PGPR promotes the recovery of submerged macrophytes via indigenous microbiome modulations under combined abiotic stress

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1. Materials and methods

1.1. 16. S rRNA gene sequencing

The 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) universal primers were used to amplify the 16S rRNA incomplete gene. The PCR product was purified, cloned into the pMD18-T vector, and then transferred to *Escherichia coli* DH5α competent cells through heat shock transformation. Sequencing was done on the positive clones.

1.2. 16. S rRNA gene high throughput sequencing technique

Briefly, the V3–V4 hypervariable regions of bacteria 16S rRNA gene were amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') [1]. PCR was performed using the following program: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. The raw sequenced reads of the 16S rRNA gene were quality-filtered by fastp version 0.20.0 [2] and merged by FLASH version 1.2.7 [3] with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, reads containing ambiguous characters were also discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of overlap region is 0.2. Reads that could not be assembled were discarded; (iii) Samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatch in primer matching. UPARSE (version 7.1 http://drive5.com/uparse/) was used to cluster operational taxonomic units (OTUs) with 97% similarity [4,5]. The resampled OTU table was used for subsequent community analysis. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 [6] against the 16S rRNA database (Silva v138) using confidence threshold of 0.7. All of the OTUs belonged to bacteria.

Figure S1. A schematic drawing in one environmental conditions.

Table S1. Environmental characteristics of the living position of submerged macrophytes and isolated PGPR strain numbers.

Location	Sediment OM (%)	Light intensity (%)	Strain source	Number of cultivated isolates	Code name
Tanglinghu (Donghu Lake,Wuhan City)	3.12 ± 0.16	100	*Najas marina*	15	A1-15
			Hydrilla verticillata	12	B1-12
Shahu Lake, Yinchuan City	2.06 ± 0.06	100	*Chara vulgaris*	25	C1-25
Maojiabu (West Lake, Hangzhou City)	15.24 ± 0.03	1	*Vallisneria natans*	30	D1-D30
	15.94 ± 0.10	4	*Vallisneria natans*	15	E1-E15
	11.51 ± 0	3	*Vallisneria natans*	30	F1-F30
		3	*Hydrilla verticillata*	9	G1-9
	12.36 ± 0.16	100	*Vallisneria natans*	31	H1-H31
		100	*Hydrilla verticillata*	22	I1-22
		56	*Potamogeton maackianus*	20	J1-20
		56	*Potamogeton wrightii*	15	K1-15
Xilihu (West Lake, Hangzhou City)	17.76 ± 0.02	34	*Vallisneria natans*	19	L1-L19
Microcosm system	17.35 ± 0.52	100	*Vallisneria natans*	9	M1-9

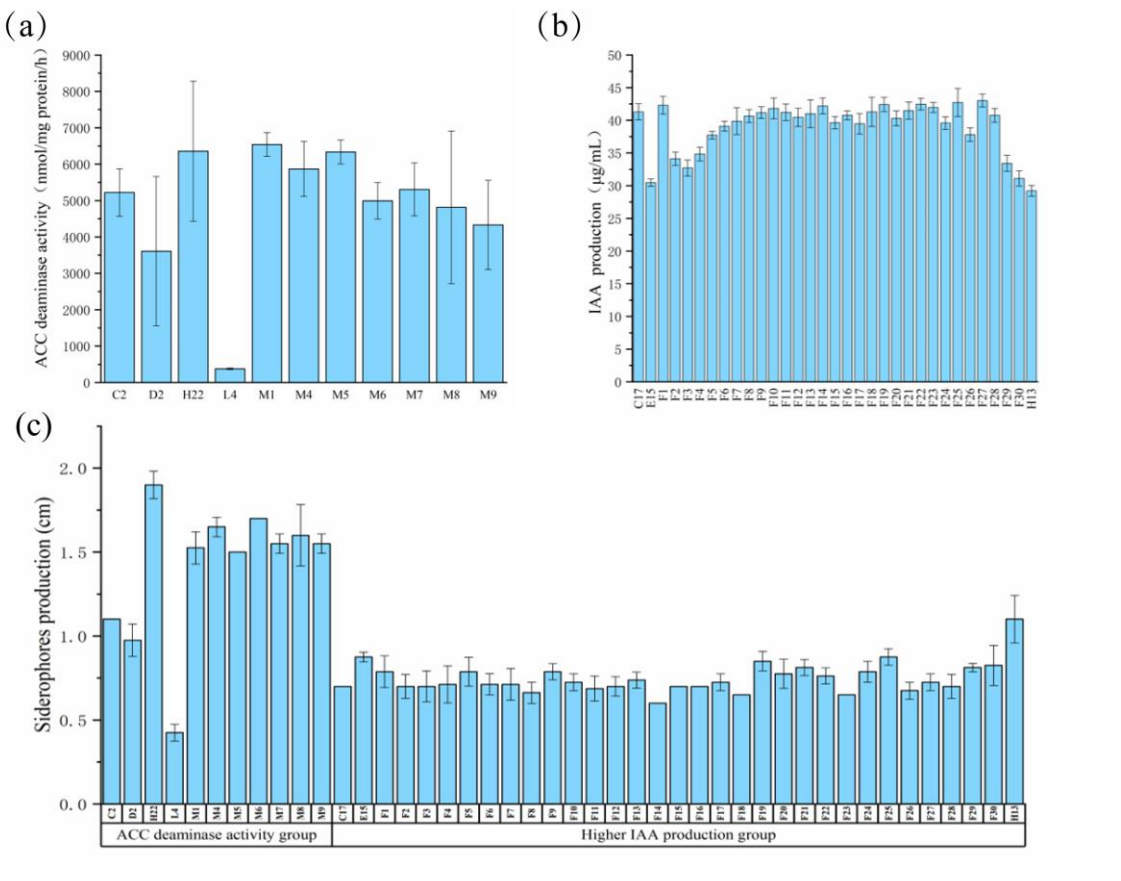

Figure S2. The growth-promoting properties of the isolated strains and correlation analysis; (a) ACC deaminase activity; (b) IAA production (> 29 μg mL^{-1}); (c) Siderophore production.

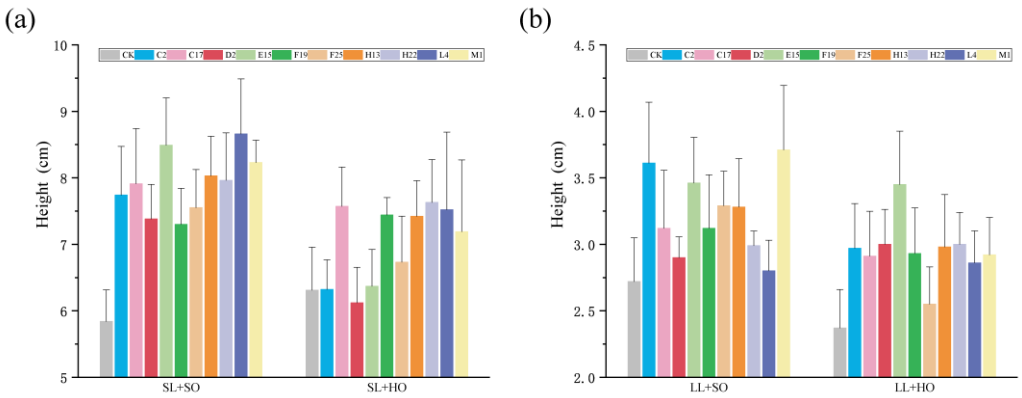

Figure S3. Shoot height of *V. natans* in different PGPR inoculation treatments under four stress conditions; (a) Suitable light intensity and suitable sediment organic matter load (SL+SO) and suitable light intensity and high sediment organic matter load (SL+HO); (b) Low light intensity and suitable 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.

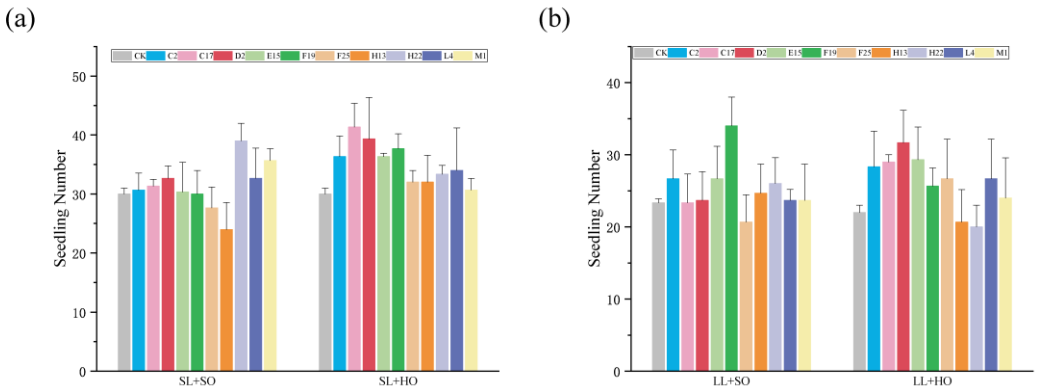

Figure S4. Seedling numbers of *V. natans* in different PGPR inoculation treatments under four environments; (a) Suitable light intensity and suitable sediment organic matter load (SL+SO) and suitable light intensity and high sediment organic matter load (SL+HO); (b) Low light intensity and suitable 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.

Table S2 The highest growth promoting effect and their significant differences under the four environments ($p < 0.05$, Univariate ANOVA).

Stress Conditions	The Optimal Strain	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Subset	
					Lower Bound	Upper Bound	1	2
SL+SO	L4	10	1.483	0.142	1.382	1.584	a	
SL+HO	H22	10	1.209	0.102	1.133	1.267		b
LL+SO	M1	10	1.364	0.178	1.237	1.491	a	
LL+HO	E15	10	1.456	0.169	1.335	1.577	a	
Sig.							1	0.1

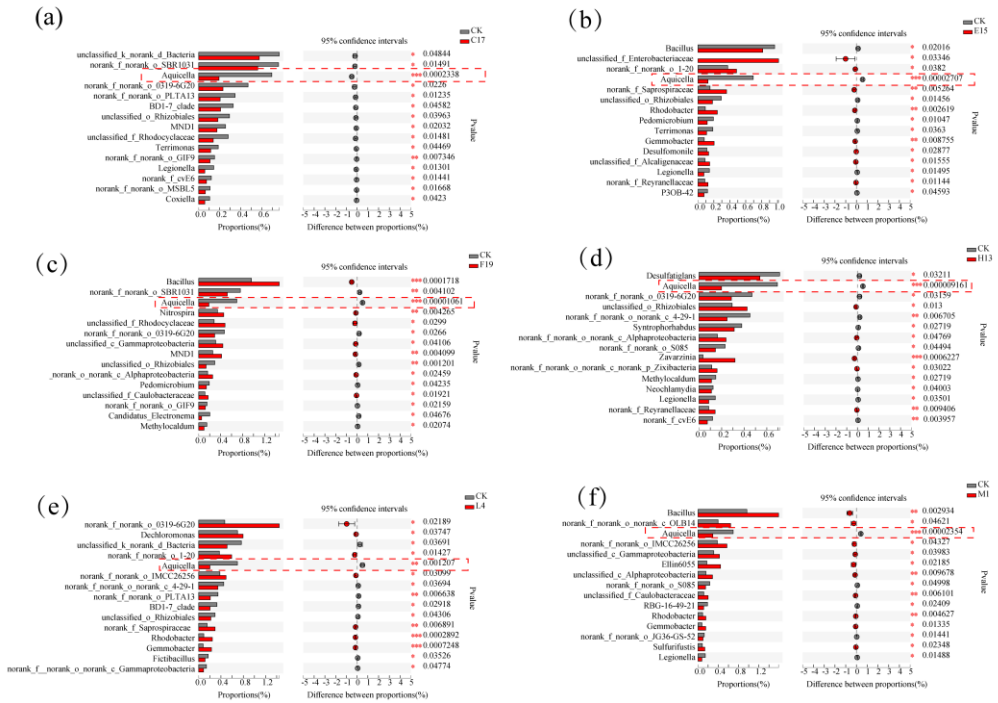

Figure S5. Relative abundance of the 15 top-ranked genera of bacteria with significant differences between inoculated group and uninoculated control group in the rhizosphere of *V. natans* seedlings under SL+SO. CK: uninoculated control; C17, E15, F19, H13, L4 and M1 present the inoculated group with the corresponding strains. Asterisk indicates significant difference between CK and the inoculated group ($p < 0.05$, Student's t test).

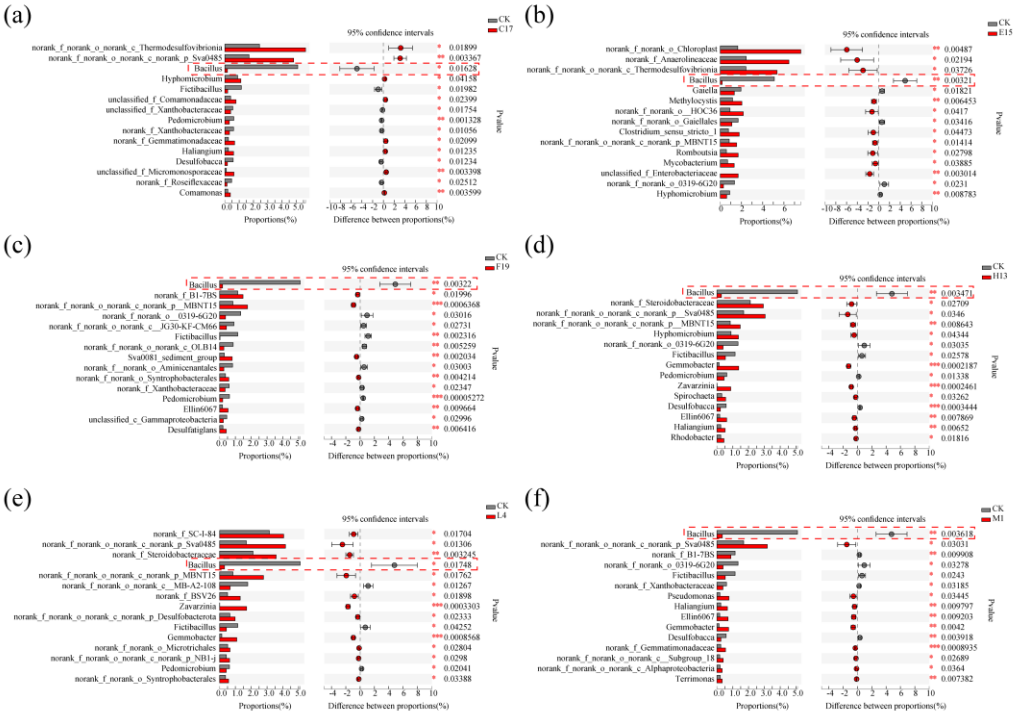

Figure S6. Relative abundance of the 15 top-ranked genera of bacteria with significant differences between inoculated group and uninoculated control group at genus level in the rhizosphere of *V. natans* seedlings under SL+HO. CK: uninoculated control; C17, E15, F19, H13, L4 and M1 present the inoculated group with the corresponding strains. Asterisk indicates significant difference between CK and the inoculated group ($p < 0.05$, Student's t test).

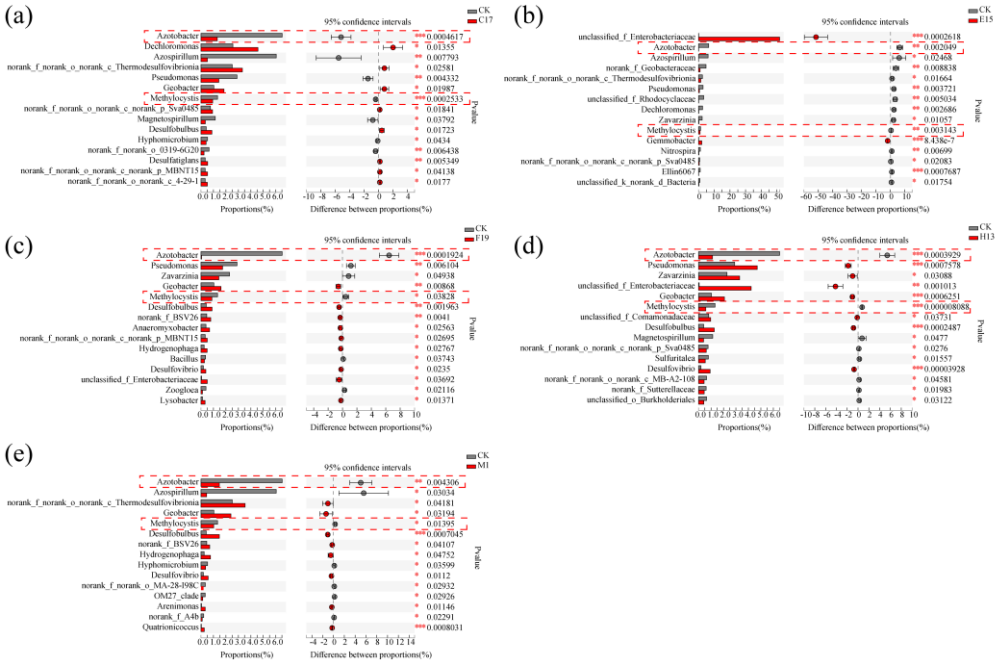

Figure S7. Relative abundance of the 15 top-ranked genera of bacteria with significant differences between inoculated group and uninoculated control group at genus level in the rhizosphere of *V. natans* seedlings under LL+SO. CK: uninoculated control; C17, E15, F19, H13 and M1 present the inoculated group with the corresponding strains. Asterisk indicates significant difference between CK and the inoculated group ($p < 0.05$, Student's t test).

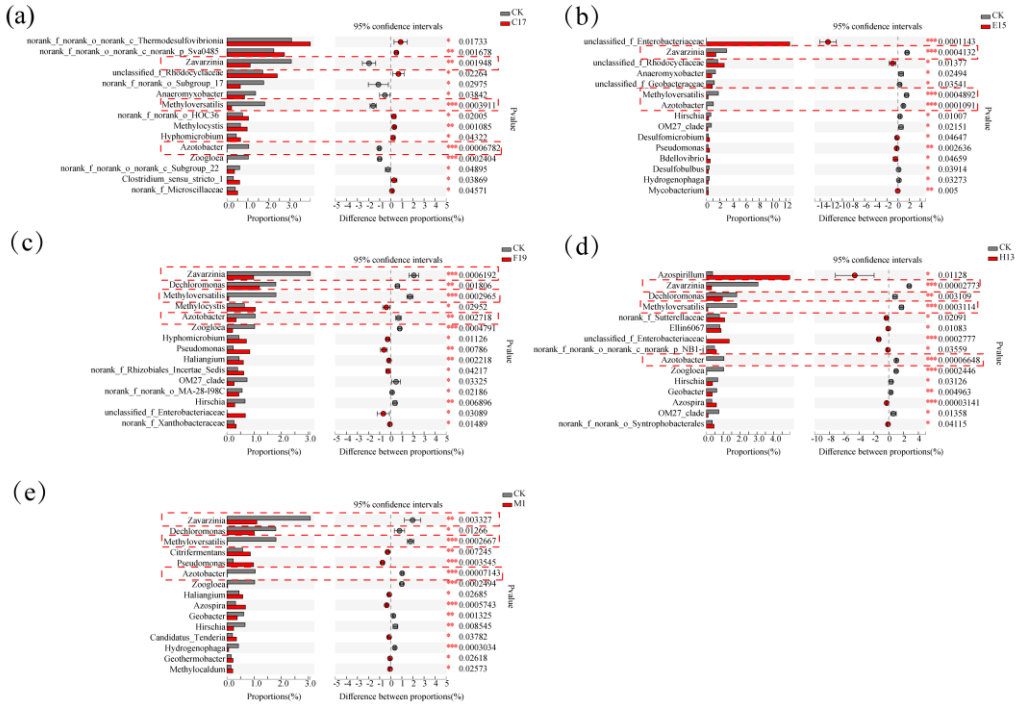

Figure S8. Relative abundance of the 15 top-ranked genera of bacteria with significant differences between inoculated group and uninoculated control group at genus level in the rhizosphere of *V. natans* seedlings under LL+HO. CK: uninoculated control; C17, E15, F19, H13 and M1 present the inoculated group with the corresponding strains. Asterisk indicates significant difference between CK and the inoculated group ($p < 0.05$, Student's t test).

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