



Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: [www.elsevier.com/locate/jhazmat](http://www.elsevier.com/locate/jhazmat)

Research Paper

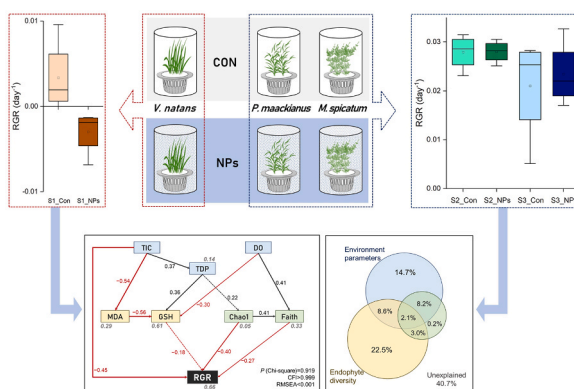
# Response strategies of stem/leaves endophyte communities to nano-plastics regulate growth performance of submerged macrophytes

Beibei Hao<sup>a</sup>, Haoping Wu<sup>b,\*</sup>, Siyi Zhang<sup>a</sup>, Bin He<sup>a,\*</sup><sup>a</sup> National-Regional Joint Engineering Research Center for Soil Pollution Control and Remediation in South China, Guangdong Key Laboratory of Integrated Agro-environmental Pollution Control and Management, Institute of Eco-environmental and Soil Sciences, Guangdong Academy of Sciences, Guangzhou 510650, China<sup>b</sup> Guangdong Key Laboratory of Ornamental Plant Germplasm Innovation and Utilization, Environmental Horticulture Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China

## HIGHLIGHTS

- Unravel NPs effects on plant growth, physiology and stem/leaves endophyte.
- NPs reduced the RGR of *V. natans* but not *P. maackianus* and *M. spicatum*.
- Endophyte and environment contribute more to plant performance than enzyme.
- Species-specific response strategies of macrophyte-endophyte to nano-plastic.
- Different phytoremediation potential of macrophytes to nano-plastic pollution.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

Editor: S.B. Miglioranza

## Keywords:

Submerged Macrophytes  
Endophytic bacteria  
Nano-plastics exposure  
Antioxidative stress

## ABSTRACT

Research on the toxicity effects of nano-plastics on submerged macrophytes has been increasing over the past several years. However, how the endophytic bacteria of submerged macrophytes respond to nano-plastics remains unknown, although they have been widely shown to help terrestrial plants cope with various environmental stressors. Here, a microcosm experiment was performed to unravel the effects of high concentration of nano-plastics (20 mg/L) on three submerged macrophyte (*Vallisneria natans*, *Potamogeton maackianus*, *Myriophyllum spicatum*) and their endophytic bacterial communities. Results indicated that nano-plastics induced antioxidative stress in plants, but significantly reduction in relative growth rate (RGR) only occurred in *V. natans* (from 0.0034 to  $-0.0029 \text{ day}^{-1}$ ), accompanied by change in the stem/leaves endophyte community composition. Further analysis suggested nano-plastics caused a reduction in environmental nutrient availability and the proportion of positive interactions between endophyte communities (43%), resulting in the lowest RGR of *V. natans*. In contrast, endophytes may help *P. maackianus* and *M. spicatum* cope with nano-plastic stress by increasing the proportion of positive correlations among communities (70% and 75%), leaving their RGR unaffected. Collectively, our study elucidates the species-specific response strategies of submerged macrophyte-

\* Corresponding authors.

E-mail addresses: [wuhaoping@gdaas.cn](mailto:wuhaoping@gdaas.cn) (H. Wu), [bhe@soil.gd.cn](mailto:bhe@soil.gd.cn) (B. He).<https://doi.org/10.1016/j.jhazmat.2023.132883>

Received 23 July 2023; Received in revised form 25 October 2023; Accepted 26 October 2023

Available online 3 November 2023

0304-3894/© 2023 Elsevier B.V. All rights reserved.

endophyte to nano-plastics, which helps to reveal the different phytoremediation potential of submerged macrophytes against nano-plastic pollution.

## 1. Introduction

Endophytic bacteria are endosymbiotic microorganisms that colonize the inter cellular and/or intracellular locations of plants [38,39]. They are ubiquitously present in the stem, roots, petioles, leaf segments, inflorescences of weeds, fruit, buds, and seeds of plants [16]. Endophytic bacterial community in plant are highly variable, depending on multiple components, such as host species, host developmental stage, inoculum density and environmental condition [16]. Numerous studies have been conducted on the interaction between terrestrial plants and endophytes, showing that endophytic bacterial generally benefit host plant growth through nutrient acquisition (including nitrogen fixation and phosphate solubilization), phytohormone and siderophore production, protection against abiotic stresses (such as salinity, drought or pollution), or phytopathogen control [38]. In wetland ecosystem, Singh et al. [43] summarizes the activity and ability of wetland plant endophytic bacteria of to enhance phytoremediation of organic contaminants in water. Several published studies have shown that the success of phytoremediation in constructed wetlands depends on the tolerance of plants to high pollutant loads and the potential of their associated endophytic bacteria to degrade these pollutants [22,43,46]. However, most of these studies focus on the synergistic relationship between emergent plants and endophyte bacteria in constructed wetland, and little is known about the interaction mechanisms between submerged macrophytes and endophytic bacterial communities in freshwater ecosystems. Especially in heavily polluted freshwater ecosystems, whether endophytes can benefit the growth of submerged macrophytes by improving their tolerance to various contaminants is still unclear.

Submerged macrophytes, as one of the main primary producers of freshwater ecosystems, play important roles in ecological services and biochemical cycles, such as nutrient cycling, water quality improvement, sediment stabilization, etc. Jeppesen et al., [23,56]. Various biotic and abiotic factors that are detrimental to the growth performance of submerged macrophytes in natural habitats may eventually pose potential hazards to their ecosystem functions [57,62]. As a worldwide environmental issue, micro(nano)plastics pollution has become an emergent contaminant in freshwater environments and has attracted widespread attention [34]. Given the importance submerged macrophytes in freshwater ecosystems, research on the environmental toxicity of micro(nano)plastics to submerged macrophytes has been increasing over the past several years [20,31,50]. These published studies have extensively elucidated submerged macrophytes growth and metabolism [49,56], leaf biofilms and phyllosphere microorganisms [17,20], and purification function [31] in response to high exposure concentrations of micro(nano)plastics. However, studies on how high concentrations of nano-plastics affect stem/leaves endophyte communities of submerged macrophytes and their relationship with host plants are still lacking.

In this study, a microcosm experiment was performed to unravel the effects of nano-plastics on growth performances and stem/leaves endophytic bacteria of three submerged macrophytes (*Vallisneria natans*, *Potamogeton maackianus*, *Myriophyllum spicatum*). Each plant species was crossed with two nano-plastics scenarios and cultivated separately under control and high concentration nano-plastic exposure conditions. After one month of cultivation, the relative growth rate, antioxidant indicators and stem/leaves endophyte community structure of submerged macrophytes in each treatment were determined. Considering the species-specific characteristics of submerged macrophytes, we hypothesized that the effects of nano-plastics on plant growth performance and physiology may differ among these three species. Due to the widely reported protective effect of endophytes on host plants, we further hypothesized that stem/leaves endophytes may reduce the negative impact

of nano-plastics on plants by altering the bacterial community structure.

## 2. Materials and methods

### 2.1. Plant material and cultivation conditions

Three submerged macrophytes, *V. natans*, *P. maackianus*, and *M. spicatum*, were collected from the same habitat conditions in a shallow lake and cultivated in aquarium for 1 month before being used in experiments. Plants shoot tips/seedlings of similar size were selected and rinsed thoroughly with deionized water and planted in a transparent vertical glass cylinder (height 25 cm; diameter 12 cm) filled with lake water (TN 1.270 mg/L, TP 0.055 mg/L). No sediment was provided during the experiment, and submerged macrophytes can only absorb and utilize nutrients from the aquatic environment. Two plant shoot tips/seedlings were fixed at the bottom of each glass cylinder with a planting basket equipped with a holder. After 5 days of pre-cultivation, the nano-plastic stock solution was added when the plants adapted to environment, and the experiment began. During the experiment, the roots of submerged macrophytes were surrounded by holders and were not in direct contact with the nano-plastic in the water column.

Three submerged macrophytes was crossed with two nano-plastic scenarios, and a total of six treatments were included in our experiment (S1\_Con, S1\_NPs, S2\_Con, S2\_NPs, S3\_Con, S3\_NPs). S1, S2, and S3 represent three submerged macrophytes *V. natans*, *P. maackianus*, and *M. spicatum*, while Con and NPs represent the cultivation conditions without and with nano-plastics addition, respectively. Polystyrene latex beads (100–500 nm) without chemical reactivity and functional groups were selected as representative of nano-plastics, purchased from Aladdin (CAS number: 9003–53–6). The morphology and size distribution of polystyrene particles were observed with a scanning electron microscope (SEM, Hitachi TM-1000, Japan) (Fig. S1). Referring to the published toxicological studies of nano-plastics on submerged macrophytes [17,20,31], nano-plastics at a concentration of 20 mg/L have negative effects on submerged macrophytes and/or their phyllosphere bacteria. Therefore, the concentration of nano-plastics was set to 20 mg/L to reveal the effect of high-concentrations nano-plastics stress on submerged macrophytes and their stem/leaves endophytic bacteria.

### 2.2. Sample collection

The simulation experiment was conducted from 17 May to 24 June 2022. When the experiment was terminated, the content of dissolved oxygen (DO) was measured in situ in all glass cylinders before collecting plant samples. At the same time, water samples were collected and brought back to the laboratory to determine environmental parameters such as total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), total organic carbon (TOC), total inorganic carbon (TIC), and the content of nano-plastics (NPs). Thereafter, plant materials in each treatment were collected. A section of plant material was selected and fixed with glutaraldehyde. Following the method of Zhang et al. [60], the accumulation of nano-plastics on the leaf surface of each plant were observed using SEM. In the laboratory, the plant materials were washed twice to remove microorganisms attached to the plant surface. Firstly, the plant material was rinsed with deionized water and then transferred to an Erlenmeyer flask containing 200 mL of sterile PBS (phosphate-buffered saline, pH 7.0). Secondly, place the Erlenmeyer flask in an ultrasonic cleaner (400 W), sonicate for 1 min, and then shake for 30 s. Repeat this step 5 times to remove microorganisms attached to the plant surface. Next, the number of ramet/leaf/node and the biomass (fresh weight) of each species were measured. Afterwards, the shoots and roots

of plants were separated using ethanol-sterilized scissors. The derooted plant materials were used to endophyte community analysis, antioxidant indicators measurement, and observation of nano-plastic internalization in plant (TEM, JEOL JEM-1230).

### 2.3. Plant RGR and antioxidant stress

According to the method of Hoffmann and Poorter [19], the relative growth rate (RGR) of the three submerged macrophytes were calculated as follows, where  $W_2$  and  $W_1$  are plant biomass (fresh weight) at times  $t_2$  and  $t_1$ , respectively.

$$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

The apical part plant material was used for antioxidant indicators measurement to assess plant antioxidative stress response to nano-plastic. The content superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione (GSH) of each submerged macrophytes were measured in the laboratory according to the manufacturer's instructions. The assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

### 2.4. Endophyte identification and bioinformatic analysis

The middle part plant material was surface sterilized with 2% (w/v) NaClO solution for 5 mins, followed by 70% ethanol for 1 min [52]. After rinsing with sterile deionized water, plant material was ground under liquid nitrogen and stored at  $-80^\circ\text{C}$  for DNA extraction [45]. The DNA of endophytic bacterial was extracted from 0.5 g stem/leaves powder sample using Cetyltrimethyl ammonium bromide (CTAB) method, and the quality of DNA samples were monitored by agarose gels. The V3-V4 region of 16 S rRNA gene was amplified using specific primer 341 F (5' -CCTAYGGGRBGCASCAG-3') and 806 R (5'-GGACTACNNGGTATCTAAT-3') [4]. Illumina NovaSeq (USA) was used for paired-end sequencing ( $2 \times 250$  bp) with the purified amplicons pooled in equimolar (WeiKe Meng Technology Group Co., Ltd., Shenzhen, China). Demultiplexed sequences were quality filtered, denoised, merged and chimera removed using the QIIME2 DADA2 plugin to obtain the feature table of amplicon sequence variant (ASV) [6]. The ASV sequences were then aligned to the pretrained GREEN-GENES 13\_8 99% database using the QIIME2 feature classifier plugin to generate a classification table [4]. Any contaminating mitochondrial and chloroplast sequences were filtered using the QIIME2 feature-table plugin.

Bioinformatics analysis was performed following the "Atacama soil microbiome tutorial" of Qiime2-docs along with customized program scripts (<https://docs.qiime2.org/2019.1/>). LEfSe and DESeq2 were used to identify the endophytic bacteria with different abundance among treatments. To estimate the microbial diversity within an individual sample, feature level alpha diversity indices, such as observed OTUs, Chao1 richness estimator, Shannon diversity index, and Faith's phylogenetics diversity index were calculated using the core-diversity plugin within QIIME2. Besides, the potential KEGG Ortholog functional profiles of microbial communities was predicted with PICRUST.

### 2.5. Statistical analysis

Two-way ANOVA and post hoc test were used to test the effects of plant species, nano-plastics and their interaction on environmental parameters, plant RGR and antioxidant indicators (SOD, MDA, GSH), and endophyte alpha diversity (Chao1, Faith\_pd, Observed\_features, Shannon\_index, Simpson\_index). T-test was performed to analyzed the effects of nano-plastic on the number of ramet/leaf/node of plants. Two-way analysis of similarity (ANOSIM) was conducted to compare the response of endophyte community composition to plant species, nano-plastics and their interactions. To visualize possible differences, non-metric multidimensional scaling (NMDS) was performed using the R

(version 4.2.1) vegan package. Before statistical analysis, data were tested for normality. If necessary, the data were logarithm or square root transformed before performing ANOVA.

Redundancy analysis (RDA) was performed to reveal the association of endophyte communities in relation to environmental factors based on relative abundances of endophyte at family level using the R package "vegan". In the bio-plots of RDA results, only the names of the top 30 abundant endophyte taxa were listed. Co-occurrence analysis was performed by calculating Spearman's correlations between predominant taxa and the network plot was used to display the significant associations among taxa (Spearman's coefficient  $|r| > 0.5$  and  $P < 0.05$ ). The analysis result was performed using R (version 4.2.1) and visualized by the Cytoscape (version 3.9.1).

Structural equation modeling (SEM) was conducted using the AMOS program (Version 20, IBM SPSS AMOS) with maximum likelihood estimation to quantify the direct and indirect effects of environmental factors, antioxidant indicators, and endophytic bacterial diversity on plant RGR. The promising explanatory factors in the model were selected based on the results of correlation analysis and stepwise multiple regression analysis. According to the results of SEM, variance decomposition was further conducted to distinguish the contribution ratio of environmental factors, antioxidant indicators, and endophytic bacterial diversity on plant RGR.

## 3. Results

### 3.1. Environmental condition

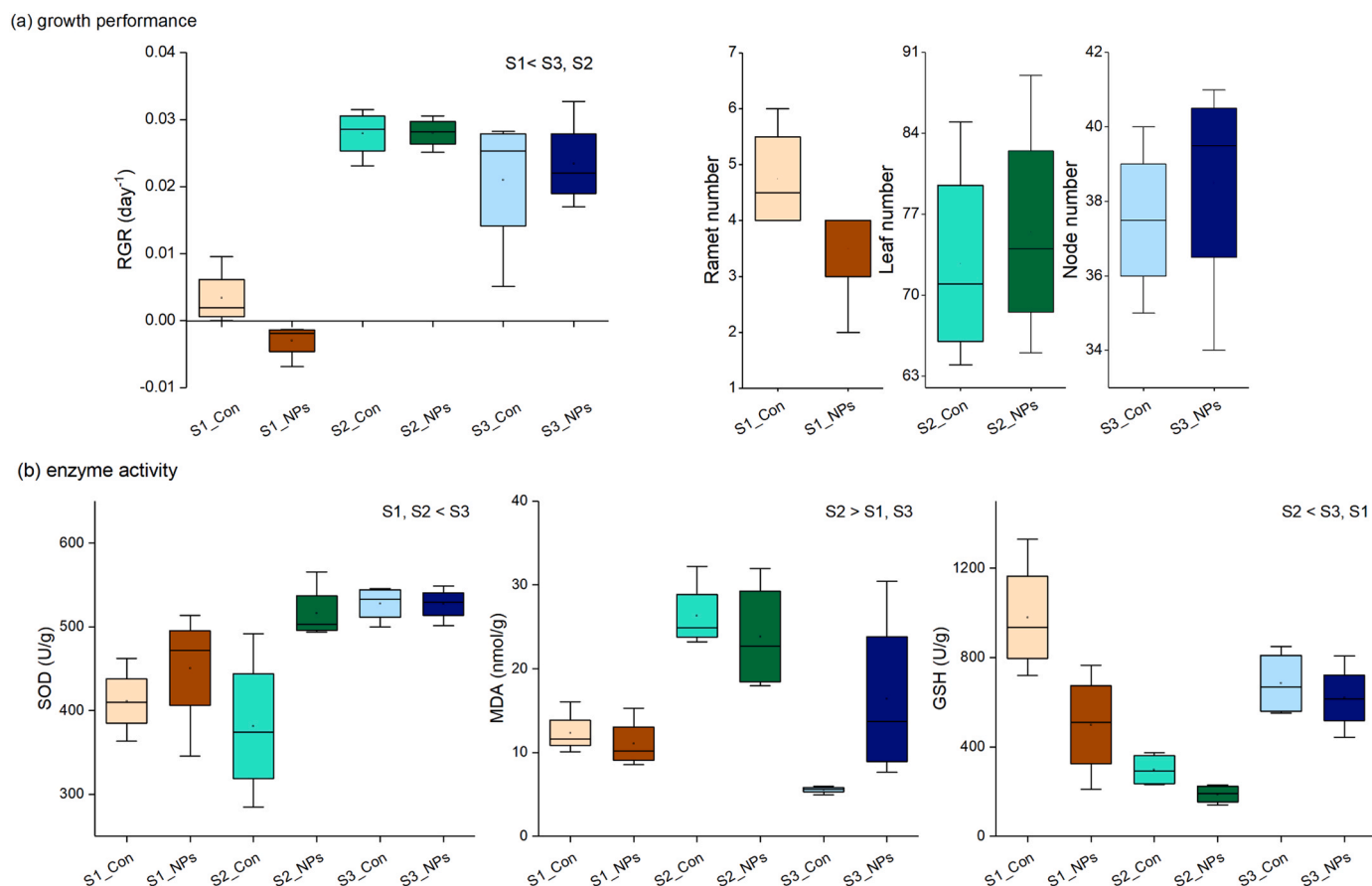
Environmental variables, such as the content of dissolved oxygen (DO), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), total organic carbon (TOC), total inorganic carbon (TIC), and nano-plastics content (NPs) in each treatment, are exhibited in Fig. S2. Two-way ANOVA results showed that TDP and TIC were significantly different among plant species (two-way ANOVA,  $P < 0.01$ ), and DO, TDN, TDP were significantly influenced by the interaction between species and nano-plastics (species\*nano-plastics).

In scenario with nano-plastics addition, the content of NPs was significantly lower in the *V. natans* treatment than in the *P. maackianus* treatment (Fig. S2). In addition, significant nano-plastics enrichment was observed on the leaf surfaces of the three submerged macrophytes, but not inside the plant stems (Fig. S3). Scanning electron microscopy (SEM) showed that nano-plastics were distributed on the leaves in the form of aggregates or dispersed particles (Fig. S3). And results of transmission electron microscopy (TEM) indicated that nano-plastics caused varying degrees of damage to the three plants, such as membrane damage in *V. natans*, chloroplast deformation in *P. maackianus*, starch accumulation in *M. spicatum* (Fig. S3).

### 3.2. Plant growth performance and antioxidative stress

The relative growth rate (RGR) of submerged macrophytes was significantly different among the three species, and the RGR of *V. natans* was significantly lower than that of *P. maackianus* and *M. spicatum* (Fig. 1a; Table 1). In addition, nano-plastics significantly reduced the RGR of *V. natans* from 0.0034 to  $-0.0029 \text{ day}^{-1}$  (T-test  $P < 0.05$ ), but had no significant influence on the ramet number of *V. natans*, leaf number of *P. maackianus*, and node number of *M. spicatum* (Fig. 1a; T-test  $P > 0.05$ ).

Enzyme indicators of submerged macrophytes were significantly different among the three species (Table 1). As shown in Fig. 1b, SOD was *V. natans*, *P. maackianus* < *M. spicatum*, MDA was *M. spicatum*, *V. natans* < *P. maackianus*, and GSH was *P. maackianus* < *M. spicatum*, *V. natans*. Significant nano-plastics effect was found on MDA, and significant species\*nano-plastics effects were found on SOD, MDA, and GSH (Table 1). Multiple comparison showed that nano-plastics significantly increased MDA in *M. spicatum* and SOD in *P. maackianus*, while



**Fig. 1.** The (a) growth performance (RGR, ramet number, leaf number, node number) and (b) antioxidant indicators (SOD, MDA, GSH) of *V. natans*, *P. maackianus*, and *M. spicatum* cultured in scenarios with and without nano-plastics addition (20 mg/L). Each treatment consisted of 4 replicates. S1\_Con and S1\_NPs represents *V. natans* cultured in treatments without and with nano-plastics addition; S2\_Con and S2\_NPs represents *P. maackianus* cultured in treatments without and with nano-plastics addition; S3\_Con and S3\_NPs represents *M. spicatum* cultured in treatments without and with nano-plastics addition.

**Table 1**

Main and interactive effects of plant species and nano-plastics on the RGR, MDA, SOD, CAT, POD, and GSH of submerged macrophytes.

indices	nano-plastics			species			species* nano-plastics		
	d.f.	F	P	d.f.	F	P	d.f.	F	P
RGR	1	0.287	0.599	2	50.398	< 0.001	2	1.212	0.321
SOD	1	2.140	0.161	2	11.499	0.001	2	2.293	0.130
MDA	1	4.440	0.049	2	15.849	< 0.001	2	9.024	0.002
GSH	1	1.412	0.250	2	5.815	0.011	2	22.044	< 0.001

significantly decreased GSH in *V. natans* (Fig. 1).

### 3.3. Endophytic bacteria

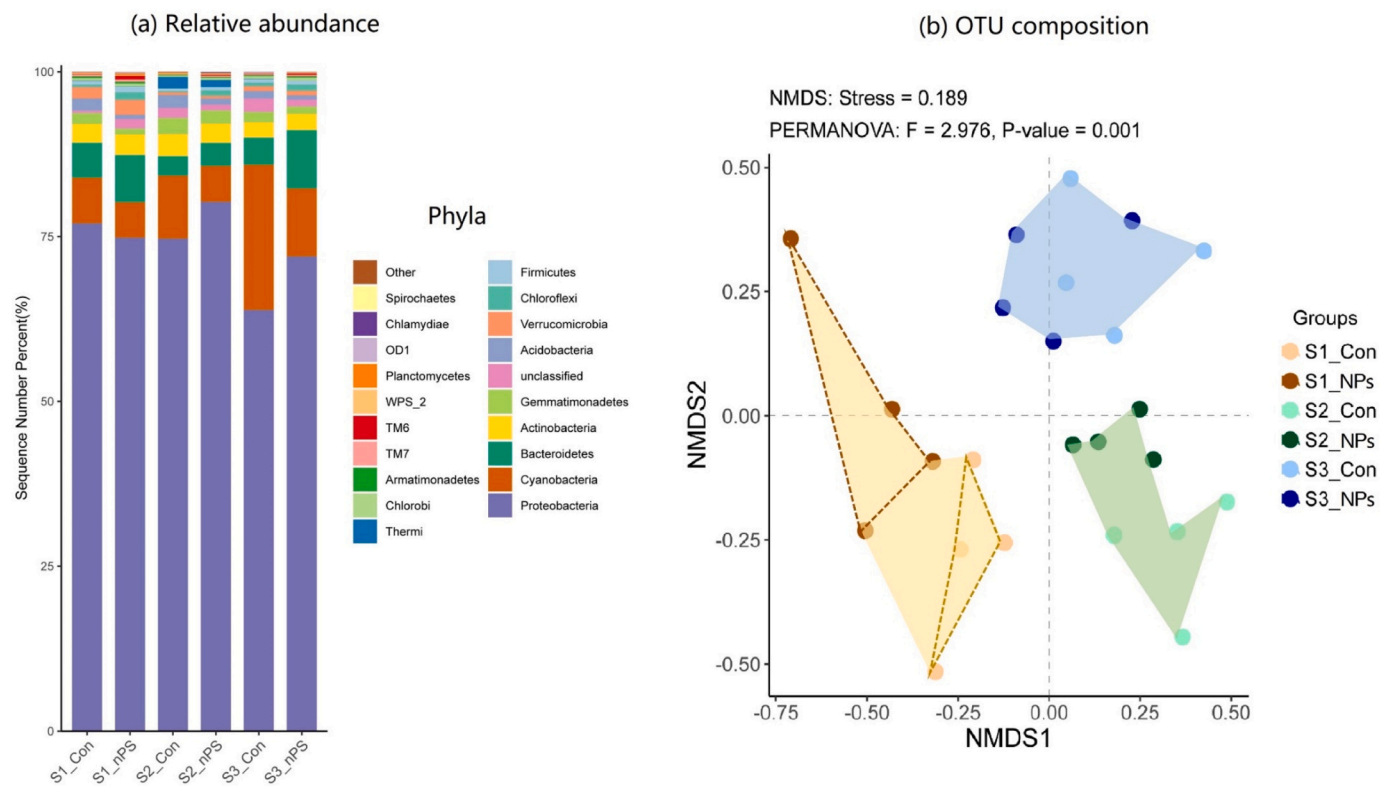
#### 3.3.1. Community composition

A total of 3761 endophytic bacterial OTUs were identified in the three submerged macrophytes. Proteobacteria and Cyanobacteria were the dominant phyla, accounting for 74% and 10% of the total endophyte abundance, respectively (Fig. 1a). The OTU composition of endophytic bacteria was significantly influenced by species and nano-plastics (Fig. 1b; two-way ANSIM,  $P < 0.001$ ), but not by species\* nano-plastics. Results of post hoc pairwise tests showed significant differences in bacterial composition between any two plant species (Fig. 1b). Furthermore, nano-plastics only significantly altered the endophytic OTU composition in *V. natans*, but not in the other two species (Fig. 1b). At family level, nano-plastics exposure resulted in an increase in the relative abundance of Hyphomicrobiaceae, Acetobacteraceae, Rhodobacteraceae, Chitinophagaceae in *V. natans* endophyte.

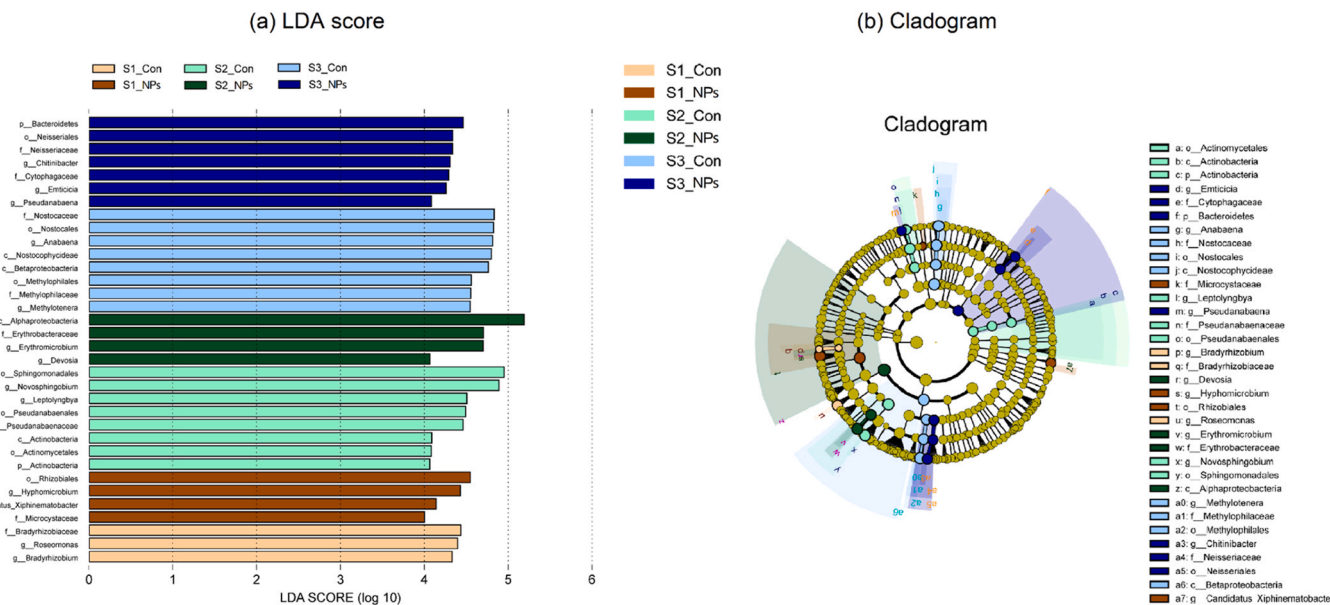
According to LEfSe analysis, endophytic bacterial biomarkers (with LDA scores > 4) were different among the six treatments as genus level (Fig. 1a). Cladogram described changes in the endophytic bacteria from phylum to genera among treatments (Fig. 1b). Further DESeq2 analysis suggested that the abundance of endophyte phyla such as Acidobacteria and Chloroflexi was significantly different between S1\_Con and S1\_NPs, as well as between S2\_Con and S2\_NPs ( $P < 0.05$ ). Fig. 2.

#### 3.3.2. Alpha diversity

The alpha diversity indices of endophytic bacteria were significantly different among the three plant species (two-way ANOVA,  $P < 0.05$ ). Multiple comparison results showed that the Chao1 and Observed features indices of *V. natans* were significantly higher than those of the other two species, and the Faith\_pd index of *V. natans* was significantly higher than that of *P. maackianus* (Fig. 4). Meanwhile, endophytic bacterial diversity was not significantly affected by nano-plastics and species\* nano-plastics (two-way ANOVA,  $P > 0.05$ ).



**Fig. 2.** The community composition of endophytic bacteria in *V. natans*, *P. maackianus*, and *M. spicatum* cultured in scenarios with and without nano-plastics addition, (a) relative abundance at phylum level and (b) non-metric multidimensional scaling (NMDS) plots of the OUT composition. Each treatment consisted of 4 replicates.



**Fig. 3.** LefSe analysis of abundance of endophytic bacteria in each treatment. (a) LDA score identified the size of differentiation among treatments with a threshold of 4. (b) Cladogram of endophytic bacterial communities.

**3.3.3. Community and environment**

Results of redundancy analysis (RDA) indicated a significant relationship between endophytic bacterial composition and environmental variables ( $P < 0.01$ ). The content of nano-plastics (NPs), TDP, and TOC were important factors that significantly affected bacterial composition (Fig. 5), explaining 14% of the variation (adjusted  $R^2$ ). Bacteria Rhodobacteraceae, Comamonadaceae, Acetobacteraceae were positively

related to NPs and were more abundant in the NPs groups than in the Con groups (Fig. 5). TDP was positively correlated with Rhizobiaceae and Caulobacteraceae which are abundant in *V. natans*. TOC, another explanatory factor of bacterial community, was negatively related to the content of NPs (Fig. 5).

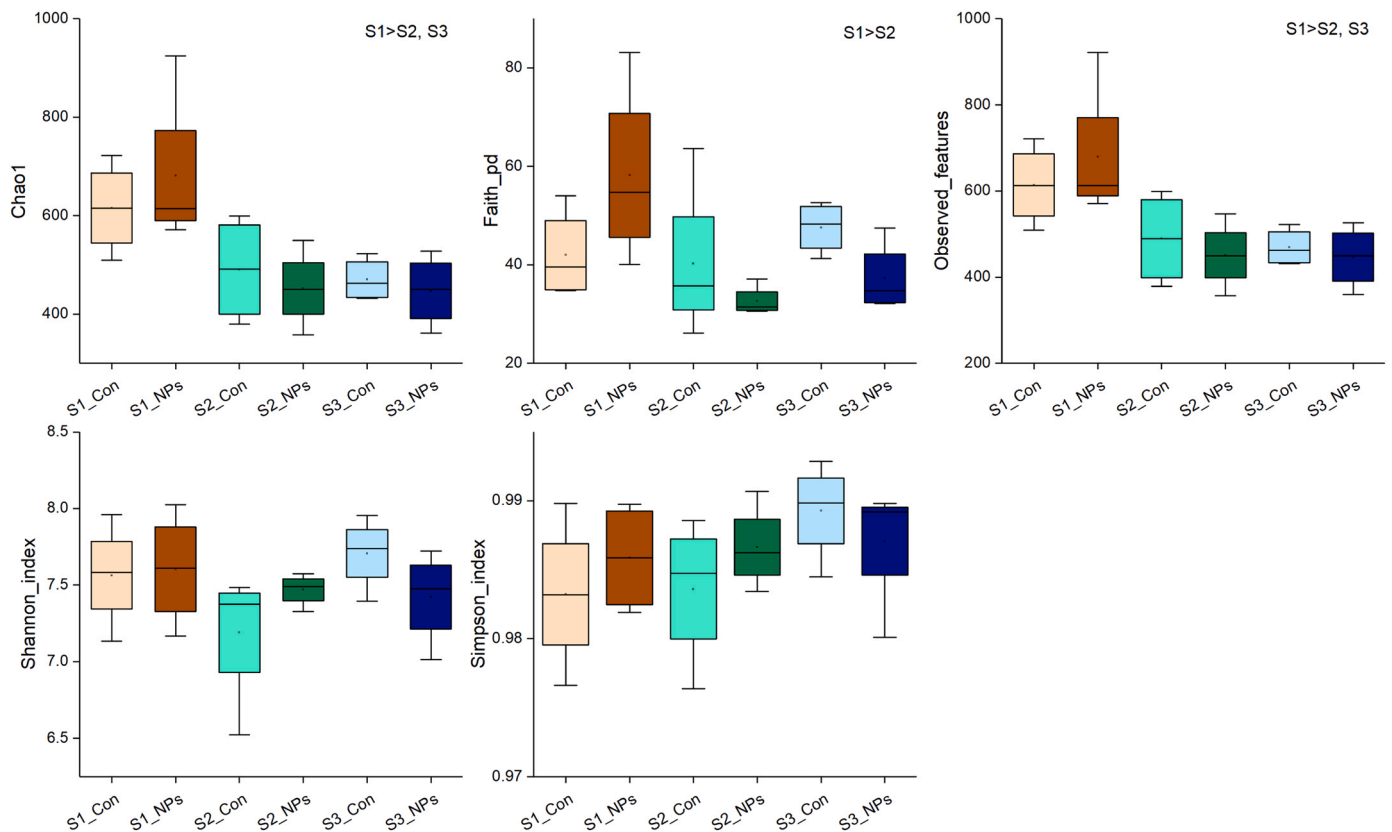


Fig. 4. The diversity indices (Chao1, Faith\_pd, Observed\_features, Shannon\_index and Simpson) of endophytic bacteria in each treatment.

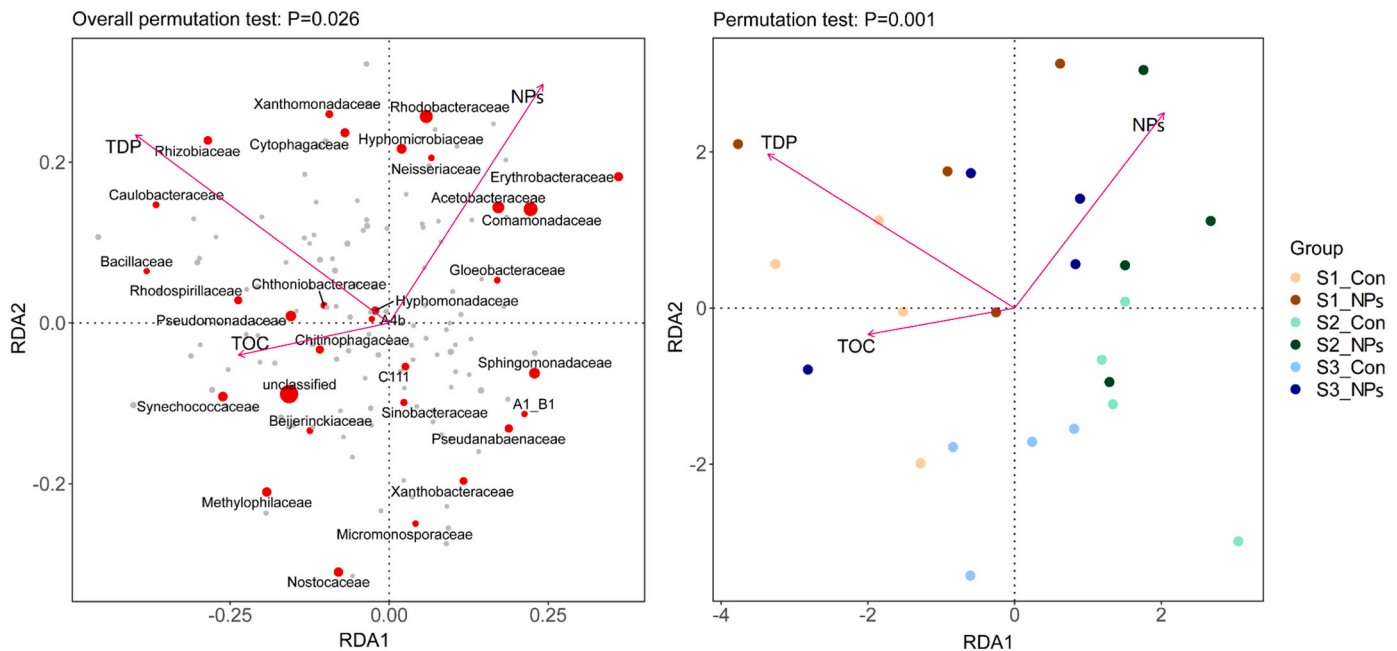
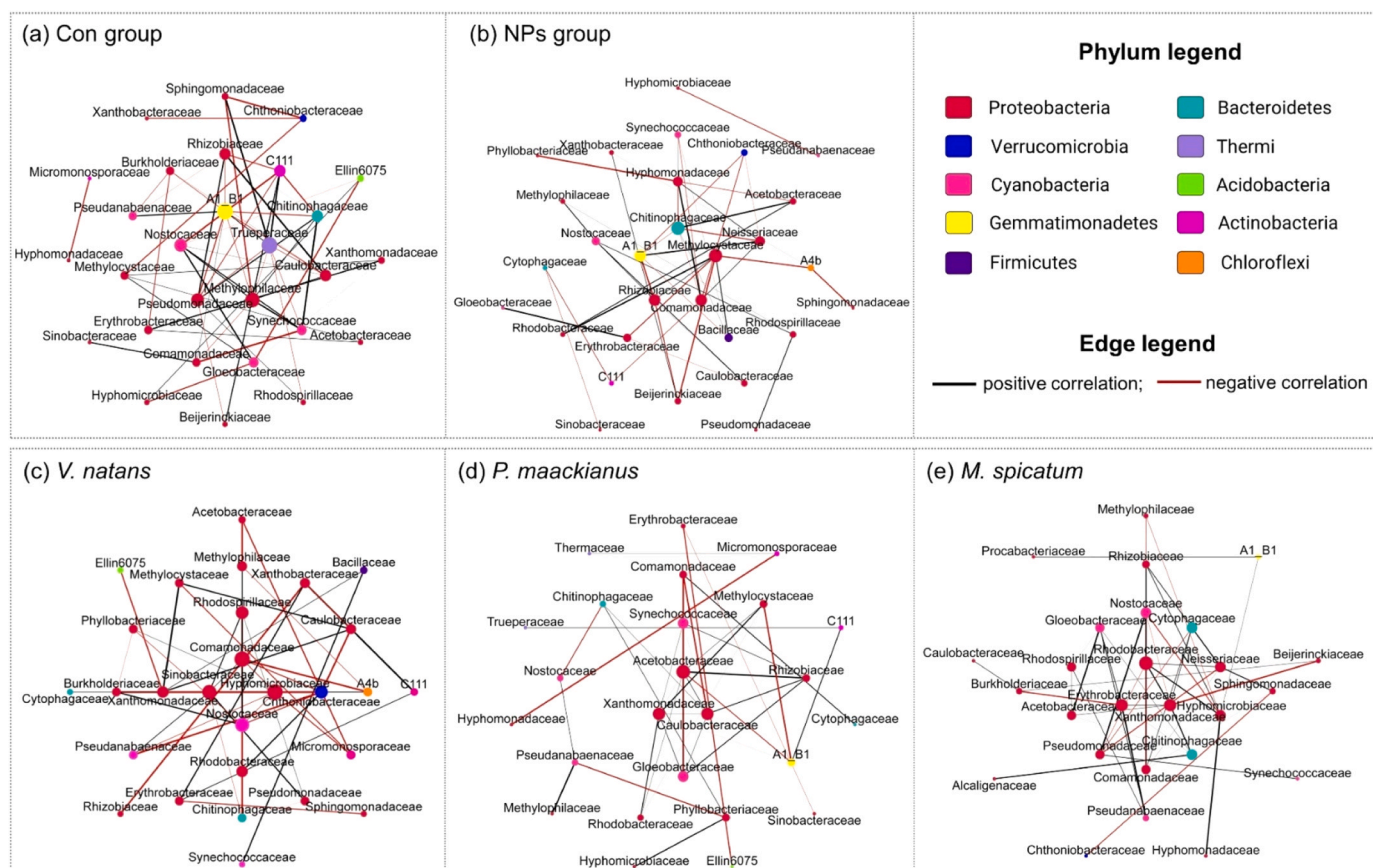


Fig. 5. Bio-plots of RDA results showing the relationships between endophytic bacteria, sampling groups and environmental variables.

### 3.3.4. Co-occurrence network

Co-occurrence network describe significant correlations among bacterial taxa in the two nano-plastics groups (Con group and NPs group) and in the three submerged macrophytes (*V. natans*, *P. maackianus*, *M. spicatum*), respectively (Fig. 6). Table 2 describes the topological indices for each association network. The average degree

and closeness centrality were both lower in the NPs group than in the Con group, while the betweenness centrality was higher in the former than in the latter. Besides, the proportion of positive correlations was similar between the Con and NPs group (Table 2). In the Con group, Trueperaceae and Nostocaceae were key nodes with the highest closeness centrality and betweenness centrality (0.51 and 0.22), respectively



**Fig. 6.** Network analysis of endophytic bacterial communities based on correlation analysis revealed different pattern of community topology structure in the (a, b) Con and NPs groups and (c, d, e) in the three plants. Each node represents one bacterial family. The size of the nodes is proportional to the number of connections. Each edge represents the correlation between bacterial families, and an edge is drawn if they share a strong and significant correlation (Spearman's coefficient  $|r| > 0.5$  and  $P < 0.01$ ). The black lines indicate positive correlations, while red lines indicate negative correlations.

**Table 2**

Topological indices of co-occurrence networks of endophyte in the Control (Con) group and nano-plastics (NPs) group, and in three different species (*V. natans*, *P. maackianus*, and *M. spicatum*).

Topological indices	nano-plastics		species		
	Con group	NPs group	<i>V. natans</i>	<i>P. maackianus</i>	<i>M. spicatum</i>
Number of nodes	28	29	28	25	25
Number of edges	59	46	51	40	51
Degree	<b>4.21</b>	<b>3.17</b>	3.64	3.20	3.92
Topological Coefficient	<b>0.39</b>	<b>0.35</b>	0.36	0.33	0.39
Betweenness Centrality	<b>0.06</b>	<b>0.09</b>	<b>0.08</b>	0.17	0.09
Closeness Centrality	0.44	0.36	<b>0.33</b>	0.45	0.34
Positive Correlations (%)	49	48	<b>43</b>	<b>70</b>	<b>75</b>
Negative Correlations (%)	51	52	<b>57</b>	<b>30</b>	<b>25</b>

(Fig. 6a). In the NPs group, A1\_B1 had the highest closeness centrality (0.43) and Rhizobiaceae had the highest betweenness centrality (0.35) (Fig. 6b).

Among the three plant species, the average degree was *M. spicatum* (3.92) > *V. natans* (3.64) > *P. maackianus* (3.20), and the maximum value of closeness and betweenness centrality appeared in the *P. maackianus* (Table 2). Besides, the proportion of positive correlations was remarkably higher than that of negative correlations in the *P. maackianus* and *M. spicatum*; conversely, for *V. natans*, the proportion of negative correlations was higher than that of positive correlations (Table 2). The key nodes with the highest closeness and betweenness centrality differed among three species. They were Chthoniobacteraceae and Caulobacteraceae in the *V. natans* group, Acetobacteraceae and Caulobacteraceae in the *P. maackianus* group, and Rhodobacteraceae

and Xanthomonadaceae in the *M. spicatum* group (Fig. 6c, d, e).

**3.3.5. Prediction of functional composition**

In addition, the sequencings of endophyte were analyzed to predict the functional composition of bacterial community inside the stem/leaf of submerged macrophytes. At level 1, more than 70% of the bacterial community was enriched in the metabolism pathway according to the KEGG database. At level 2, amino acid metabolisms were the most abundant bacterial pathway in endophyte accounting for more than 10% in all treatments, while Biosynthesis of terpenoids and steroids pathway was most abundant at level 3 (Fig. S4). However, pathway ANOVA results showed no significant difference in the relative abundance of sequences among treatments ( $P > 0.05$ ).

### 3.4. Structural equation modeling

According to the results of correlations analysis and stepwise multiple regression analysis, environment variables TIC, DO and TDP, endophytic bacterial diversity indices Chao1 and Faith, antioxidant indices MDA and GSH were selected as the promising explanatory factors of RGR for SEM analysis (Fig. 7a). In SEM, three environmental variables explained 61% of the variance in GSH, and 5% and 33% of the variance in Chao1 and Faith, respectively. The target variable RGR was directly and negatively influenced by TIC (path coefficient =  $-0.45$ ), GSH ( $-0.18$ ), Chao1 ( $-0.40$ ) and Faith ( $-0.27$ ), explaining 66% of the variance in RGR. In addition, RGR was also indirectly affected by DO ( $-0.05$ ), TIC ( $-0.12$ ), TDP ( $-0.18$ ), Chao1 ( $-0.11$ ) and MDA ( $0.10$ ), which are not shown in Fig. 7a.

Further analysis of variance decomposition showed that environment, endophyte and enzyme together explained 59% of the variance in RGR, and the three corresponding factors explained 34%, 36% and 14% (Fig. 7b). Environmental\*endophytes (interactions between environment and endophytes), environmental\*enzymes, and endophyte\*enzymes explained 11%, 10%, and 5% of the variance in RGR, respectively. Additionally, 2% of the variance was accounted for by environment\*endophyte\*enzyme (Fig. 7b).

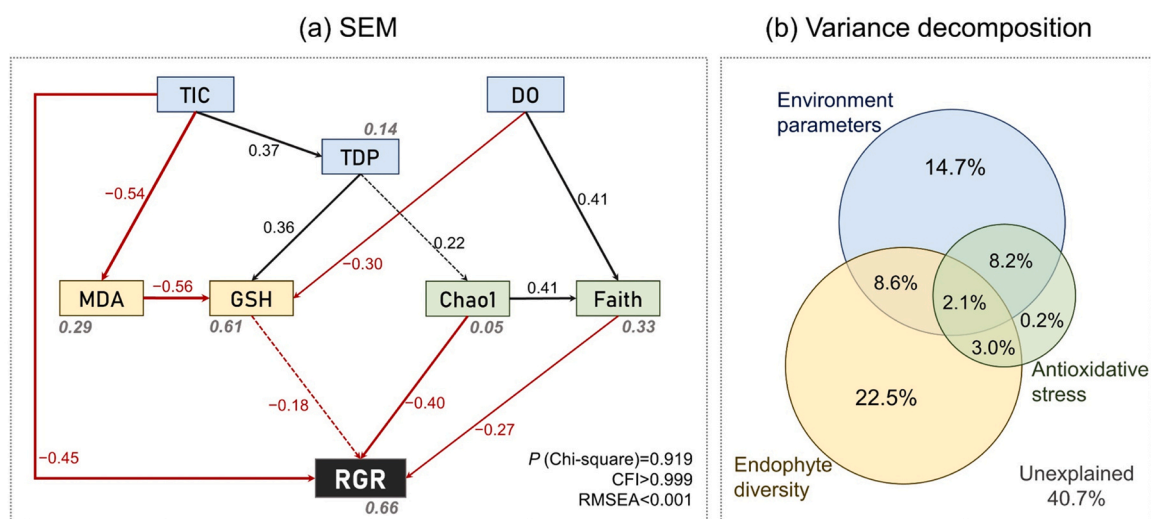
## 4. Discussion

### 4.1. Effects of nano-plastics on plants' performance vary among species

Multiple studies have reported the effects of nano-plastics on the growth performance, photosynthesis, and metabolic and antioxidant system of submerged macrophytes, with results ranged from adverse [31, 49,57], positive [29] to neutral [20,62]. This can be attributed to the inconsistencies in nano-plastic properties (e.g. type, size, concentrations), tested species, and the environmental conditions [28,33,5]. Overall, however, it is recognized that the adverse effects of nano-plastics on aquatic organisms increase with decreasing particle size and increasing concentration [44]. For instance, nano-plastics with particle sizes of less than 100 nm and concentrations greater than 10 mg/L have been widely shown to negatively impact aquatic organisms [32,58].

In this study, after 35 days of exposure to high concentrations of nano-plastics (100–500 nm, 20 mg/L), the responses of plant relative growth rate (RGR) and antioxidant indicators to nano-plastics varied significantly among the three submerged macrophytes. For *V. natans*, nano-plastics significantly reduced GSH content and concomitantly reduced RGR. GSH plays a major role in the detoxification of reactive oxygen species (ROS) and its deficiency exposes the cell to a risk of damage [42,63]. This could be one possible reason for reduced RGR in *V. natans*. By contrast, nano-plastics exposure significantly increased the SOD activity in *P. maackianus* and MDA content in *M. spicatum*. SOD acts as the first step in active oxygen-scavenging system and plays an important role in maintaining balance and protecting the cells from oxidative damage [40,56], while MDA is an end product of lipid peroxidation and can reveal the degree of cell membrane damage and plant resilience [37]. Although both *P. maackianus* and *M. spicatum* were subjected to varying degrees of antioxidative stress, their relative growth rates were not reduced by nano-plastics exposure. These results indicate that nano-plastic induced antioxidative stress is not the main factor explaining RGR in submerged macrophytes in this study. This conjecture is consistent with the results of structural equation model (SEM) and variance proportion (Fig. 7).

Nano-plastics can influence plants externally or internally [33]. Multiple studies have shown that plants can take up nano-plastics by roots and transport them to stems and leaves by the transpiration [33, 51], especially plastic particles less than 50 nm [7,8]. In this study, TEM images indicated that no significant internalization of nano-plastics into the stems/leaves of submerged macrophytes was observed (Fig. S3), which may be attributed to the large nano-plastics size (100–500 nm). The size of nano-plastics is a major factor determining whether they can be internalized, as plant epidermis and cell wall make it difficult for larger nano-plastics to penetrate the tissues of vascular plants [27,54]. On the other hand, the roots of the submerged macrophytes in this study were isolated from the nano-plastics in the water column, so it was difficult for the nano-plastic to be absorbed through the roots and transported to the stems and leaves. Currently, research on the nano-plastics effect on submerged macrophytes is relatively lacking [33]. And among these limited studies, only Yuan et al. [59] reported that nano-plastics can accumulate in the intercellular spaces of *Vallisneria denseserrulata* and be transported from roots to leaves through the



**Fig. 7.** (a) Structural equation model (SEM) describing the effects of environmental variables (TIC, DO, TDP), antioxidant indicators (MDA, GSH), and endophytic bacterial diversity (Chao1, Faith) on the RGR of submerged macrophytes in all treatments. Single-headed arrows represent unidirectional causal relationships. Solid lines indicate significant ( $p < 0.05$ ) effects, and dashed lines indicate nonsignificant effects. Numbers near the lines are standardized path coefficients. Black lines and numbers represent positive effect, while red ones represent negative effect. The thickness of the arrows is proportional to the magnitude of the standardized path coefficients. The italicized gray numbers adjacent to the boxes indicate the R<sup>2</sup> values explained by these factors. (b) Proportion of variance in RGR explained by environment, enzyme, bacteria, and their interactions.

apoplast pathway and vascular bundles. Most other studies agree that nano-plastics can be absorbed and enriched on the surface of submerged macrophytes stems/leaf surface and negatively affect plant growth and metabolism [47,49,55]. Consistent with the findings of most studies, nano-plastics adsorbed on the surface of submerged macrophytes in this study may be one of the main factors that negatively affects plant growth performance (e. g. cell membrane damage, chloroplast deformation observed in TEM images).

#### 4.2. Species-specific response of endophyte communities to nano-plastics

Previous study have reported that submerged macrophytes growing in sediments highly contaminated with polycyclic aromatic hydrocarbons (PAHs), could recruited specific microbial taxa, such as *Herbaspirillum*, into the rhizosphere and root interior for toxicant degradation [53]. In this study, stem/leaves endophytic bacteria exhibited species-specific responses to nano-plastics when of submerged macrophytes were exposed to aquatic environment highly polluted by nano-plastics. Nano-plastics significantly altered the OUT composition of endophyte in *V. natans*, but not in *P. maackianus* and *M. spicatum*. Consistent with the results of Fu et al. [14], nano-plastics also significantly increased the abundance of Chloroflexi in *V. natans* endophytes, a phylum that was shown to be resistant to nano-plastics. Additionally, the relative abundance of Hyphomicrobiaceae, Acetobacteraceae, Rhodobacteraceae, Synechococcaceae, and Pseudomonadaceae in *V. natans* endophytes also increased with nano-plastics exposure. These bacteria taxa have the capability to degrade polystyrene [21,25] and other toxic environmental pollutants [11,12,3], such as persistent organic pollutants, heavy metal, etc.

Nano-plastics exposure also reduced the strength of associations between submerged macrophytes endophyte communities, as evidenced by reductions in the number of edges, average degree, and topological coefficient in co-occurrence network analysis (Table 2). Similar results have been reported by Shi et al. [41] who found that nano-plastics decreased the soil microbial network complexity. Meanwhile, the slight increased proportion of negative links in the NPs groups indicated that nano-plastics increased the competition intensity among endophytic communities [10,61]. In particular, the endophyte community of *V. natans*, as it had the highest proportion of negative correlations in the network analysis. By contrast, in the endophyte network of *M. spicatum* and *P. maackianus*, positive correlations outweighed negative ones, suggesting that mutual cooperation dominate interactions between endophytic communities. Furthermore, *M. spicatum* exhibited the highest network complexity among the three plants.

The concept of closeness and betweenness centrality can be applied to ecological networks to identify keystone nodes [15,24]. Closeness centrality measures the proximity of a species to all other species, while betweenness centrality describes the importance of a species as a connector in the community [9,13]. In general, the larger the centrality value, the more important the node is in the network [30]. In this study, co-occurrence analysis of submerged macrophyte endophytes showed that although most of the nodes were derived from Proteobacteria, keystone endophyte taxa differed not only between the Con and NPs groups, but also among the three plants. These results suggest that for submerged macrophytes exposed to high concentrations of nano-plastics, both stem/leaf endophyte composition and ecological associations between endophyte communities exhibit species-specific responses to nano-plastics.

#### 4.3. Environment and endophyte are the main factors affecting plant RGR

Structural equation modeling (SEM) and variance decomposition results indicate that the relative growth rate (RGR) of the three submerged macrophytes was affected and explained to varying degree by endophyte diversity (36%), environmental indicators (34%) and anti-oxidant indicators (14%). As an important determinant of plant growth,

growth limitation of submerged macrophytes by total inorganic carbon (TIC) is a common phenomenon [48]. The strong negative correlation between TIC and RGR suggested that submerged macrophytes growth was also inhibited by TIC in our study. TIC limitation directly affected plant RGR by reducing photosynthesis on the one hand, and indirectly affected plant RGR by inducing antioxidative stress on the other hand. Moreover, plant RGR was also indirectly affected by other environmental parameters, such as total dissolved phosphorus (TDP) and dissolved oxygen (DO), which negatively affect RGR by inducing antioxidative stress and/or altering endophyte diversity.

The relationship between endophyte and plant is described as a balanced symbiotic continuum ranging from mutualism through commensalism to parasitism [1,18,38]. Mutualistic symbionts benefit their host and frequently promote plant growth, commensal endophytes live on host metabolites without affecting the host plant's performance [36]. And parasitic endophytes reduce plant fitness and cause harm to the host when exposing the host plant to biotic or abiotic stress [26]. In this study, the RGR of the three submerged macrophytes was directly negatively influenced by the endophyte diversity indices (Fig. 7a). *V. natans* had the highest endophyte Chao1 index and Faith index, but its RGR was significantly lower than that of *P. maackianus* and *M. spicatum*. Furthermore, the number of unclassified OTUs in *V. natans* endophyte was increased by nano-plastics, concomitant with a significant decrease in host RGR. These results indicated that the diversity and abundance of parasitic endophytes may be relatively high in *V. natans* and further exacerbated under conditions of high nano-plastics exposure, thereby ultimately exerting a pronounced inhibitory effect on the growth of *V. natans*.

In addition, the species-specific response of host endophyte community structure to nano-plastics could be another potential reason affecting plant RGR, as variance decomposition indicated that 11% of the variance in RGR was explained by interactions between environment and endophyte (Fig. 7b). The contents of nano-plastics (NPs), TDP and TOC, as important environmental factors affecting the composition of endophytes (Fig. 5), had relatively low values in the aquatic environment treated by *V. natans* exposure to nano-plastics (S1\_NPs). Coupled with the lowest TIC and TDN content, these may be symptoms of nutrient deficiency in the aquatic environment of S1\_NPs. When nutrients are insufficient in the environment, some bacterioplankton taxa may invade the interior of the host plant to have access to more resources and greater protection from stress [2,35]. These newly invasive bacterial taxa may compete nutrients with pre-existing endophytes in *V. natans*, resulting in increased negatively correlated proportions and more stable endophyte networks, but at the expense of reduced host RGR. However, for endophyte in *P. maackianus* and *M. spicatum*, nano-plastics may promote positive interactions between endophyte communities, although they did not affect their composition and diversity. This may also be a potential mechanism by which endophyte communities protect host plants from nano-plastics stress, thus RGR was not significantly reduced in both species. In summary, our results demonstrated that, the different response strategies of stem/leaves endophyte communities to nano-plastics may regulate the growth performance of submerged macrophytes, but the mechanisms underlying the differences in species response strategies remains to be further investigated.

## 5. Conclusion

Our study demonstrates that high concentrations of nano-plastics have species-specific effects on the growth, physiology, and stem/leaves endophyte community of submerged macrophytes. *V. natans* turned out to be more sensitive to nano-plastics than *P. maackianus* and *M. spicatum*, as evidenced by reductions in plant RGR and changes in endophyte community composition and co-occurrence correlations. Further analysis revealed that the response strategies of endophyte community to nano-plastics and change in aquatic environmental

conditions induced by nano-plastics were important determinants for plant RGR. These findings highlight the potentially determinative role of endophyte communities in modulating the growth performance of submerged macrophytes to nano-plastics. This reveals the different phytoremediation potential of submerged macrophytes in nano-plastic contaminated water environment. Future research should focus on the interaction mechanism between endophytic bacterial and submerged macrophytes.

### Environmental Implication

- The hazards of nano-plastic to freshwater aquatic organisms have been widely documented. Given the importance of submerged macrophytes, the environmental toxicity of nano-plastic to submerged macrophytes will directly affect their structuring roles in ecological services and biochemical cycles.
- Endophytic bacteria protect host plants from abiotic stresses, which has been widely demonstrated in terrestrial plants. However, whether the symbiotic relationship between endophytes and submerged macrophytes can enhance plant performance against nano-plastic environmental pollution remains unknown.
- This study highlights the species-specificity of submerged macrophyte-endophyte interactions and reveals their phytoremediation potential in nano-plastic contaminated water environments.

### CRedit authorship contribution statement

**Beibei Hao:** Designed the study, conducted the experiments, analyzed the data and drafted the manuscript. **Haoping Wu:** Conducted the experiments, analyzed the data and revised the manuscript. **Siyi Zhang:** Analyzed the data, reviewed the manuscript. **Bin He:** Reviewed and revised the manuscript.

### Declaration of Competing 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.

### Data Availability

Data will be made available on request.

### Acknowledgements

The study was financially supported by the National Natural Science Foundation of China (52000041, 42177065, 41930865), National Key R&D Program Project (2023YFC3205700), the Science and Technology Planning Project of Guangdong Province (2019B121201004), GDAS' Project of Science and Technology Development (2020GDASYL-20200102013, 2023GDASZH-2023010103), Special Fund for Scientific Innovation Strategy-construction of High Level Academy of Agriculture Science (R2022YJ-YB3005), Special Funds for Agricultural Industry Development of Guangdong Academy of Agricultural Sciences (202228), Guangdong Basic and Applied Basic Research Foundation (2023A1515012037) and Asia-Pacific Network for Global Change Research APN project (CRRP2020-03MY-He).

### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2023.132883](https://doi.org/10.1016/j.jhazmat.2023.132883).

### References

- [1] Aly, A.H., Debbab, A., Proksch, P., 2011. Fungal endophytes: unique plant inhabitants with great promises. *Appl Microbiol Biotechnol* 90, 1829–1845.
- [2] Beattie, G.A., Lindow, S.E., 1999. Bacterial colonization of leaves: a spectrum of strategies. *Phytopathology* 89 (5), 353–359.
- [3] Bhatt, P., Pandey, S.C., Joshi, S., Chaudhary, P., Pathak, V.M., Huang, Y., Wu, X., Zhou, Z., Chen, S., 2022. Nanobioremediation: a sustainable approach for the removal of toxic pollutants from the environment. *J Hazard Mater* 427, 128033.
- [4] Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., Gregory Caporaso, J., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* 6 (1), 1–17.
- [5] Boots, B., Russell, C.W., Green, D.S., 2019. Effects of microplastics in soil ecosystems: above and below ground. *Environ Sci Technol* 53 (19), 11496–11506.
- [6] Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 13 (7), 581–583.
- [7] Capozzi, F., Carotenuto, R., Giordano, S., Spagnuolo, V., 2018. Evidence on the effectiveness of mosses for biomonitoring of microplastics in fresh water environment. *Chemosphere* 205, 1–7.
- [8] Chae, Y., An, Y.-J., 2020. Nanoplastic ingestion induces behavioral disorders in terrestrial snails: trophic transfer effects via vascular plants. *Environ Sci: Nano* 7 (3), 975–983.
- [9] Colladon, A.F., Vagaggini, F., 2017. Robustness and stability of enterprise intranet social networks: The impact of moderators. *Inf Process Manag* 53 (6), 1287–1298.
- [10] Coyte, K.Z., Schluter, J., Foster, K.R., 2015. The ecology of the microbiome: networks, competition, and stability. *Science* 350 (6261), 663–666.
- [11] Fakhar, A., Gul, B., Gurmani, A.R., Khan, S.M., Ali, S., Sultan, T., Chaudhary, H.J., Rafique, M., Rizwan, M., 2022. Heavy metal remediation and resistance mechanism of *Aeromonas*, *Bacillus*, and *Pseudomonas*: A review. *Crit Rev Environ Sci Technol* 52 (11), 1868–1914.
- [12] Fedeson, D.T., Saake, P., Calero, P., Nikel, P.I., Ducat, D.C., 2020. Biotransformation of 2, 4-dinitrotoluene in a phototrophic co-culture of engineered *Synechococcus elongatus* and *Pseudomonas putida*. *Microb Biotechnol* 13 (4), 997–1011.
- [13] Freeman, L.C., 2002. Centrality in social networks: Conceptual clarification. *Social network: critical concepts in sociology*. In: Londres, I. Routledge, pp. 238–263.
- [14] Fu, S., Ding, J., Zhang, Y., Li, Y., Zhu, R., Yuan, X., Zou, H., 2018. Exposure to polystyrene nanoplastic leads to inhibition of anaerobic digestion system. *Sci Total Environ* 625, 64–70.
- [15] González, A.M.M., Dalsgaard, B., Olesen, J.M., 2010. Centrality measures and the importance of generalist species in pollination networks. *Ecol Complex* 7 (1), 36–43.
- [16] Gouda, S., Das, G., Sen, S.K., Shin, H.-S., Patra, J.K., 2016. Endophytes: a treasure house of bioactive compounds of medicinal importance. *Front Microbiol* 7, 1538.
- [17] Hao, B., Wu, H., You, Y., Liang, Y., Huang, L., Sun, Y., Zhang, S., He, B., 2023. Bacterial community are more susceptible to nanoplastics than algae community in aquatic ecosystems dominated by submerged macrophytes. *Water Res* 232, 119717.
- [18] Haridoim, P.R., Van Overbeek, L.S., Berg, G., Pirttilä, A.M., Compant, S., Campisano, A., Döring, M., Sessitsch, A., 2015. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev* 79 (3), 293–320.
- [19] Hoffmann, W.A., Poorter, H., 2002. Avoiding bias in calculations of relative growth rate. *Ann Bot* 90 (1), 37–42.
- [20] Hong, J., Huang, X., Wang, Z., Luo, X., Huang, S., Zheng, Z., 2022. Combined toxic effects of enrofloxacin and microplastics on submerged plants and epiphytic biofilms in high nitrogen and phosphorus waters. *Chemosphere* 308, 136099.
- [21] Hou, L., Xi, J., Liu, J., Wang, P., Xu, T., Liu, T., Qu, W., Lin, Y.B., 2022. Biodegradability of polyethylene mulching film by two *Pseudomonas* bacteria and their potential degradation mechanism. *Chemosphere* 286, 131758.
- [22] Hussain, Z., Arslan, M., Malik, M.H., Mohsin, M., Iqbal, S., Afzal, M., 2018. Integrated perspectives on the use of bacterial endophytes in horizontal flow constructed wetlands for the treatment of liquid textile effluent: phytoremediation advances in the field. *J Environ Manag* 224, 387–395.
- [23] Jeppesen, E., Søndergaard, M., Søndergaard, M., Christoffersen, K., 2012. The structuring role of submerged macrophytes in lakes. & Business Media. Springer Science.
- [24] Jordán, F., Liu, Wc, Davis, A.J., 2006. Topological keystone species: measures of positional importance in food webs. *Oikos* 112 (3), 535–546.
- [25] Kim, H.R., Lee, H.M., Yu, H.C., Jeon, E., Lee, S., Li, J., Kim, D.-H., 2020. Biodegradation of polystyrene by *Pseudomonas* sp. isolated from the gut of superworms (larvae of *Zophobas atratus*). *Environ Sci Technol* 54 (11), 6987–6996.
- [26] Kogel, K.-H., Franken, P., Hüchelhoven, R., 2006. Endophyte or parasite—what decides? *Curr Opin Plant Biol* 9 (4), 358–363.
- [27] Li, H., Lu, X., Wang, S., Zheng, B., Xu, Y., 2021. Vertical migration of microplastics along soil profile under different crop root systems. *Environ Pollut* 278, 116833.
- [28] Li, X., He, E., Xia, B., Liu, Y., Zhang, P., Cao, X., Zhao, L., Xu, X., Qiu, H., 2021. Protein corona-induced aggregation of differently sized nanoplastics: impacts of protein type and concentration. *Environ Sci: Nano* 8 (6), 1560–1570.
- [29] Lian, J., Wu, J., Xiong, H., Zeb, A., Yang, T., Su, X., Su, L., Liu, W., 2020. Impact of polystyrene nanoplastics (PSNPs) on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *J Hazard Mater* 385, 121620.

- [30] Liu, W., Li, X., Liu, T., Liu, B., 2019. Approximating betweenness centrality to identify key nodes in a weighted urban complex transportation network. *J Adv Transp* 2019.
- [31] Liu, Y., Wei, L., Yu, H., Cao, X., Peng, J., Liu, H., Qu, J., 2022. Negative impacts of nanoplastics on the purification function of submerged plants in constructed wetlands: responses of oxidative stress and metabolic processes. *Water Res* 227, 119339.
- [32] Lyons, D.M., Burić, P., 2022. Behavior, fate, and toxicity of engineered nanoparticles in estuarine and coastal environments. *Toxicol Nanopart Nanomater Hum, Terr Aquat Syst* 79–100.
- [33] Mateos-Cárdenas, A., van Pelt, F.N., O'Halloran, J., Jansen, M.A., 2021. Adsorption, uptake and toxicity of micro- and nanoplastics: effects on terrestrial plants and aquatic macrophytes. *Environ Pollut* 284, 117183.
- [34] Mendoza, L.M.R., Balcer, M., 2019. Microplastics in freshwater environments: a review of quantification assessment. *TrAC Trends Anal Chem* 113, 402–408.
- [35] Mercado-Blanco, J., 2014. Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture. Springer, pp. 25–32.
- [36] Mishra, S., Bhattacharjee, A., Sharma, S., 2021. An ecological insight into the multifaceted world of plant-endophyte association. *Crit Rev Plant Sci* 40 (2), 127–146.
- [37] Nimptsch, J., Pflugmacher, S., 2007. Ammonia triggers the promotion of oxidative stress in the aquatic macrophyte *Myriophyllum mattogrossense*. *Chemosphere* 66 (4), 708–714.
- [38] Papik, J., Folkmanova, M., Polivkova-Majorova, M., Suman, J., Uhlik, O., 2020. The invisible life inside plants: Deciphering the riddles of endophytic bacterial diversity. *Biotechnol Adv* 44, 107614.
- [39] Reinhold-Hurek, B., Hurek, T., 2011. Living inside plants: bacterial endophytes. *Curr Opin Plant Biol* 14 (4), 435–443.
- [40] Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L.M., Bebianno, M.J., 2017. Microplastics effects in *Scrobicularia plana*. *Mar Pollut Bull* 122 (1–2), 379–391.
- [41] Shi, J., Sun, Y., Wang, X., Wang, J., 2022. Microplastics reduce soil microbial network complexity and ecological deterministic selection. *Environ Microbiol* 24 (4), 2157–2169.
- [42] Sies, H., 1999. Glutathione and its role in cellular functions. *Free Radic Biol Med* 27 (9–10), 916–921.
- [43] Singh, T., Awasthi, G., Tiwari, Y., 2022. Recruiting endophytic bacteria of wetland plants to phytoremediate organic pollutants. *Int J Environ Sci Technol* 19 (9), 9177–9188.
- [44] Sjollem, S.B., Redondo-Hasselerharm, P., Leslie, H.A., Kraak, M.H., Vethaak, A.D., 2016. Do plastic particles affect microalgal photosynthesis and growth? *Aquat Toxicol* 170, 259–261.
- [45] Sun, Y., Li, X., Ding, C., Pan, Q., Wang, J., 2023. Host species and microplastics differentiate the crop root endophytic antibiotic resistome. *J Hazard Mater* 442, 130091.
- [46] Syranidou, E., Christofilopoulos, S., Gkavrou, G., Thijs, S., Weyens, N., Vangronsveld, J., Kalogerakis, N., 2016. Exploitation of endophytic bacteria to enhance the phytoremediation potential of the wetland helophyte *Juncus acutus*. *Front Microbiol* 7, 1016.
- [47] Tang, N., Li, X., Gao, X., Liu, X., Xing, W., 2022. The adsorption of arsenic on micro- and nano-plastics intensifies the toxic effect on submerged macrophytes. *Environ Pollut* 311, 119896.
- [48] Vadstrup, M., MADSEN, T.V., 1995. Growth limitation of submerged aquatic macrophytes by inorganic carbon. *Freshw Biol* 34 (3), 411–419.
- [49] van Weert, S., Redondo-Hasselerharm, P.E., Diepens, N.J., Koelmans, A.A., 2019. Effects of nanoplastics and microplastics on the growth of sediment-rooted macrophytes. *Sci Total Environ* 654, 1040–1047.
- [50] Wang, Q., Meng, L., Liu, W., Zeb, A., Shi, R., Lian, Y., Su, C., 2023. Single and combined effects of polystyrene nanoplastics and Cd on submerged plants *Ceratophyllum demersum* L. *Sci Total Environ* 872, 162291.
- [51] Wang, W., Yuan, W., Xu, E.G., Li, L., Zhang, H., Yang, Y., 2022. Uptake, translocation, and biological impacts of micro (nano) plastics in terrestrial plants: Progress and prospects. *Environ Res* 203, 111867.
- [52] Xiong, C., Zhu, Y.G., Wang, J.T., Singh, B., Han, L.L., Shen, J.P., Li, P.P., Wang, G. B., Wu, C.F., Ge, A.H., 2021. Host selection shapes crop microbiome assembly and network complexity. *N Phytol* 229 (2), 1091–1104.
- [53] Yan, H., Yan, Z., Wang, L., Hao, Z., Huang, J., 2022. Toward understanding submersed macrophyte *Vallisneria natans*-microbe partnerships to improve remediation potential for PAH-contaminated sediment. *J Hazard Mater* 425, 127767.
- [54] Yin, L., Wen, X., Huang, D., Du, C., Deng, R., Zhou, Z., Tao, J., Li, R., Zhou, W., Wang, Z., 2021. Interactions between microplastics/nanoplastics and vascular plants. *Environ Pollut* 290, 117999.
- [55] Yu, G., Huang, S., Luo, X., Zhao, W., Zheng, Z., 2022. Single and combined toxicity effects of nanoplastics and bisphenol F on submerged the macrophyte *Hydrilla verticillata*. *Sci Total Environ* 814, 152564.
- [56] Yu, H., Qi, W., Cao, X., Wang, Y., Li, Y., Xu, Y., Zhang, X., Peng, J., Qu, J., 2022. Impact of microplastics on the foraging, photosynthesis and digestive systems of submerged carnivorous macrophytes under low and high nutrient concentrations. *Environ Pollut* 292, 118220.
- [57] Yu, H., Zhang, X., Hu, J., Peng, J., Qu, J., 2020. Ecotoxicity of polystyrene microplastics to submerged carnivorous *Utricularia vulgaris* plants in freshwater ecosystems. *Environ Pollut* 265, 114830.
- [58] Yuan, W., Zhou, Y., Liu, X., Wang, J., 2019. New perspective on the nanoplastics disrupting the reproduction of an endangered fern in artificial freshwater. *Environ Sci Technol* 53 (21), 12715–12724.
- [59] Yuan, W., Xu, E.G., Li, L., Zhou, A., Peijnenburg, W.J., Grossart, H.-P., Liu, W., Yang, Y., 2023. Tracing and trapping micro- and nanoplastics: Untapped mitigation potential of aquatic plants? *Water Res*, 120249.
- [60] Zhang, Q., Qu, Q., Lu, T., Ke, M., Zhu, Y., Zhang, M., Zhang, Z., Du, B., Pan, X., Sun, L., 2018. The combined toxicity effect of nanoplastics and glyphosate on *Microcystis aeruginosa* growth. *Environ Pollut* 243, 1106–1112.
- [61] Zhou, H., Gao, Y., Jia, X., Wang, M., Ding, J., Cheng, L., Bao, F., Wu, B., 2020. Network analysis reveals the strengthening of microbial interaction in biological soil crust development in the Mu Us Sandy Land, northwestern China. *Soil Biol Biochem* 144, 107782.
- [62] Zhou, J., Liu, X., Jiang, H., Li, X., Li, W., Cao, Y., 2022. Antidote or Trojan horse for submerged macrophytes: role of microplastics in copper toxicity in aquatic environments. *Water Res* 216, 118354.
- [63] Zouainia, S., Djebar, M.R., Sbartaï, H., Cherait, A., Khene, L., Kahli, H., Berrebbah, H., 2016. Toxicol Impact assessment Cadmium Aquat Macrophyte: elodea Can Stud Univ" Vasile Goldis" Arad Ser Stiint Vietii (Life Sci Ser) 26 (3), 375.