Supplementary Materials

Effects of enrofloxacin on nutrient removal by a floating treatment wetland planted with *Iris Pseudacorus*: response and resilience of rhizosphere microbial communities

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Content

References

SI 2.2.2.1 Determination of chlorophyll content

Briefly, with 1 gram of fresh leaves were cut into pieces and ground with a mortar and pestle. The ground samples were incubated in the 80% acetone for 24 hours at 4°C and then centrifuged at 2500 rpm for 5 minutes. The supernatant was transfer to a 100 ml flask with 80% acetone. The absorbance of this solution was measured spectrophotometrically at a wavelength of 645 nm and 663 nm in triplicate, with 80% acetone used as blank. The chlorophyll a, b and total chlorophyll were calculated using the following formulas.

$$mg\ chlorophyll\ a\ per\ gram\ tissue = \frac{12.7(A663) - 2.69\ (A645)\ \times V}{1000\ \times W}$$

$$mg\ chlorophyll\ b\ per\ gram\ tissue = \frac{22.9\ (A645) - 4.68\ (A663)\ \times V}{1000\ \times W}$$

$$mg\ Total\ chlorophyll\ per\ gram\ tissue = \frac{20.2\ (A645) - 8.02\ (A663)\ \times V}{1000\ \times W}$$

Where A is the absorbance at specific wavelength, V is the final volume of chlorophyll extract in 80% acetone, and W is the fresh weight of tissues extracted.

SI 2.2.3.1 Microbial analysis.

A fast DNA Spin Kit for soil (MP, USA) was used to extract the bacterial DNA according to the manufacturer's protocol. Subsequently, polymerase chain reaction (PCR) amplification of the total bacterial 16S rRNA was conducted using the primers 338F-806R (Cao et al., 2016), as the PCR reaction conditions and primer sequences were presented in Table S1. After the Purified amplicons were pooled in equimolar, the PCR products were processed with Illumina Miseq PE300 platform (Illumina, San Diego, USA) for pair-end sequencing (2×250) in a Bio-Pharm Technology Company (Majorbio, Shanghai, China), while the operational taxonomic units (OTU) were formed by clustering of high-quality sequences at 97% Sequence similarity using UPARSE software (version 7.1) (Sun et al., 2019).

Table S1. PCR primer sequences and reaction conditions

Primer sequences:	Primer 338F (5′-ACTCCTACGGGAGGCA GCAG-3′)
	Primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′)
Reaction conditions:	Denaturation at 95 °C for 3 min
	Denaturation 27 cycles of 95 °C for 30 seconds
	Annealing at 55 °C for 30 seconds
	Annealing at 72 °C at 45 seconds
	Final step for 10 min at 72 °C and 10 °C until the user halted the process
A mixture with a final volume of 20 μL was used to conduct the polymerase chain reaction (PCR)	

Table S2. Total content and concentration of N and P in plant tissue

	N content in plants [mg]		N concentration in plant [mg/g]	
	Tank 1	**Tank 2**	**Tank 1**	**Tank 2**
Shoots	1718.85	1329.55	21.74	24.08
Roots	5536.09	2739.05	46.05	34.07
Total	**7254.94**	**4068.59**	**67.79**	**58.15**
	P content in plants [mg]		P concentration in plant [mg/g]	
	Tank 1	**Tank 2**	**Tank 1**	**Tank 2**
Shoots	219.23	142.80	2.77	2.59
Roots	812.76	278.41	6.76	3.46
Total	**1031.99**	**421.21**	**9.53**	**6.05**

Table S3. Average dissolved oxygen levels during the experimental period

Weeks	Dissolved Oxygen [mg/l]	
	Tank 1	Tank 2
1	4.09	3.09
2	3.95	3.03
3	6.05	4.70
4	5.39	5.79
5	4.93	5.61
6	6.02	6.68

Table S4. Richness and diversity estimation of total bacterial 16S rRNA gene in Rhizosphere from pyrosequencing analysis

			Richness estimators		Diversity estimators	
Sample	OTU	Coverage	Chao1	Ace	Shannon	Simpson
T1I	613.67±5.36[a]	0.99±0.00033	739.49±8.09[a]	763.57±6.37[a]	3.99±0.047[a]	0.06±0.0055[a]
T1F	489.00±7.77[b]	1.00±0.0012	641.58±31.04[a]	669.52±61.33[a]	2.96±0.24[b]	0.18±0.0348[a, b]
T2I	453.33±27.81[b]	0.99±0.00033	613.66±36.49[a]	612.56±33.69[a]	2.80±0.27[b]	0.24±0.060[b]
T2F	471.67±19.81[b]	1.00±0.00067	633.75±33.62[a]	616.77±20.76[a]	3.54±0.076[a, b]	0.08±0.011[a, b]

Note: different letters (a, b) indicate significant differences between the different values of each indicator.

(T1I: Tank 1 Initial; T1F: Tank 1 Final; T2I: Tank 2 Initial; T2F: Tank 2 Final.)

Table S5. Relative abundance (%) of genera related to nitrogen removal

Genera	Tank 1 initial	Tank 1 Final	Tank 2 Initial	Tank 2 Final
Genera related to nitrification process				
Nitrospira	0.0081 ±0.00076	0.0072 ±0.0044	0	0.0044 ±0.0032
Ellin6067	0	0.0025 ±0.00057	0.0012 ±0.0012	0
Sum of Genera related to nitrification process				
	0.0081 ±0.00076	0.0096 ±0.0049	0.0012 ±0.012	0.0044 ±0.0032
Genera related to denitrification process				
Acidovorax	10.81 ±0.75	2.75 ±0.38	3.54 ±0.29	3.36 ±0.26
Acinetobacter	0.0083 ±0.0048	0	0	0
Aeromonas	0.038 ±0.0061	1.03 ±0.31	0.083 ±0.0088	0.30 ±0.12
Azospirillum	0.083 ±0.083	0.0042 ±0.0022	0.0074 ±0.0021	0.0034 ±0.00043
Bacillus	0.23 ±0.049	0.010 ±0.0023	0.21 ±0.036	0.014 ±0.0031
Dechloromonas	0.011 ±0.0091	0	0.0062 ±0.0045	0.0012 ±0.0012
Desulfovibrio	0.0012 ±0.0012	0.0025 ±0.0013	0.0085 ±0.0053	0
Flavobacterium	0.58 ±0.19	0.67 ±0.090	1.50 ±0.50	0.59 ±0.036
Hydrogenophaga	2.46 ±0.12	0.21 ±0.19	1.06 ±0.17	0.90 ±0.074
Hyphomicrobium	0.75 ±0.080	0.080 ±0.012	0.28 ±0.069	0.030 ±0.0048
Pseudomonas	0.016 ±0.012	4.98 ±3.40	0.10 ±0.0090	22.86 ±3.80
Rhodobacter	3.98 ±0.32	0.16 ±0.15	1.44 ±0.29	0.66 ±0.069
Rhodoplanes	0.0060 ±0.0026	0	0.0049 ±0.0033	0.0017 ±0.0017
Sulfuritalea	0.0011 ±0.0011	0	0.0012 ±0.0012	0
Vogesella	0.0011 ±0.0011	0	0.0049 ±0.0013	0
Xanthomonas	0	0	0.011 ±0.011	0
Sum of Genera related to denitrification process				
	18.99 ±1.63	9.89 ±4.54	8.25 ±1.41	28.73 ±4.37

Table S6. Relative abundance (%) of genera related to Biological Phosphorus removal

Genera	Tank 1 Initial	Tank 1 Final	Tank 2 Initial	Tank 2 Final
Acinetobacter	0.0083 ±0.0048	0	0	0
Candidatus Accumulibacter	0.0036 ±0.0036	0.013 ±0.0080	0.0086 ±0.0044	0.0037 ±0.0021
Dechloromonas	0.011 ±0.0091	0	0.0062 ±0.0045	0.0012 ±0.0012
Microlunatus	0.0021 ±0.0021	0.0079 ±0.0051	0.0025 ±0.0025	0.014 ±0.0046
Pseudomonas	0.016 ±0.012	4.98 ±3.40	0.10 ±0.0090	22.86 ±3.80

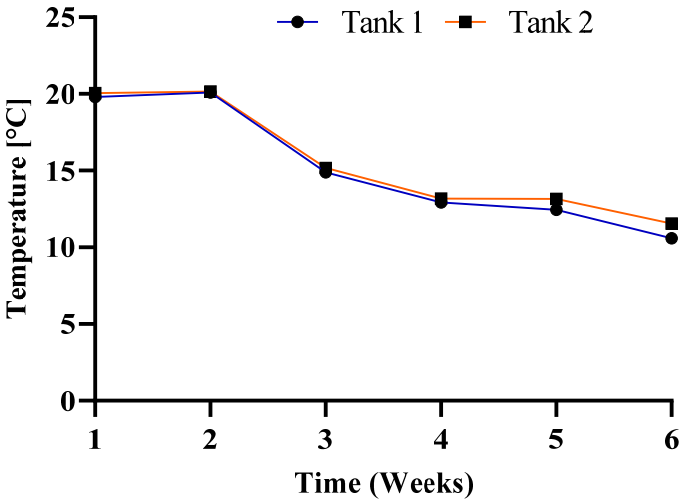


Figure S1. Average temperature per week during the entire experiment.

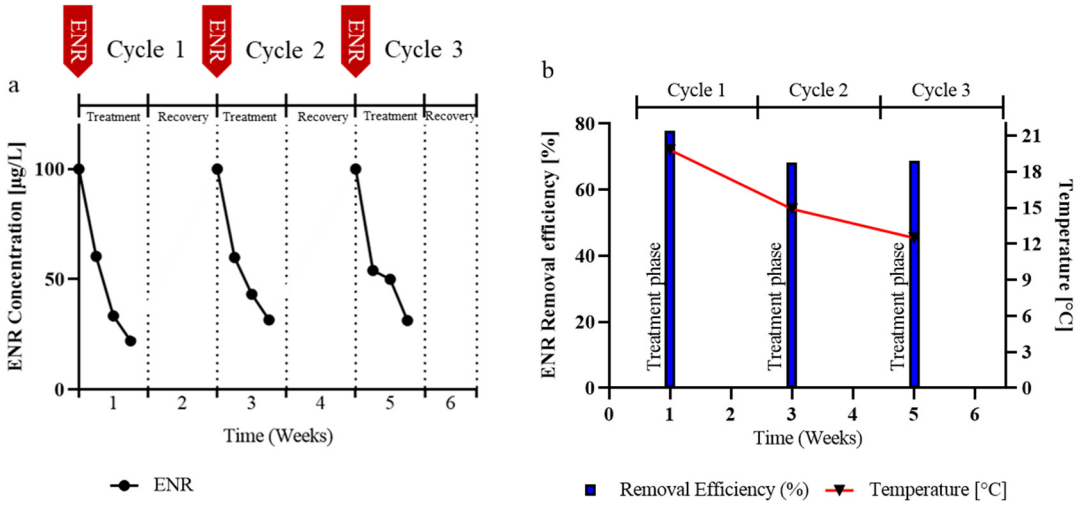
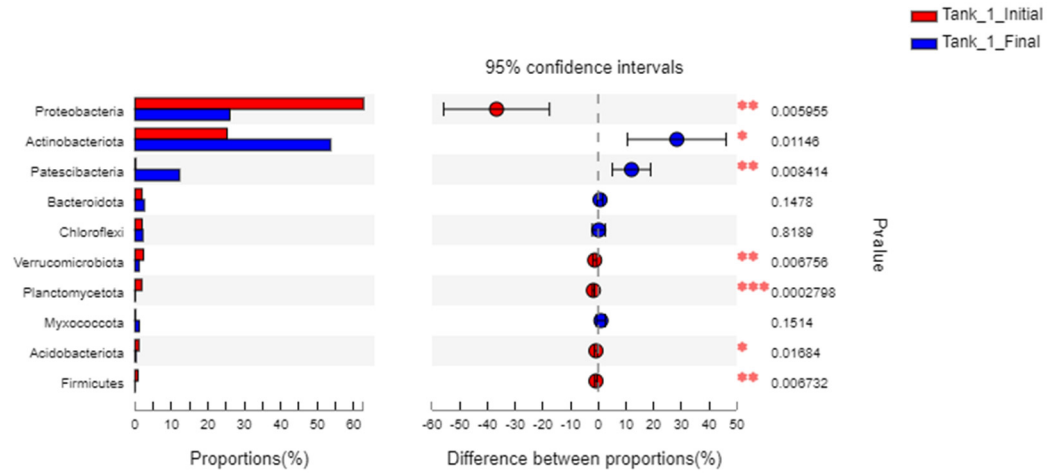
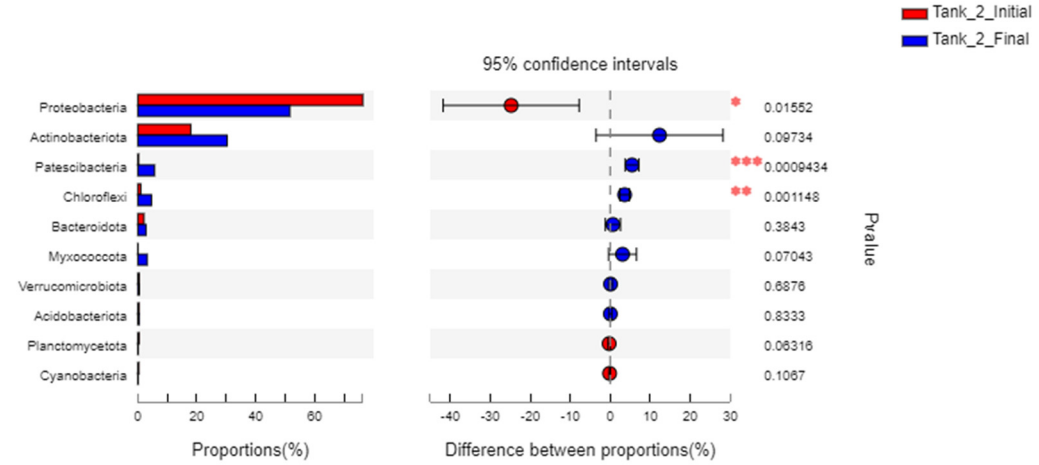


Figure S2. Changes of ENR removal during the different cycles (a) and the relation between ENR removal efficiency and temperature (b)

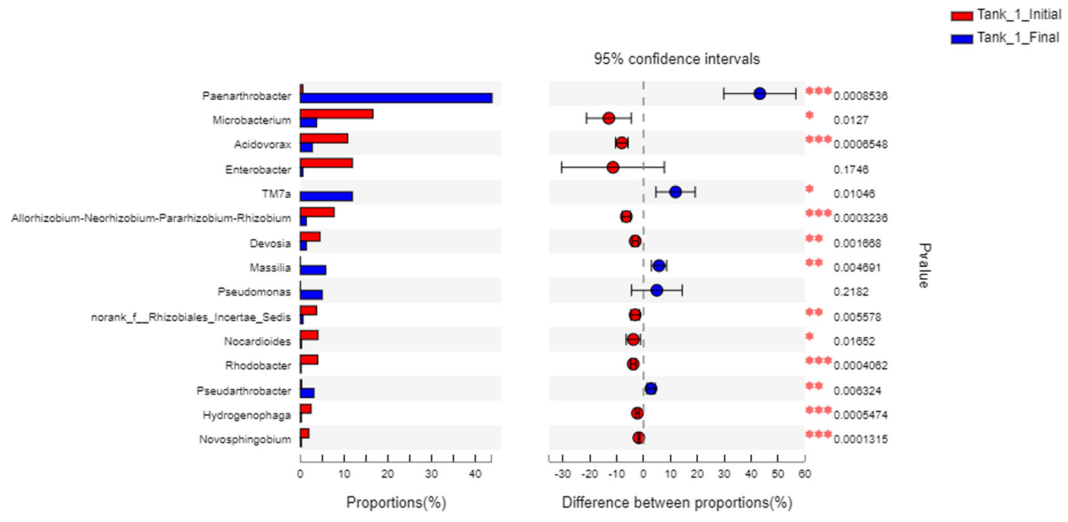
a


Tank_1_Initial
Tank_1_Final
95% confidence intervals
Proteobacteria
Actinobacteriota
Patescibacteria
Bacteroidota
Chloroflexi
Verrucomicrobiota
Planctomycetota
Myxococcota
Acidobacteriota
Firmicutes
0.005955
0.01146
0.008414
0.1478
0.8189
0.006756
0.0002798
0.1514
0.01684
0.006732
Pvalue
Proportions(%)
Difference between proportions(%)

b


Tank_2_Initial
Tank_2_Final
95% confidence intervals
Proteobacteria
Actinobacteriota
Patescibacteria
Chloroflexi
Bacteroidota
Myxococcota
Verrucomicrobiota
Acidobacteriota
Planctomycetota
Cyanobacteria
0.01552
0.09734
0.0009434
0.001148
0.3843
0.07043
0.6876
0.8333
0.06316
0.1067
Pvalue
Proportions(%)
Difference between proportions(%)

c


Tank_1_Initial
Tank_1_Final
95% confidence intervals
Paenarthrobacter
Microbacterium
Acidovorax
Enterobacter
TM7a
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
Devosia
Massilia
Pseudomonas
norank_f__Rhizobiales_Incertae_Sedis
Nocardioides
Rhodobacter
Pseudarthrobacter
Hydrogenophaga
Novosphingobium
0.0008536
0.0127
0.0006548
0.1746
0.01046
0.0003236
0.001668
0.004691
0.2182
0.005578
0.01652
0.0004062
0.006324
0.0005474
0.0001315
Pvalue
Proportions(%)
Difference between proportions(%)

d

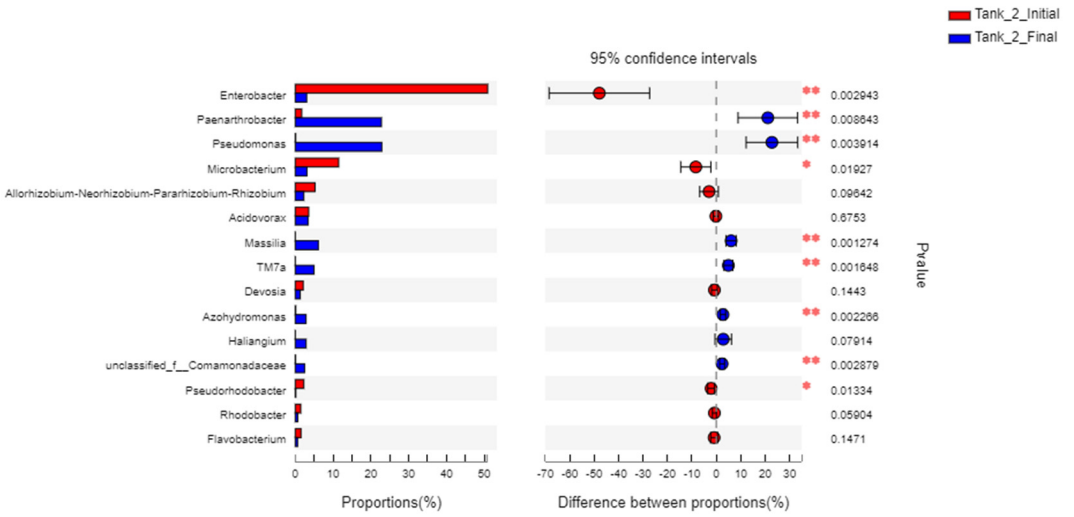


Figure S3. Student's t-test bar plot on phylum level (a, b) and genus level (c, d) for the initial and final sample of tanks 1 and 2. *: p<0.05; **: p<0.01; ***:p<0.001.

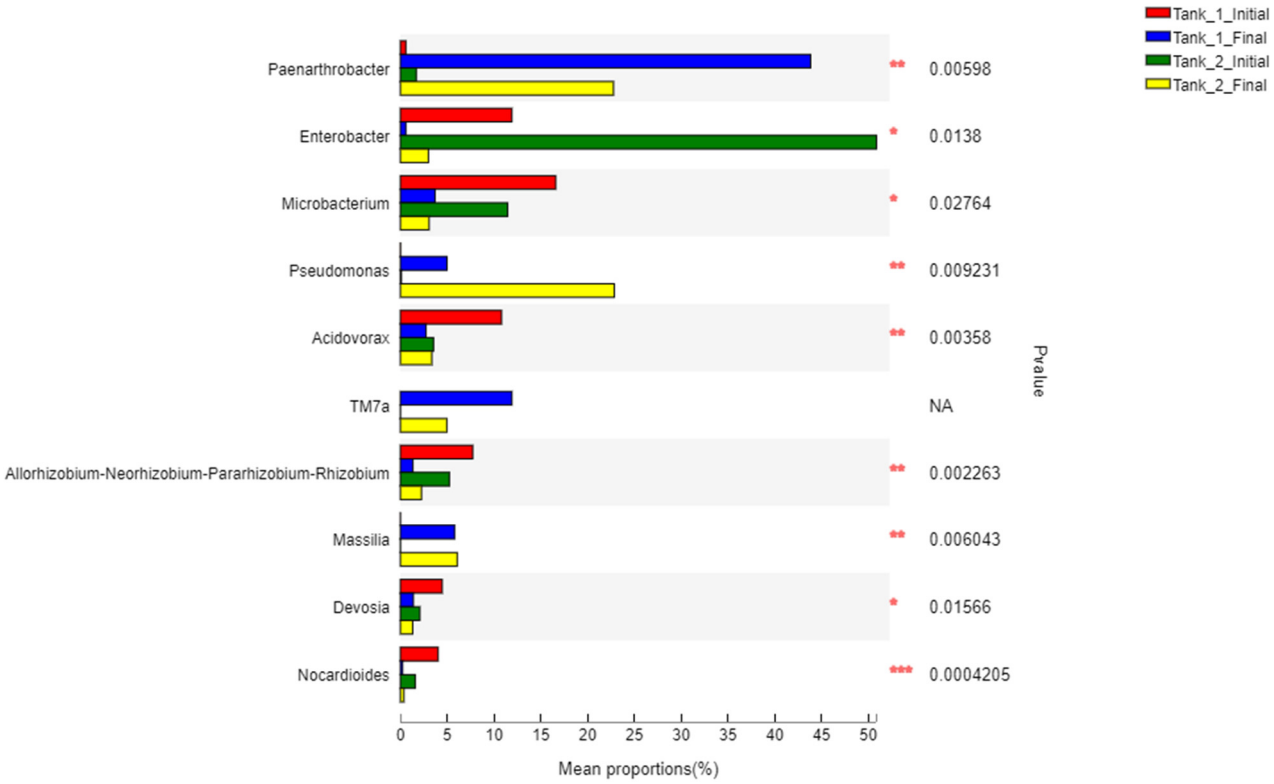


Figure S4. One-way ANOVA bar plot for the initial and final samples of both tanks.

***: p<0.05; **: p<0.01; ***:p <0.001.**

References

Cao, S., Du, R., Li, B., Ren, N., Pen, yongzhen, 2016. High-throughput profiling of microbial community structures in an ANAMMOX-UASB reactor treating high-strength wastewater. Appl Microbiol Biotechnol 11.

Sun, S., Liu, J., Zhang, M., He, S., 2019. Simultaneous improving nitrogen removal and decreasing greenhouse gas emission with biofilm carriers addition in ecological floating bed. Bioresource Technology 292, 121944. https://doi.org/10.1016/j.biortech.2019.121944