the value of IAA-producing PGPR in reducing the abiotic stress of plants [9,15,40]. In this study, the amounts of produced IAA vary greatly among the bacterial strains. A total of 98 isolates could produce IAA in the presence of Tryptophan, 33 isolates of which were observed to possess higher IAA production, ranging from 29.22 to 43.01 μg mL^{-1} (Figure S2), while others produced only 1–11 μg mL^{-1}. There were no overlapping strains among strains with ACC deaminase activity and higher IAA production. They were divided into the ACC deaminase activity group and the higher IAA production group. This result was in line with the findings of Hanaka et al. [42], who reported that IAA concentration and ACC deaminase activity were conversely related. This phenomenon may be based on the activation of ACC synthase through IAA, which may inhibit ACC deaminase activity [43,44]. As an important element for plant growth, the majority of the iron in soil cannot be directly absorbed by plants. The siderophores produced by bacteria can chelate with iron to make it more available [45,46]. In this study, except L4, the strains in the group of ACC deaminase activity group had a higher siderophore production (orange halo diameter of 0.98–1.9 cm), while the higher IAA production group had a lower siderophore production (0.6–1.1 cm) (Figure S2).

3.1.2. Strains' Identification and Selection

The strains with better comprehensive PGP properties were identified via 16S rRNA gene sequencing. Based on the results of 16S rRNA gene sequencing, the same species were eliminated. Finally, ten strains were chosen to test their effects on seed germination and the early growth of *V. natans* under different stress conditions. The ten isolates belonged to *Pseudomonas* spp., *Citrobacter* spp., *Achromobacter* spp., *Enterobacter* spp., *Klebsiella* spp. and *Stenotrophomonas* spp., and their PGP properties are shown in Table 1. Three *Pseudomonas* strains displayed various PGP properties, including the production of ACC deaminase, IAA, siderophore. Moon and Ali screened two *Pseudomonas* strains from the rhizosphere of coastal sand dune plants, which showed comprehensive PGP properties, consistent with the results of this study [47]. The ten strains' adjacency phylogenetic tree is shown in Figure 1. Most of these strains were derived from the *V. natans*' rhizosphere in different habitats, except for C2 and C17, which were from *Chara vulgaris* (Table S1).

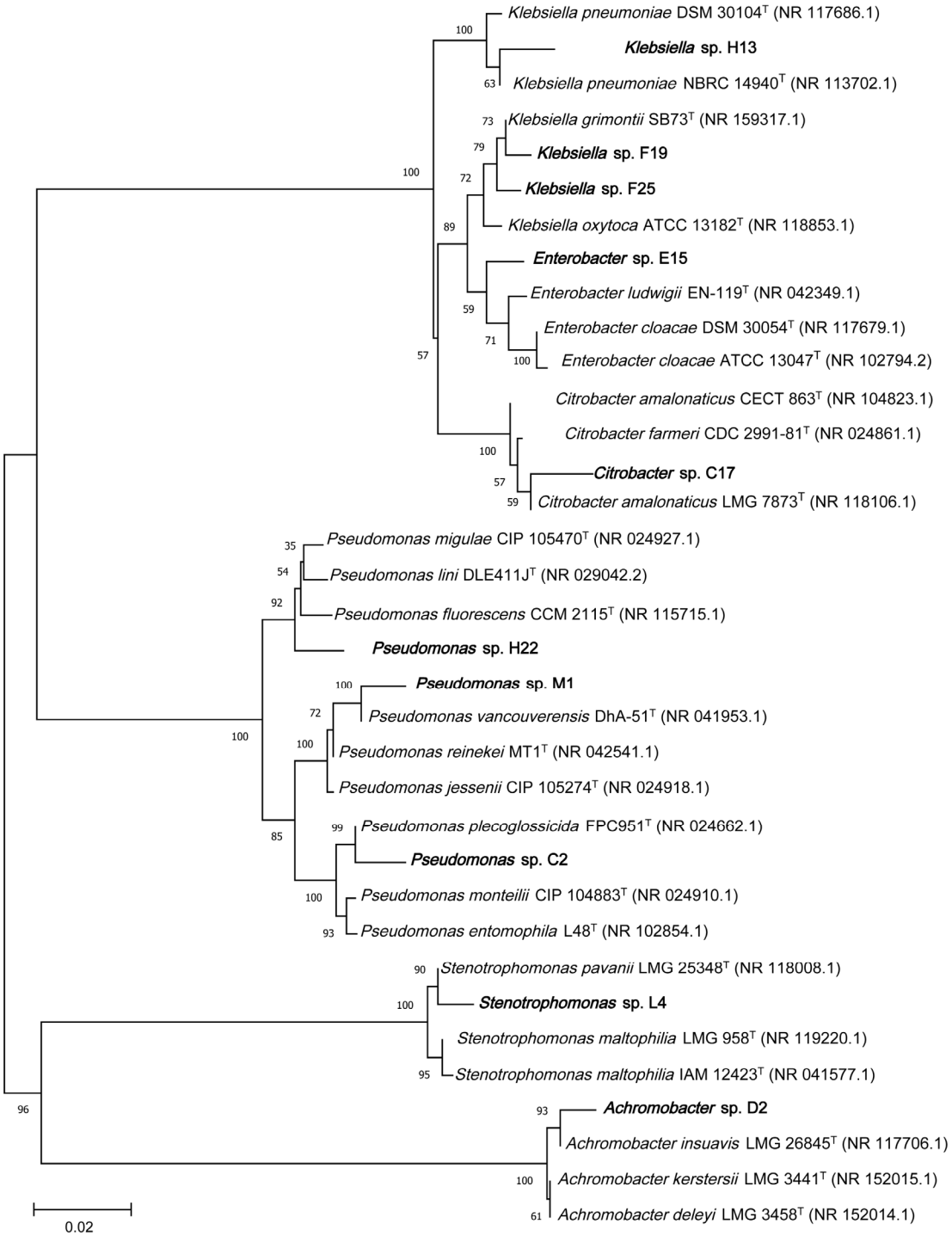

Figure 1. Adjacency phylogenetic tree of the ten selected PGPR strains. Selected strains are shown in bold.

3.2. PGPR's Effect on Seed Germination and Early Growth of V. natans

Low light intensity stress significantly inhibited the seed germination and early growth of *V. natans*, and the double stress of low light intensity and high sediment organic matter load made the inhibition more obvious (Figures S3 and S4). The shoot height of *V. natans* seedlings without PGPR inoculation was 5.84 ± 0.47 cm, 6.31 ± 0.65 cm, 2.72 ± 0.33 cm and 2.37 ± 0.29 cm under SL + SO, SL + HO, LL + SO and LL + HO, respectively, and the seedlings' number was 30 ± 1, 30 ± 1, 23 ± 1, 22 ± 1, respectively. Similar to our study, under low light intensity and high sediment organic matter load stress conditions,

the inhibition of submerged plants has been reported by Schelske et al. [5] and Chappuis et al. [48]. Although, in our preliminary experiment, the total leaf length of the adult *V. natans* in the high sediment organic matter load reduced by about a 1/3 at 40 days when compared to the suitable conditions (C. Wang, unpublished), the growth of *V. natans* seedlings did not show growth inhibition. PGPR inoculation showed different effects on *V. natans*' growth under four different stress conditions (Figure 2). Under single stress of low light intensity (LL + SO), M1 (*Pseudomonas vancouverensis*) with higher ACC deaminase activity showed the largest growth promotion effect (relative height = 1.36 ± 0.18). Under double stress of low light intensity and high sediment organic matter load (LL + HO), E15 (*Enterobacter ludwigii*) with higher IAA production had the largest growth-promoting effect (relative height = 1.46 ± 0.17) (Figure 2). The results clearly indicated that the two isolates can be of great value in enhancing *V. natans* seedlings' growth under the corresponding stress condition. More importantly, the maximum growth-promoting efficiency of PGPR inoculation under single low light stress and double stress conditions (LL + SO and LL + HO) was comparable to that under suitable conditions (SL + SO) ($p > 0.05$) (Table S2). These findings suggested that the two bacterial strains (E15 and M1) which belong to the genera *Enterobacter* and *Pseudomonas* would be interesting candidates as commercial microbial agents for the artificial restoration of submerged macrophytes. In agriculture, *Enterobacter* spp. and *Pseudomonas* spp. have been applied as agronomic inoculants for crops such as chickpea [20] and rice [49] in order to improve their quality or yield. Although PGPR inoculation did not generally increase the germination rate of *V. natans* seeds, the maximum number of seedlings could reach 41 ± 4 (Figure S4). These findings collectively showed that the selected PGPR inoculation is a feasible method for promoting *V. natans* seedlings' growth under low light intensity and (or) high sediment organic matter load. Therefore, more PGPR strains from submerged macrophytes could be found in order to investigate their potential to assist in the recovery of submerged macrophytes. These strains could be anaerobes suitable for growing in rhizosphere under hypoxic or anaerobic conditions, as well as antagonistic bacteria against the phytopathogen [50,51]. In addition, combining different strains may be more effective in promoting the growth of plant [52], which should be taken into account in future studies.

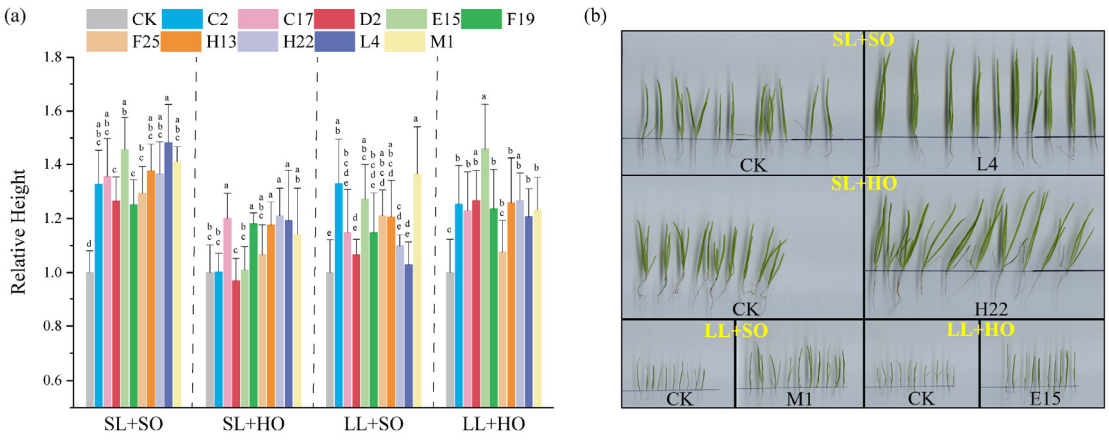

Figure 2. Relative shoot height of *V. natans* in different PGPR inoculation treatments (**a**). Morphological differences of *V. natans* with or without PGPR inoculation (**b**). CK: non-inoculation control; C2, C17, D2, E15, F19, F25, H13, H22, L4 and M1 present the inoculation treatments with the corresponding strains. Different letters indicate statistically significant differences ($p < 0.05$, Univariate ANOVA). Ten replicates of each treatment were used for height measurement.

3.3. Indigenous Microbiome Response of V. natans Seedlings to PGPR Inoculants

3.3.1. PGPR Inoculation Increased the Relative Abundance of *Unclassified_f_Enterobacteriaceae* in Indigenous Microbiome

In order to achieve plant growth promotion by producing plant hormones or siderophores, the PGPR needs to keep the number of active cells at a reasonable level [53]. The results of bacterial 16S rRNA gene profiling showed that PGPR inoculation adjusted the relative abundance of *unclassified_f_Enterobacteriaceae* in *V. natans* seedlings' rhizosphere. In this study, very few *unclassified_f_Enterobacteriaceae* were detected in the rhizosphere of non-inoculated *V. natans* seedlings under the four stress conditions; however, after inoculated with E15 (*Enterobacter ludwigii*), the relative abundance reached 1.013%, 1.684%, 51.730% and 12.610% under SL + SO, SL + HO, LL + SO and LL + HO, respectively (Figures S5–S8). The similarity of the corresponding OTU sequence and the 16S rRNA gene sequences of E15 was 99.77%. In addition, under LL + SO and LL + HO, the relative abundance of *unclassified_f_Enterobacteriaceae* in the rhizosphere of H13 (*Klebsiella quasivariicola*) inoculated *V. natans* seedlings reached 4.228% and 1.036%, respectively (Figures S7 and S8). The similarity of the corresponding OTU sequence and the 16S rRNA gene sequences of H13 was 98.14%. Therefore, strains E15 and H13 could colonize the rhizosphere of *V. natans* seedlings and became the dominant taxa (relative abundance greater than 1%). The components of root exudates and innate immune response may regulate the colonization process [54]; this requires further investigation. These species may promote the growth of *V. natans* seedlings by producing IAA and siderophores. Under low light intensity stress, PGPR may also promote the growth of *V. natans* seedlings by increasing chlorophyll content and photosynthesis. Previous studies reported that PGPR capable of dissolving phosphorus, and producing IAA and siderophores, can promote photosynthesis and increase the yield of runner bean [55]. In addition, the siderophores generated by PGPR can increase the activity of iron-containing enzymes such as catalase and peroxidase. The enhancement of these enzymes' activity can increase the content of chlorophyll, and then promote the photosynthesis of plants [56]. Under high sediment organic matter load, the anaerobic degradation of cellulose and lignin produce a variety of soluble organic substances. These substances and other possible phytotoxins (metals, gases, and dissolved sulfides) in anaerobic sediment could form a hostile environment for plant growth [6]. The capacity of aquatic plants to tolerate a hostile sediment environment may depend on oxygen transport from shoots to roots, since oxygen, in addition to supporting root respiration, helps to detoxify. Therefore, PGPR inoculation may promote photosynthesis, which is the important pathway of oxygen production, and then alleviate the stress of high sediment organic matter load.

3.3.2. Rhizobacterial α-Diversity and Its Correlations with Shoot Height of *V. natans* Seedlings

In the present study, compared with the non-inoculated control, separate inoculation of the most selected strains increased the α-diversity of the rhizosphere bacterial community of the *V. natans* seedlings. Under SL + SO, the Simpson diversity index of the rhizosphere microbial communities was 0.983–0.996 (Figure 3a). Except for C17- and H13-inoculated seedlings, the Simpson diversity index of the other PGPR-inoculated seedlings was higher than that of the non-inoculated control. Under SL + HO, the Simpson diversity index was 0.994–0.998 (Figure 3b). Except for E15, the Simpson diversity index in PGPR inoculation seedlings was higher than that of the control. Under LL + SO and LL + HO, the Simpson diversity index was 0.831–0.995 and 0.988–0.998, respectively (Figure 3c,d). In these two environments, when compared to control, the Simpson diversity index of E15- and H13-inoculated seedlings was lower, but the Simpson diversity index of the other PGPR-treated seedlings was higher. In addition, after excluding the inoculated groups with a Simpson diversity index lower than that of the control, the rhizosphere bacterial α-diversity and shoot height of *V. natans* seedlings showed a significant positive correlation under SL + SO, LL + SO and LL + HO (R_{adj}^2 = 0.396–0.527, $p < 0.05$) (Figure 3a–d), which indicated that PGPR may help *V. natans* seedlings grow by increasing the rhizosphere

bacterial α-diversity. Chen et al. [57] also showed that rhizosphere bacterial α-diversity was significantly positively correlated with maize grain yield. *Bacillus amyloliquefaciens* could compensate a pathogen attack on lettuce by regulating the indigenous microbial α-diversity [24]. This pattern of action has been found in another study of *Bacillus subtilis* inoculation to improve cucumber biocontrol capacity [58].

3.3.3. Structure of Rhizobacterial Communities, and Their Correlations with Shoot Height

The PGPR inoculation in the current study clearly altered the bacterial communities structure of the *V. natans* seedlings' rhizosphere in the four environmental conditions (Figure 4). The diversion of community structure was more significantly under high sediment organic matter load. The PCA plots showed that the rhizosphere bacterial communities of the PGPR-inoculated group and the control group were divided in all the stress conditions. At the same time, linear regression analysis of PC1 in PCA and shoot height of *V. natans* seedlings showed a significant positive correlation both in SL + HO ($R_{adj}^2 = 0.659$, $p < 0.05$) and LL + HO ($R_{adj}^2 = 0.580$, $p < 0.05$) (Figure 4b,d), indicating that under high sediment organic matter load, the greater the difference in rhizosphere bacterial community structure between PGPR inoculation groups and non-inoculated control, the greater the growth promoting effect. Changes in microbial community structure may be caused by specific functional species. The indigenous microbiome analysis revealed that some species' relative abundance was down-regulated in all PGPR treatment groups under the same stress condition (Table 3). The relative abundance of *Aquicella* spp. and *Bacillus* spp. was significantly decreased ($p < 0.05$) after PGPR inoculation under SL + SO and SL + HO, respectively (Figures S5 and S6). Under LL + SO, the relative abundance of *Azotobacter* spp. and *Methylocystis* spp. was significantly down-regulated ($p < 0.05$) (Figure S7). Under LL + HO, the relative abundance of *Zavarzinia* spp., *Methyloversatilis* spp. and *Azotobacter* spp. was significantly decreased ($p < 0.05$) (Figure S8). Interestingly, although the down-regulated species in the four environmental conditions were not identical, they were all involved in the carbon and nitrogen (C/N) cycling in the rhizosphere of *V. natans* seedlings (Table 3). This phenomenon may be caused by rhizosphere ecological niche competition, where inoculated PGPR replaces indigenous microorganisms in the rhizosphere [21]. Targeted shifts toward microbiota that are beneficial to plants may also be part of the regulation of microbial community structure. Zhang et al.'s study indicated PGPR consortium could greatly increase the abundance of *Ramlibacter* spp. and *Comamonas* spp. which commonly have antifungal effects and relieve the disease symptoms of *Phytophthora capsici* infected sweet pepper [33].

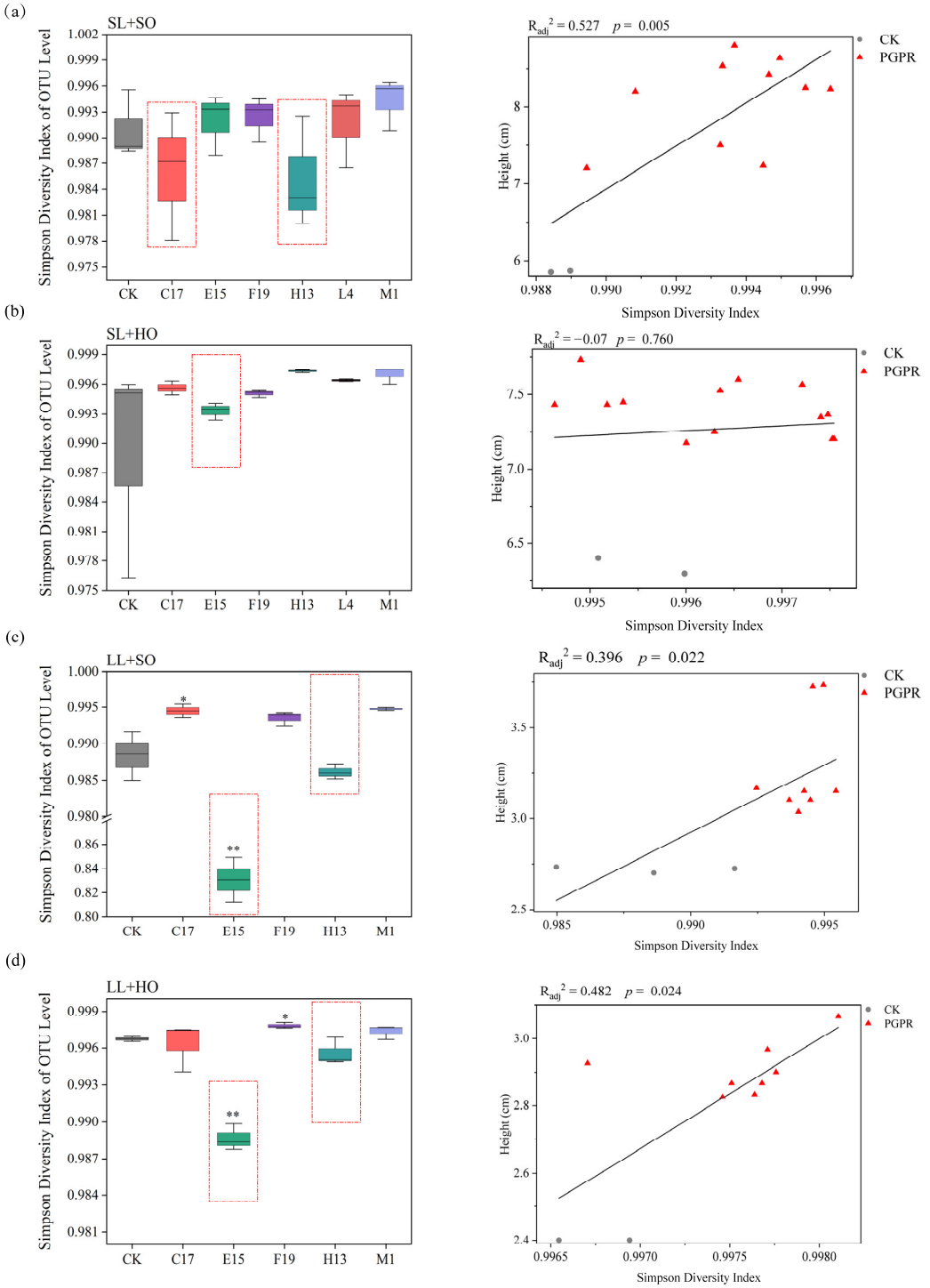

Figure 3. Rhizobacterial Simpson diversity index and its correlations with shoot height of *V. natans* under four stress conditions; (**a**) Suitable light intensity and suitable sediment organic matter load (SL + SO); (**b**) Suitable light intensity and high sediment organic matter load (SL + HO); (**c**) Low light intensity and suitable sediment organic matter load (LL + SO); (**d**) Low light intensity and high sediment organic matter load (LL + HO). CK: non-inoculation control; C17, E15, F19, H13, L4 and M1 present the inoculation treatments with the corresponding strains. Asterisk indicates significant difference between CK and the inoculated group (* $p < 0.05$, ** $p < 0.01$, Student's *t*-test). Three replicates of each treatment were used for bacterial 16S rRNA gene profiling.

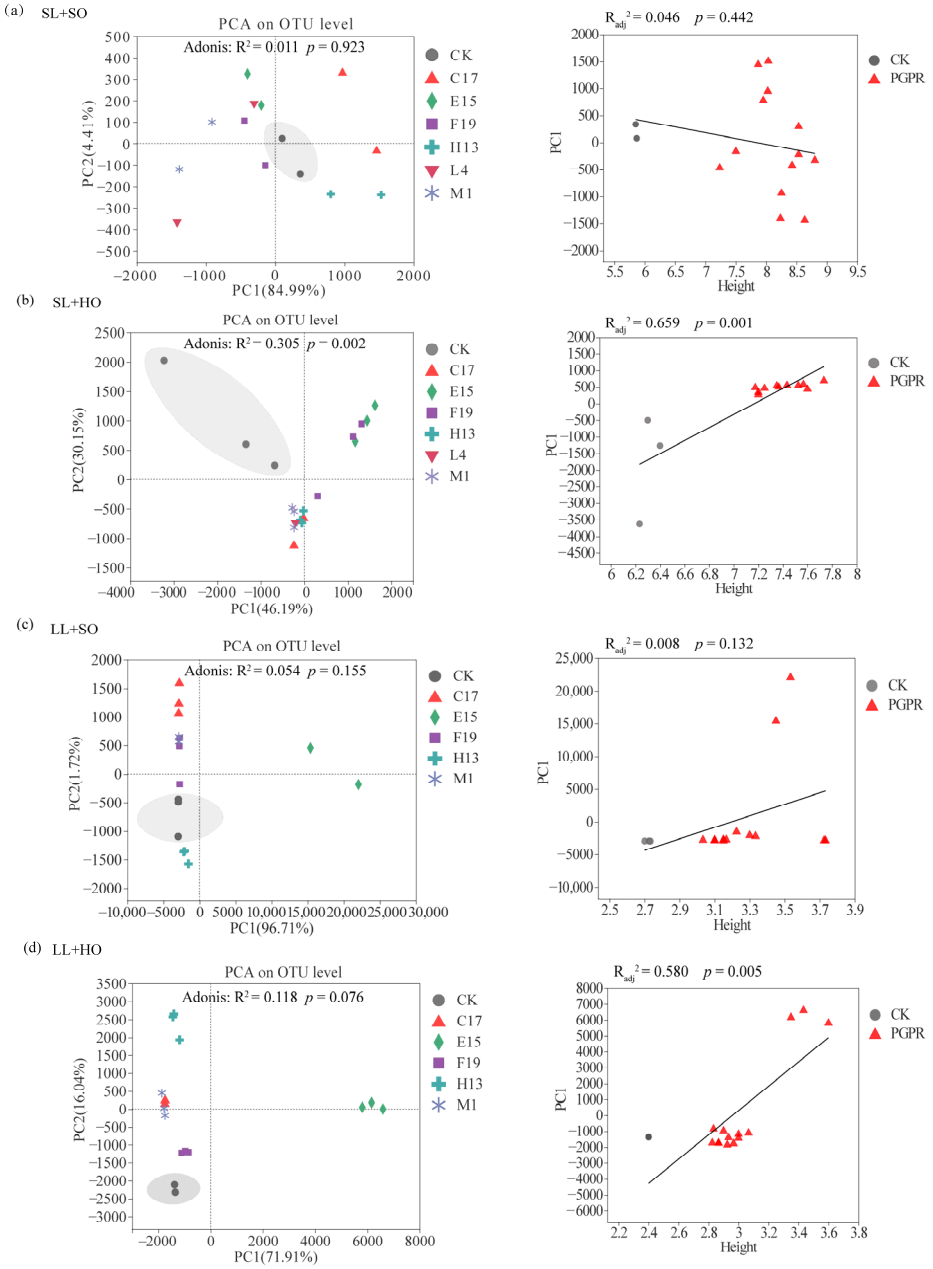

Figure 4. Principal component analysis (PCA) of the structure of bacterial communities and the correlations with shoot height of *V. natans* under four stress conditions; (**a**) Suitable light intensity and suitable sediment organic matter load (SL + SO); (**b**) Suitable light intensity and high sediment organic matter load (SL + HO); (**c**) Low light intensity and suitable sediment organic matter load (LL + SO); (**d**) Low light intensity and high sediment organic matter load (LL + HO). The gray circle is marked as the control group without bacteria addition, and the PGPR-inoculated group is outside the circle. Adonis was used to analyze the differences between the two groups: the non-inoculated control and the PGPR-inoculated group. Three replicates of each treatment were used for bacterial 16S rRNA gene profiling.

Table 3. Significantly down-regulated species after PGPR inoculation in the four environmental conditions and their ecological functions.

Environmental Conditions	Species	Function
SL + SO	*Aquicella*	Nitrogen fixation (KEGG).
SL + HO	*Bacillus*	Heterotrophic nitrate denitrification [59]; Nitrogen fixation [40].
LL + SO	*Azotobacter*	Nitrogen fixation [60].
	Methylocystis	Nitrogen fixation and aerobic methanooxidation bacteria [61].
LL + HO	*Zavarzinia*	Benzene and baphthalene degradation and aerobic carboxidotrophic [62].
	Methyloversatilis	Possess a wide range of metabolic capacities; denitrification and nitrogen fixation [63].
	Azotobacter	Nitrogen fixation [60].

4. Conclusions

This study showed inoculation with the selected PGPR strains is a feasible method for promoting *V. natans* seedlings' growth under low light intensity and (or) high sediment organic matter load. On the one hand, these stains may promote the early growth of *V. natans* by producing ACC deaminase, IAA and siderophore. On the other hand, the regulation of a rhizosphere bacterial community structure by changing the relative abundance of inoculated strain or bacteria related to C/N cycling, and increasing the α-diversity of bacterial community, may be another mechanism through which PGPR promoted the growth of *V. natans* seedlings. The strains (E15 and M1) could be interesting targets for the creation of new industrial microbial agents for artificial restoration of submerged macrophytes. This finding provides an effective and environmentally friendly strategy for restoring submerged macrophytes under low light intensity and (or) high sediment organic matter load. This study expands on the application of plant–microbe interaction in the field of freshwater ecological restoration.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15030590/s1, Figure S1: A schematic drawing in one environmental conditions; Figure S2: The growth-promoting properties of the isolated strains and correlation analysis; (a) ACC deaminase activity; (b) IAA production (>29 μg mL^{-1}); (c) Siderophore production; Figure S3: Shoot height of *V. natans* in different PGPR inoculation treatments under four stress conditions; (a) Suitable light intensity and suitable sediment organic matter load (SL+SO) and suitable light intensity and high sediment organic matter load (SL + HO); (b) Low light intensity and suitable sediment organic matter load (LL + SO) and low light intensity and high sediment organic matter load (LL + HO). CK: non-inoculation control; C2, C17, D2, E15, F19, F25, H13, H22, L4 and M1 present the inoculation treatments with the corresponding strains; Figure S4: Seedling numbers of *V. natans* in different PGPR inoculation treatments under four environments. Explanations as in Figure S3; Figures S5–S8: Relative abundance of the 15 top-ranked genera of bacteria with significant differences between inoculated group and uninoculated control group in the rhizosphere of *V. natans* seedlings under four environments. CK: uninoculated control; C17, E15, F19, H13, L4 and M1 present the inoculated group with the corresponding strains. Asterisk indicates significant difference between CK and the inoculated group ($p < 0.05$, Student's t test); Table S1: Environmental characteristics of the living position of submerged macrophytes and isolated PGPR strain numbers; Table S2: The highest growth promoting effect and their significant differences under the four environments ($p < 0.05$, Univariate ANOVA). The details of 16S rRNA gene sequencing; The details of 16S rRNA gene high throughput sequencing technique. References [64–69] is cited in the supplementary materials.

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Data Availability Statement: The corresponding author can provide data used to support this study's findings upon reasonable request. The 16S rRNA gene sequences of the selected strains were uploaded to the NCBI GenBank database, and the accession number is listed in Table 1. The 16S rRNA gene high throughput raw sequence data were uploaded to the NCBI Sequence Read Archive (SRA) database under accession number PRJNA867510. These raw sequence data are publicly accessible at https://www.ncbi.nlm.nih.gov/ (accessed on 7 July 2022).

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References

1. Ho, J.C.; Michalak, A.M.; Pahlevan, N. Widespread global increase in intense lake phytoplankton blooms since the 1980s. *Nature* **2019**, *574*, 667–670. [CrossRef] [PubMed]
2. Sayer, C.D.; Burgess, A.; Kari, K.; Davidson, T.A.; Peglar, S.; Yang, H.; Rose, N. Long-term dynamics of submerged macrophytes and algae in a small and shallow, eutrophic lake: Implications for the stability of macrophyte-dominance. *Freshw. Biol.* **2010**, *55*, 565–583. [CrossRef]
3. Sand-Jensen, K.; Pedersen, N.L.; Thorsgaard, I.; Moeslund, B.; Borum, J.; Brodersen, K.P. 100 years of vegetation decline and recovery in Lake Fure, Denmark. *J. Ecol.* **2008**, *96*, 260–271. [CrossRef]
4. Sand-Jensen, K.; Madsen, T.V. Minimum light requirements of submerged fresh-water macrophytes in laboratory growth experiments. *J. Ecol.* **1991**, *79*, 749–764. [CrossRef]
5. Schelske, C.L.; Lowe, E.F.; Kenney, W.F.; Battoe, L.E.; Brenner, M.; Coveney, M.F. How anthropogenic darkening of Lake Apopka induced benthic light limitation and forced the shift from macrophyte to phytoplankton dominance. *Limnol. Oceanogr.* **2010**, *55*, 1201–1212. [CrossRef]
6. Barko, J.W.; Smart, R.M. Effects of Organic Matter Additions to Sediment on the Growth of Aquatic Plants. *J. Ecol.* **1983**, *71*, 161. [CrossRef]
7. Wu, J.; Cheng, S.; Liang, W.; He, F.; Wu, Z. Effects of sediment anoxia and light on turion germination and early growth of *Potamogeton crispus*. *Hydrobiologia* **2009**, *628*, 111–119. [CrossRef]
8. Bai, G.; Zhang, Y.; Yan, P.; Yan, W.; Kong, L.; Wang, L.; Wang, C.; Liu, Z.; Liu, B.; Ma, J.; et al. Spatial and seasonal variation of water parameters, sediment properties, and submerged macrophytes after ecological restoration in a long-term (6 year) study in Hangzhou west lake in China: Submerged macrophyte distribution influenced by environmental variables. *Water Res.* **2020**, *186*, 116379. [CrossRef]
9. Lugtenberg, B.; Kamilova, F. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* **2009**, *63*, 541–556. [CrossRef]
10. Liu, F.; Xing, S.; Ma, H.; Du, Z.; Ma, B. Plant growth-promoting rhizobacteria affect the growth and nutrient uptake of *Fraxinus americana* container seedlings. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 4617–4625. [CrossRef]
11. Costa-Gutierrez, S.B.; Adler, C.; Espinosa-Urgel, M.; de Cristóbal, R.E. Pseudomonas putida and its close relatives: Mixing and mastering the perfect tune for plants. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 3351–3367. [CrossRef] [PubMed]
12. Han, L.; Zhang, H.; Xu, Y.; Li, Y.; Zhou, J. Biological characteristics and salt-tolerant plant growth-promoting effects of an ACC deaminase-producing *Burkholderia pyrrocinia* strain isolated from the tea rhizosphere. *Arch. Microbiol.* **2021**, *203*, 2279–2290. [CrossRef] [PubMed]
13. Haroon, U.; Khizar, M.; Liaquat, F.; Ali, M.; Akbar, M.; Tahir, K.; Batool, S.S.; Kamal, A.; Chaudhary, H.J.; Munis, M.F.H. Halotolerant Plant Growth-Promoting Rhizobacteria Induce Salinity Tolerance in Wheat by Enhancing the Expression of SOS Genes. *J. Plant Growth Regul.* **2022**, *41*, 2435–2448. [CrossRef]
14. Zarei, T.; Moradi, A.; Kazemeini, S.A.; Akhgar, A.; Rahi, A.A. The role of ACC deaminase producing bacteria in improving sweet corn (*Zea mays* L. var saccharata) productivity under limited availability of irrigation water. *Sci. Rep.* **2020**, *10*, 20361. [CrossRef] [PubMed]
15. Guo, J.; Muhammad, H.; Lv, X.; Wei, T.; Ren, X.; Jia, H.; Atif, S.; Hua, L. Prospects and applications of plant growth promoting rhizobacteria to mitigate soil metal contamination: A review. *Chemosphere* **2020**, *246*, 125823. [CrossRef]
16. Kumar, A.; Tripti; Maleva, M.; Bruno, L.B.; Rajkumar, M. Synergistic effect of ACC deaminase producing *Pseudomonas* sp. TR15a and siderophore producing Bacillus aerophilus TR15c for enhanced growth and copper accumulation in *Helianthus annuus* L. *Chemosphere* **2021**, *276*, 130038. [CrossRef]
17. Ali, S.; Kim, W.-C. Plant Growth Promotion Under Water: Decrease of Waterlogging-Induced ACC and Ethylene Levels by ACC Deaminase-Producing Bacteria. *Front. Microbiol.* **2018**, *9*, 1096. [CrossRef]

18. 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]
19. Ahemad, M.; Kibret, M. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *J. King Saud Univ. Sci.* **2014**, *26*, 1–20. [CrossRef]
20. Ferreira, C.M.H.; Soares, H.M.V.M.; Soares, E.V. Promising bacterial genera for agricultural practices: An insight on plant growth-promoting properties and microbial safety aspects. *Sci. Total Environ.* **2019**, *682*, 779–799. [CrossRef]
21. Berg, G.; Kusstatscher, P.; Abdelfattah, A.; Cernava, T.; Smalla, K. Microbiome Modulation—Toward a Better Understanding of Plant Microbiome Response to Microbial Inoculants. *Front. Microbiol.* **2021**, *12*, 650610. [CrossRef] [PubMed]
22. Passera, A.; Vacchini, V.; Cocetta, G.; Shahzad, G.-I.; Arpanahi, A.A.; Casati, P.; Ferrante, A.; Piazza, L. Towards Nutrition-Sensitive Agriculture: An evaluation of biocontrol effects, nutritional value, and ecological impact of bacterial inoculants. *Sci. Total Environ.* **2020**, *724*, 138127. [CrossRef] [PubMed]
23. Kong, H.G.; Song, G.C.; Sim, H.-J.; Ryu, C.-M. Achieving similar root microbiota composition in neighbouring plants through airborne signalling. *ISME J.* **2020**, *15*, 397–408. [CrossRef] [PubMed]
24. Erlacher, A.; Cardinale, M.; Grosch, R.; Grube, M.; Berg, G. The impact of the pathogen *Rhizoctonia solani* and its beneficial counterpart *Bacillus amyloliquefaciens* on the indigenous lettuce microbiome. *Front. Microbiol.* **2014**, *5*, 175. [CrossRef]
25. Ravanbakhsh, M.; Sasidharan, R.; Voesenek, L.; Kowalchuk, G.A.; Jousset, A. ACC deaminase-producing rhizosphere bacteria modulate plant responses to flooding. *J. Ecol.* **2017**, *105*, 979–986. [CrossRef]
26. Wang, C.; Wang, H.; Li, Y.; Li, Q.; Yan, W.; Zhang, Y.; Wu, Z.; Zhou, Q. Plant growth-promoting rhizobacteria isolation from rhizosphere of submerged macrophytes and their growth-promoting effect on *Vallisneria natans* under high sediment organic matter load. *Microb. Biotechnol.* **2021**, *14*, 726–736. [CrossRef]
27. Bai, G.; Liu, Y.; Liu, Z.; Kong, L.; Tang, Y.; Ding, Z.; Zou, Y.; Wang, C.; Zhang, C.; Chen, D.; et al. Effects of Lake Geo-Engineering on Plankton in a Typical Shallow Urban Lake: Evidence Based on 10-year Data. *ACS EST Eng.* **2022**, *3*, 105–120. [CrossRef]
28. Penrose, D.M.; Glick, B.R. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant.* **2003**, *118*, 10–15. [CrossRef]
29. Dworkin, M.; Foster, J.W. Experiments with some microorganisms which utllize ethane and hydrogen. *J. Bacteriol.* **1958**, *75*, 592–603. [CrossRef]
30. Gordon, S.A.; Weber, R.P. Colorimetric estimation of indoleacetic acid. *Plant Physiol.* **1951**, *26*, 192–195. [CrossRef]
31. Schwyn, B.; Neilands, J. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* **1987**, *160*, 47–56. [CrossRef] [PubMed]
32. Loginow, W.; Wisniewski, W.; Gonet, S.S.; Ciescinska, B. Fractionation of organic carbon based on susceptibility to oxidation. *Pol. J. Soil Sci. (Pol.)* **1987**, *20*, 47–52.
33. Zhang, L.-N.; Wang, D.-C.; Hu, Q.; Dai, X.-Q.; Xie, Y.-S.; Li, Q.; Liu, H.-M.; Guo, J.-H. Consortium of Plant Growth-Promoting Rhizobacteria Strains Suppresses Sweet Pepper Disease by Altering the Rhizosphere Microbiota. *Front. Microbiol.* **2019**, *10*, 1668. [CrossRef] [PubMed]
34. Nuzzo, A.; Satpute, A.; Albrecht, U.; Strauss, S.L. Impact of Soil Microbial Amendments on Tomato Rhizosphere Microbiome and Plant Growth in Field Soil. *Microb. Ecol.* **2020**, *80*, 398–409. [CrossRef]
35. Simpson, E.H. Measurement of diversity. *Nature* **1949**, *163*, 668. [CrossRef]
36. Legendre, P.; Desdevises, Y. Independent contrasts and regression through the origin. *J. Theor. Biol.* **2009**, *259*, 727–743. [CrossRef]
37. Zhang, Y.; Zhang, H.; Liu, Q.; Duan, L.; Zhou, Q. Total nitrogen and community turnover determine phosphorus use efficiency of phytoplankton along nutrient gradients in plateau lakes. *J. Environ. Sci.* **2022**, *124*, 699–711. [CrossRef]
38. Moon, Y.S.; Ali, S. Possible mechanisms for the equilibrium of ACC and role of ACC deaminase-producing bacteria. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 877–887. [CrossRef]
39. Moon, Y.-S.; Ali, S. A fruitful decade of bacterial ACC deaminase biotechnology: A pragmatic approach towards abiotic stress relief in plants. *Theor. Exp. Plant Physiol.* **2022**, *34*, 109–129. [CrossRef]
40. Oleńska, E.; Małek, W.; Wójcik, M.; Swiecicka, I.; Thijs, S.; Vangronsveld, J. Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: A methodical review. *Sci. Total Environ.* **2020**, *743*, 140682. [CrossRef]
41. Matsuda, R.; Handayani, M.L.; Sasaki, H.; Takechi, K.; Takano, H.; Takio, S. Production of indoleacetic acid by strains of the epiphytic bacteria *Neptunomonas* spp. isolated from the red alga *Pyropia yezoensis* and the seagrass *Zostera marina*. *Arch. Microbiol.* **2017**, *200*, 255–265. [CrossRef] [PubMed]
42. Hanaka, A.; Ozimek, E.; Majewska, M.; Rysiak, A.; Jaroszuk-Ściseł, J. Physiological Diversity of Spitsbergen Soil Microbial Communities Suggests Their Potential as Plant Growth-Promoting Bacteria. *Int. J. Mol. Sci.* **2019**, *20*, 1207. [CrossRef] [PubMed]
43. Shaharoona, B.; Bibi, R.; Arshad, M.; Zahir, Z.A.; Zia, U.H. 1-Aminocylopropane-1-carboxylate (ACC) deaminase rhizobacteria extenuates ACC-induced classical triple response in etiolated pea seedlings. *Pak. J. Bot.* **2006**, *38*, 1491–1499.
44. Nascimento, F.X.; Rossi, M.J.; Glick, B.R. Ethylene and 1-aminocyclopropane-1-carboxylate (ACC) in plant-bacterial interactions. *Front. Plant Sci.* **2018**, *9*, 114. [CrossRef]
45. Zhang, X.; Zhang, D.; Sun, W.; Wang, T. The Adaptive Mechanism of Plants to Iron Deficiency via Iron Uptake, Transport, and Homeostasis. *Int. J. Mol. Sci.* **2019**, *20*, 2424. [CrossRef]

46. Kramer, J.; Oezkaya, O.; Kuemmerli, R. Bacterial siderophores in community and host interactions. *Nat. Rev. Microbiol.* **2020**, *18*, 152–163. [CrossRef]
47. Moon, Y.-S.; Ali, S. Isolation and identification of multi-trait plant growth–promoting rhizobacteria from coastal sand dune plant species of Pohang beach. *Folia Microbiol.* **2022**, *67*, 523–533. [CrossRef]
48. Chappuis, E.; Lumbreras, A.; Ballesteros, E.; Gacia, E. Deleterious interaction of light impairment and organic matter enrichment on Isoetes lacustris (Lycopodiophyta, Isoetales). *Hydrobiologia* **2015**, *760*, 145–158. [CrossRef]
49. Mitra, S.; Pramanik, K.; Sarkar, A.; Ghosh, P.K.; Soren, T.; Maiti, T.K. Bioaccumulation of cadmium by Enterobacter sp. and enhancement of rice seedling growth under cadmium stress. *Ecotoxicol. Environ. Saf.* **2018**, *156*, 183–196. [CrossRef]
50. Barbé, S.; Figàs-Segura, A.; Benada, M.; Navarro-Herrero, I.; Sampaio, T.M.; Biosca, E.G.; Marco-Noales, E. Plant-associated microbiota as a source of antagonistic bacteria against the phytopathogen *Erwinia amylovora*. *Environ. Microbiol. Rep.* **2022**, *14*, 559–569. [CrossRef]
51. Prakash, O. Lack of kinship with anaerobes is a kind of short-sightedness of agricultural and environmental microbiologists. *Environ. Microbiol. Rep.* **2022**, *14*, 330–332. [CrossRef] [PubMed]
52. Ali, S.; Moon, Y.-S.; Hamayun, M.; Khan, M.A.; Bibi, K.; Lee, I.-J. Pragmatic role of microbial plant biostimulants in abiotic stress relief in crop plants. *J. Plant Interact.* **2022**, *17*, 705–718. [CrossRef]
53. Martínez-Viveros, O.; Jorquera, M.; Crowley, D.; Gajardo, G.; Mora, M. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *J. Soil Sci. Plant Nutr.* **2010**, *10*, 293–319. [CrossRef]
54. Knights, H.E.; Jorrin, B.; Haskett, T.L.; Poole, P.S. Deciphering bacterial mechanisms of root colonization. *Environ. Microbiol. Rep.* **2021**, *13*, 428–444. [CrossRef]
55. Stefan, M.; Munteanu, N.; Stoleru, V.; Mihasan, M.; Hritcu, L. Seed inoculation with plant growth promoting rhizobacteria enhances photosynthesis and yield of runner bean (*Phaseolus coccineus* L.). *Sci. Hortic.* **2013**, *151*, 22–29. [CrossRef]
56. Kavino, M.; Harish, S.; Kumar, N.; Saravanakumar, D.; Samiyappan, R. Effect of chitinolytic PGPR on growth, yield and physiological attributes of banana (*Musa* spp.) under field conditions. *Appl. Soil Ecol.* **2010**, *45*, 71–77. [CrossRef]
57. Chen, L.; Hao, Z.; Li, K.; Sha, Y.; Wang, E.; Sui, X.; Mi, G.; Tian, C.; Chen, W. Effects of growth-promoting rhizobacteria on maize growth and rhizosphere microbial community under conservation tillage in Northeast China. *Microb. Biotechnol.* **2020**, *14*, 535–550. [CrossRef]
58. Tao, C.; Li, R.; Xiong, W.; Shen, Z.; Liu, S.; Wang, B.; Ruan, Y.; Geisen, S.; Shen, Q.; Kowalchuk, G.A. Bioorganic fertilizers stimulate indigenous soil Pseudomonas populations to enhance plant disease suppression. *Microbiome* **2020**, *8*, 137. [CrossRef]
59. Kim, J.K.; Park, K.J.; Cho, K.S.; Nam, S.-W.; Park, T.-J.; Bajpai, R. Aerobic nitrification–denitrification by heterotrophic Bacillus strains. *Bioresour. Technol.* **2005**, *96*, 1897–1906. [CrossRef]
60. Gauri, S.S.; Mandal, S.M.; Pati, B.R. Impact of Azotobacter exopolysaccharides on sustainable agriculture. *Appl. Microbiol. Biotechnol.* **2012**, *95*, 331–338. [CrossRef]
61. Bao, Z.; Okubo, T.; Kubota, K.; Kasahara, Y.; Tsurumaru, H.; Anda, M.; Ikeda, S.; Minamisawa, K. Metaproteomic Identification of Diazotrophic Methanotrophs and Their Localization in Root Tissues of Field-Grown Rice Plants. *Appl. Environ. Microbiol.* **2014**, *80*, 5043–5052. [CrossRef] [PubMed]
62. Rochman, F.F.; Sheremet, A.; Tamas, I.; Saidi-Mehrabad, A.; Kim, J.-J.; Dong, X.; Sensen, C.W.; Gieg, L.M.; Dunfield, P.F. Benzene and Naphthalene Degrading Bacterial Communities in an Oil Sands Tailings Pond. *Front. Microbiol.* **2017**, *8*, 1845. [CrossRef] [PubMed]
63. Smalley, N.E.; Taipale, S.J.; De Marco, P.; Doronina, N.V.; Kyrpides, N.; Shapiro, N.; Woyke, T.; Kalyuzhnaya, M. Functional and genomic diversity of methylotrophic Rhodocyclaceae: Description of *Methyloversatilis discipulorum* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2015**, *65*, 2227–2233. [CrossRef] [PubMed]
64. Yang, H.; Wu, J.; Huang, X.; Zhou, Y.; Zhang, Y.; Liu, M.; Liu, Q.; Ke, S.; He, M.; Fu, H.; et al. ABO genotype alters the gut microbiota by regulating GalNAc levels in pigs. *Nature* **2022**, *606*, 358–367. [CrossRef] [PubMed]
65. Chen, S.F.; Zhou, Y.Q.; Chen, Y.R.; Gu, J. Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, *34*, 884–890. [CrossRef] [PubMed]
66. Magoč, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963. [CrossRef] [PubMed]
67. Stackebrandt, E.; Goebel, B.M. A place for DNA-DNA reassociation and 16S ribosomal-RNA sequence-analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **1994**, *44*, 846–849. [CrossRef]
68. Edgar, R.C. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10*, 996–998. [CrossRef]
69. Wang, J.; Yu, D. Influence of sediment fertility on morphological variability of *Vallisneria spiralis* L. *Aquat. Bot.* **2007**, *87*, 127–133. [CrossRef]