Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

Effects of vermiculite on the growth process of submerged macrophyte *Vallisneria spiralis* and sediment microecological environment

Guoliang Bai[1,4,**], *Feng Luo*[1,2,**], *Yilingyun Zou*[1,3], *Yunli Liu*[1,3], *Rou Wang*[1,3], *Hang Yang*[2], *Zisen Liu*[1], *Junjun Chang*[5], *Zhenbin Wu*[1,3], *Yi Zhang*[1,3,*]

[1] *State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China*
[2] *School of Resources and Environmental Engineering, Wuhan University of Technology, Wuhan 430070, China*
[3] *University of Chinese Academy of Sciences, Beijing 100049, China*
[4] *School of Environmental Studies, China University of Geosciences, Wuhan 430074, China*
[5] *School of Ecology and Environmental Science, Yunnan University, Kunming 650091, China*

ARTICLE INFO

Article history:
Received 21 July 2021
Revised 18 August 2021
Accepted 18 August 2021
Available online 11 January 2022

Keywords:
Vermiculite
Vallisneria spiralis
Sediment
Growth and ecophysiological indicators
Microenvironment

ABSTRACT

Ecological restoration is one of the hot technologies for the reconstruction of eutrophic lake ecosystems in which the restoration and propagation of submerged plants is the key and difficult step. In this paper, the effect of vermiculite on the growth process of *Vallisneria spiralis* and sediment microenvironment were investigated, aiming to provide a theoretical basis for the application of vermiculite in aquatic ecological restoration. Results of growth indexes demonstrated that 5% and 10% vermiculite treatment groups statistically promote the growth of Vallisneria spiralis compared to the control. Meanwhile, the results of ecophysiological indexes showed that photosynthetic pigment, soluble sugar content, superoxide dismutase (SOD), and catalase (CAT) activity of 5% and 10% group were increased compared with the control while the malondialdehyde (MDA) content exhibited the opposite result ($p < 0.05$), which illustrated that vermiculite can improve the resistance of plants and delay the aging process of Vallisneria spiralis. In addition, result of PCA (Principal Component Analysis) demonstrated 5% and 10% group has improved the sediment physical conditions and create more ecological niche for microorganisms directly, and then promoted the growth of plants. The dissolution results showed that vermiculite can dissolve the constant and trace elements needed for plant growth. Furthermore, the addition of vermiculite

* Corresponding author.
E-mail: zhangyi@ihb.ac.cn (Y. Zhang).
** These authors contributed equally to this work.

https://doi.org/10.1016/j.jes.2021.08.038

increased the diversity of microorganisms in the sediments, and promoted the increase of plant growth-promoting bacteria and phosphorus-degrading bacteria. This study could provide a technique reference for the further application of vermiculite in the field of ecological restoration.

Introduction

In recent years, with the development of economy and society, ecological problems such as eutrophication have become more prominent (Abell et al., 2019). The exogenous nutrients have been effectively reduced with the implementation of various control measures (Kopacek et al., 2005), while the lake eutrophication problems were not effectively improved, the main cause of this phenomenon is through the accumulation of a long time stored in the lake sediment nitrogen and phosphorus and other nutrients release from sediment (Huang et al., 2015). Therefore, reducing phosphorus load of sediment has been considered as one of the key technologies to control eutrophication (Chen et al., 2018).

Submerged macrophyte act as the main primary producers of shallow lakes, occupy the main interface of water and sediment in lakes, and regulate the material, energy cycle and transfer of lake ecosystems (Zhen et al., 2020). The plants can absorb phosphorus and regulate the migration and transformation of phosphorus in sediments and overlying water during the growth process (Zhao et al., 2012; Li et al., 2020). Therefore, the transformation of eutrophic lakes into healthy grass-type lakes usually requires the reconstruction of aquatic vegetation dominated by submerged macrophyte (Gao et al., 2017). Aquatic vegetation restoration has gradually become the research hotspot of ecological remediation technology due to its low cost, environmental friendliness, and lasting effect (Song et al., 2018).

Sediment of shallow lakes is an important factor that affect the normal growth and reproduction of submerged macrophyte. It is the basic condition for rooting and stable growth of aquatic plants, and provides a large amount of nutrients for plants (Xie et al., 2005). However, the sediments in many eutrophic lakes are not suitable for the growth of submerged macrophyte, which results in the global decline of submerged macrophyte in many eutrophic lakes (Zhu et al., 2016). Thus, improving the physical and chemical conditions of sediment to promote the growth of submerged macrophyte has the vital significance in the ecological restoration process of eutrophic lakes (Tang et al., 2014; Human et al., 2015).

Sediment restoration techniques mainly include sediment dredging, aeration, and chemical addition (Devesa-Rey et al., 2011; Egemose et al., 2010). These methods often have problems such as consuming a lot of manpower and material resources and easily causing secondary pollution (Zhong et al., 2008). Therefore, the *in-situ* improvement technology of sediments has received more and more attention. Natural minerals such as bentonite (Liu et al., 2021), zeolite (Yang et al., 2014), and attapulgite (Yin and Kong, 2015) are common substrate improving materials, and environmental friendliness is a common feature of these materials.

Vermiculite is a natural clay mineral that is widely distributed all over the world. And it has a strong adsorption capacity and cation exchange capacity due to the special layered structure (Rama et al., 2019). Vermiculite has been widely applied as adsorbing material in sewage treatment due to the characteristics of inexpensive, environment friendly, high cation exchange capacities and strong sorption properties. Some studies reported that vermiculite can adsorb nitrogen, phosphorus, and heavy metal elements in sewage (Moraes et al., 2019; Da Silva et al., 2003). However, reports of its application as a sediment modifier to aquatic ecological restoration are relatively rare. The knowledge about the effect of vermiculite on submerged macrophyte ecophysiological characters and sediment microecological environment is limited.

In this study, we proposed hypothesis that vermiculite could promote the growth process of *Vallisneria spiralis*, and performed from the following contents: (1) compare the effect of different vermiculite mixing ratio on the growth of *Vallisneria spiralis*; (2) investigate the effect of different vermiculite mixing ratio on ecophysiological characters of *Vallisneria spiralis*; (3) determine the effect of vermiculite on microorganisms and sediment environment; (4) determine the relationship between ecophysiological characters of *Vallisneria spiralis*, vermiculite mixing ratio and environmental variables. The results of this study are expected to provide technical foundation for the application of vermiculite to ecological restoration project in eutrophic lakes.

1. Materials and methods

1.1. *Study site and sediment collection*

In May 2019, approximately 10 cm thickness surface sediment samples were collected 3 times randomly using a Peterson grab sampler from the center location of Xiaonanhu Lake (30°13′45′′N, 120°8′32′′E), one sub-lake of West Lake located in Hangzhou, Zhejiang Province, China, and then mixed for subsequent experiment. The ORP (oxidation reduction potential) and pH of sediment samples were measured *in situ* by ORP depolarization automatic analyzer (FJA-6, Nanjing Chuan-Di Instrument & Equipment Co., Ltd, China). The sediment samples were sealed in plastic bags and transported to the laboratory, dried at 55°C for 24 hr, and then sieved with a stand 100-mesh sieve for further testing and cultivating submerged macrophyte (Liu et al., 2017). The basic physical and chemical properties of sediment that collected from Xiaonanhu lake are: pH 6.86 ± 0.02, OM 13.1% ± 0.26%, TN 7750 ± 32.25 mg/kg, TP 1313 ± 24.79 mg/kg, Eh 52.6 ± 3.48 mV. Nutrient content of sediment exhibited a high level and the pollution degree is serious.

1.2. Experimental materials

Vallisneria spiralis is a perennial and common submersed macrophyte species, thrives in most shallow lakes in China, and has been widely used as an ecological engineering species of aquatic ecosystem restoration due to the uptake of plant tissue for excess nutrients in the water body (Han et al., 2018). The *Vallisneria spiralis* in this experiment was purchased from Hangzhou, China, and washed with deionized water to remove impurities. The purchased submerged plants were incubated in a constant temperature room with a temperature of 25°C, a light intensity of 4000 lx, and a light-dark ratio of 14:10 for 7 days. When transplanting into the prepared aquariums, the healthy and equal-size plant were selected and approximately pruned with uniform standard that keep the length of leaves and root were 15 cm and 3 cm, respectively. The vermiculite rough used in the experiment is produced in Lingshou County, Shijiazhuang City, Hebei Province, China, and its particle size is 2–4 mm. After washing with pure water and soaking for 24 hr, the vermiculite was mixed into Xiaonanhu lake sediments for incubating submerged plant later.

1.3. Batch experiments

1.3.1. Effect of vermiculite on the growth of Vallisneria spiralis

The experiment was carried out in aquariums (20 cm in diameter, 30 cm in heigh, 15 cm in width). 30 plants *Vallisneria spiralis* were planted in each aquarium, filled the aquariums with water and keep the water level constant during the experiment. A total of 7 different types of sediments were set up, including a control group (0%) that only containing West Lake sediments, and 6 treatment groups. The mass proportions of vermiculite in the sediments were 1%, 5%, 10%, 20%, 50%, and 100%, respectively, set three replicates in each group. The experimental devices were placed in a constant temperature room in Wuhan, China, from June 25, 2019 to November 15, 2019. The indoor temperature was about 25°C, indoor atmospheric pressure was about 101.325 Pa. All the data were the average of three independent replicates in this experiment.

The experiment was carried out for half a year. The growth, ecophysiological indexes of plants were measured once every half a month in the early stage, and once a month in the later stage after stabilization. During the experiment, three plants were sampled at random. After washing, the height of the plant was measured with a tape measure, and the biomass was weighed using a scale. 2 g leaves were collected to measure ecophysiological indicators, the chlorophyll content of leaves was detected by the Lichtenthaler-Arnon method, malondialdehyde (MDA) content was determined by the thiobarbituric acid method, superoxide dismutase (SOD) and catalase (CAT) activity were determined by guaiacol method, soluble sugar content is determined by the anthrone colorimetric method (Yang et al., 2011).

1.3.2. Sediment sampling and laboratory analysis

In order to investigate the influence of vermiculite on the physical and chemical properties, oxidation reduction potential (ORP), pH, organic materials (OM), total phosphorus (TP), total nitrogen (TN) were measured in this study. Sediment samples for determining sediment physical and chemical were collected once every half a month in the early stage, and once a month in the later stage after stabilization. The sediment ORP and pH of sediment samples were measured *in situ* by ORP depolarization automatic analyzer (FJA-6, Nanjing Chuan-Di Instrument & Equipment Co., Ltd, China). Sediment organic materials (OM) content was measured by an ignition method. The sediment total nitrogen and total phosphorus were measured by the alkaline potassium persulfate digestion method and molybdenum blue method, respectively.

1.3.3. Microcosm experiments

The sediment samples used for microcosm analysis were collected with improved medical syringes (3 cm in diameter; 12 cm in length) at the initial and final period of the experiment, respectively. The samples were placed in sterile centrifuge tubes (10 mL) and stored in an ultra-low temperature refrigerator at -80°C. Afterwards, Shanghai Bioengineering Co., Ltd. was commissioned to detect the microbial community structure based on the Illumina MiSeq high-throughput sequencing method. The specific process is that after extracting genomic DNA from the sample, amplify the V3-V4 region of 16S rDNA with barcode-specific primers. The primer sequences are 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC). Then the PCR amplified product was cut and recovered, and quantified with a QuantiFluor TM fluorometer. The purified amplified products were mixed in equal amounts, and the sequencing adapters were connected to construct a sequencing library, which was sequenced on Hiseq2500 PE250.

1.3.4. Material dissolution test

200 g of vermiculite samples were placed in 350 mL tissue culture bottles, then added 300 mL deionized water, placed these bottles in a greenhouse at 25°C. Aqueous solution was taken out for determination of the dissolved elements on day 1, 3, 5, 10, 15, 20, and 30, respectively. The Prodigy 7 ICP-OES/ICP-AES (Leeman Labs Inc, USA) for analytical detection has a continuous coverage of wavelengths ranging from 165 nm to 1100 nm, with an optical resolution of 0.007 nm (at 200 nm), the degree of precision is 2%.

1.4. Data analyses

The obtained data was calculated by Microsoft excel, and all graphics were manufactured by Origin 2018 except PCA (Principal Component Analysis) graphic by CANOCO 5.0. The statistical significance level was set to $p < 0.05$, and the Tukey HSD test method of two-way ANOVA was used to determine the differences of each treatment group. Significant differences of ecophysiological characters of *Vallisneria spiralis* among different vermiculite content group, and among different sampling days were showed in Appendix A Tables S1–S3. The mean values of data and standard deviation were expressed as mean

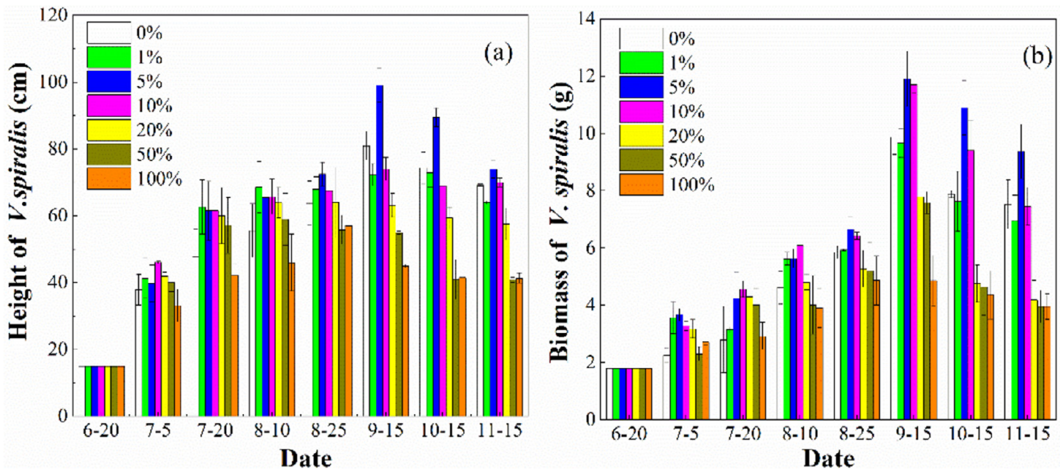

Fig. 1 – Effect of vermiculite on growth index of *Vallisneria spiralis* (Mean value ± SD, *n* = 3). a: height, b: biomass. 1%, 5%, 10%, 20%, 50%: groups with different the mass proportion of vermiculite in the sediment; 0%: control group.

± SD and the sample number was three. The above statistical analyses were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA; Version 19.0). PCA (Principal Component Analysis) was carried out to determine the relationship between ecophysiological characters of *Vallisneria spiralis*, vermiculite mixing ratio and environmental variables.

2. Results

2.1. *Effect of vermiculite on the growth indexes of* Vallisneria spiralis

The tissue height and biomass of submerged plant were measured during the experimental period, which can directly represent growth status of plants.

Fig. 1 showed that the growth variation of *Vallisneria spiralis* along with incubation time. July to September is a period of vigorous growth of *Vallisneria spiralis*, and the fastest growth rate from 25th August to 15th September, and the plant height and biomass of groups reached the highest value in 15th September, and then declined gradually. From Fig. 1a and Appendix A Table S1, the plant height of the treatment group with 5% vermiculite mixing ratio was higher than that of the control group (0%) during the whole experimental period, and was significantly higher than the control group in the later period of the experiment ($p < 0.05$). In 15th September, the plant height of the 5% group was the largest, and the height was 122.2% of the control group and the increase was 266.7% compared with 20th June. When the vermiculite mixing ratio exceeds 20% during 20th June to 10th August, the growth improvement of vermiculite on plant was not significant, but the improve effect has lost, and the plant height of the 50% and 100% groups declined faster than the control after 10th August.

Fig. 1b showed that the change of biomass has the same trend as the change of plant height. In 15th September, the biomass of 5% and 10% groups were 121% and 119.3% of the control group, which were significantly higher than the control group ($p < 0.05$), while the 20%, 50%, and 100% groups were significantly lower than the control group ($p < 0.05$).

2.2. *Effect of vermiculite on ecophysiological indexes of* Vallisneria spiralis

2.2.1. *Effect of vermiculite on chlorophyll content of* Vallisneria spiralis

It can be seen from Fig. 2 that the variation trend of chlorophyll *a* (Chl-*a*), chlorophyll *b* (Chl-*b*) and total chlorophyll (Chl-*a*+Chl-*b*) content is consistent throughout the experiment cycle, and the content in August and September was higher than that in other periods. But in 15th October, chlorophyll was reduced and the plants entered the stage of senescence, which is consistent with the change of growth index. In the stage of plant senescence, the chlorophyll content in the 5% and 10% groups were higher than that in the control group, while the chlorophyll content in the 20%, 50%, and 100% groups were significantly lower than that in the control group ($p < 0.05$).

2.2.2. *Effect of vermiculite on malondialdehyde content of* Vallisneria spiralis

From Fig. 3a, the MDA content of plants reached the maximum in 20th July, the possible reason is that submerged plant was not fully adapted to the new environment as planted. Compared with the control group, the mixing ratio of 1%, 5%, and 10% groups were lower, and the groups with the vermiculite mixing ratio more than 10% were significantly higher ($p < 0.05$). The MDA content of 20%, 50%, and 100% groups were 153.2%, 164.0%, and 140.9% of the control group, respectively.

2.2.3. *Effect of vermiculite on the enzyme activity of* Vallisneria spiralis

Fig. 3c showed that although the content of MDA in plants has increased, CAT was also produced in plants to resist the damage, CAT activity in 5% was the highest. In 20th July, the CAT activity of the 5% group was 118.9%, 145.3%, 139.3%, and 180.9% of the control group, 20%, 50%, and 100% group, respectively. During the whole experiment period, MDA content in 5% and

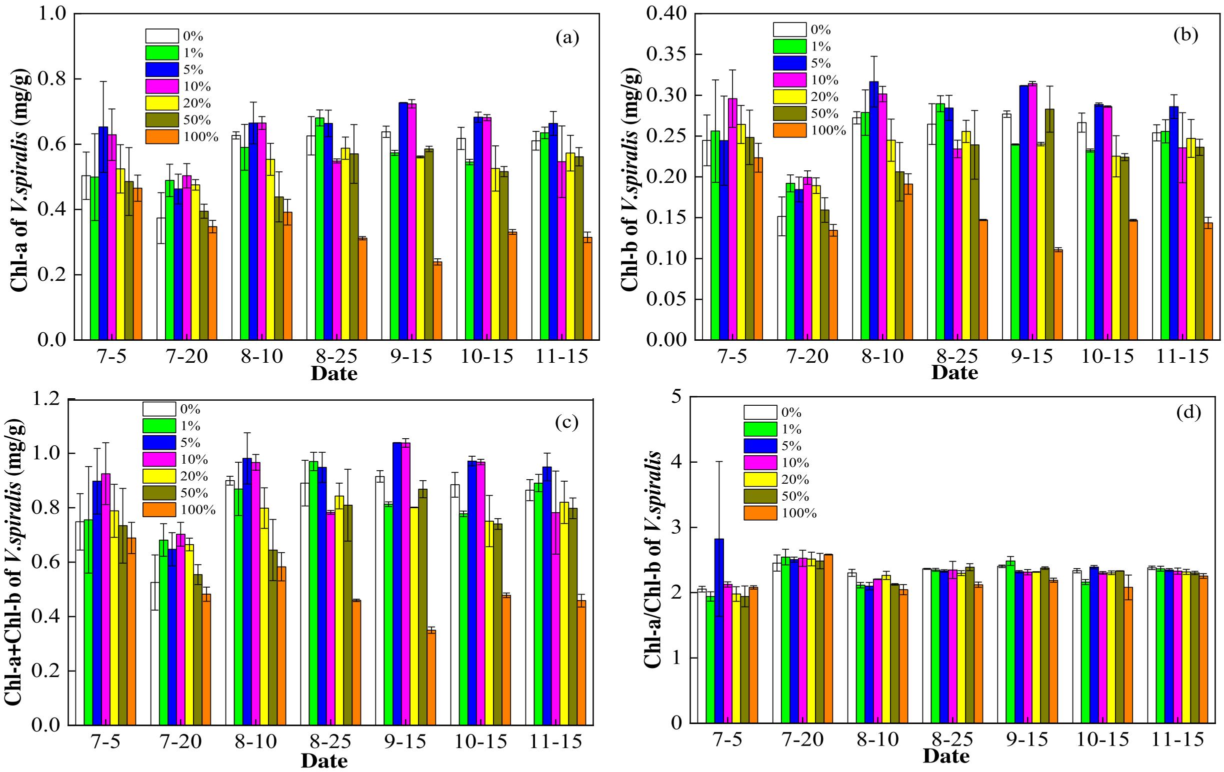

Fig. 2 – Effect of vermiculite on chlorophyll content of *Vallisneria spiralis* (Mean value ± SD, *n* = 3). a: Chl-*a*, b: Chl-*b*, c: Chl-*a*+Chl-*b*, d: Chl-*a*/Chl-*b*. 1%, 5%, 10%, 20%, 50%: groups with different the mass proportion of vermiculite in the sediment; 0%: control group.

10% groups were always lower than that in other groups, and the activity of SOD and CAT was always higher. In November, the plant produces a lot of SOD to resist the decay process, and Fig 3b also showed that the SOD activity of 5% and 10% groups were significantly higher than that of the control group ($p < 0.05$).

2.2.4. *Effect of vermiculite on the soluble sugar content of* Vallisneria spiralis

In Fig. 3d, the soluble sugar content of each group has decreased significantly in the first month after the plant was planted. The content of each group has increased to a certain extent after August, indicating that *Vallisneria spiralis* is gradually adapting to the new sediment environment. During the whole experimental period, the soluble sugar content of the 1%, 5%, and 10% groups were always higher than that of the control group, and the content of the 5% group increased significantly in October ($p < 0.05$).

2.3. *Redundancy analysis*

Principal component analysis was carried out to determine the correlation between vermiculite mixing ratio, *Vallisneria spiralis* ecophysiological indexes, and main environmental variables in Fig. 4. PCA results showed that the first and second ordination axes of the PCA eigenvalues were 0.5112 and 0.1714, and the explained variation of the first and second axis were 51.12% and 68.27%, respectively. All submerged plant ecophysiological indexes except for MDA, and sediment chemical properties were negatively correlated with vermiculite mixing ratio, while all of sediment physical properties exhibited positive correlation.

2.4. *Vermiculite dissolution analysis*

The dissolution results of vermiculite were showed in Fig. 5. In the 30-day dissolution test, vermiculite mainly dissolves constant elements such as K, Ca, Mg. The dissolution amount of K and Ca increased with the increase of time. 30 days later, the dissolution amounts of the two elements reached 3.5287 mg/L and 11.1625 mg/L, indicating that vermiculite can continuously dissolve the essential elements needed for plant growth. It can be seen from Fig. 5b that vermiculite can also dissolve trace elements including Fe, Mn, Zn, Cu, B, etc., which are needed for plant growth. The amount of Fe dissolution showed a decreasing trend with the increase of time, while the amount of Zn increased from the 15th day, and the amount of dissolution of B maintained at a relatively stable level. Among these dissolved elements, K and B participate in the development of plant branches and roots, and Mg act as a significant role in chlorophyll synthesis of plants. In addition, vermiculite also dissolves trace amounts of heavy metals, but all dissolution concentration of heavy metal was far below the harmful concentration.

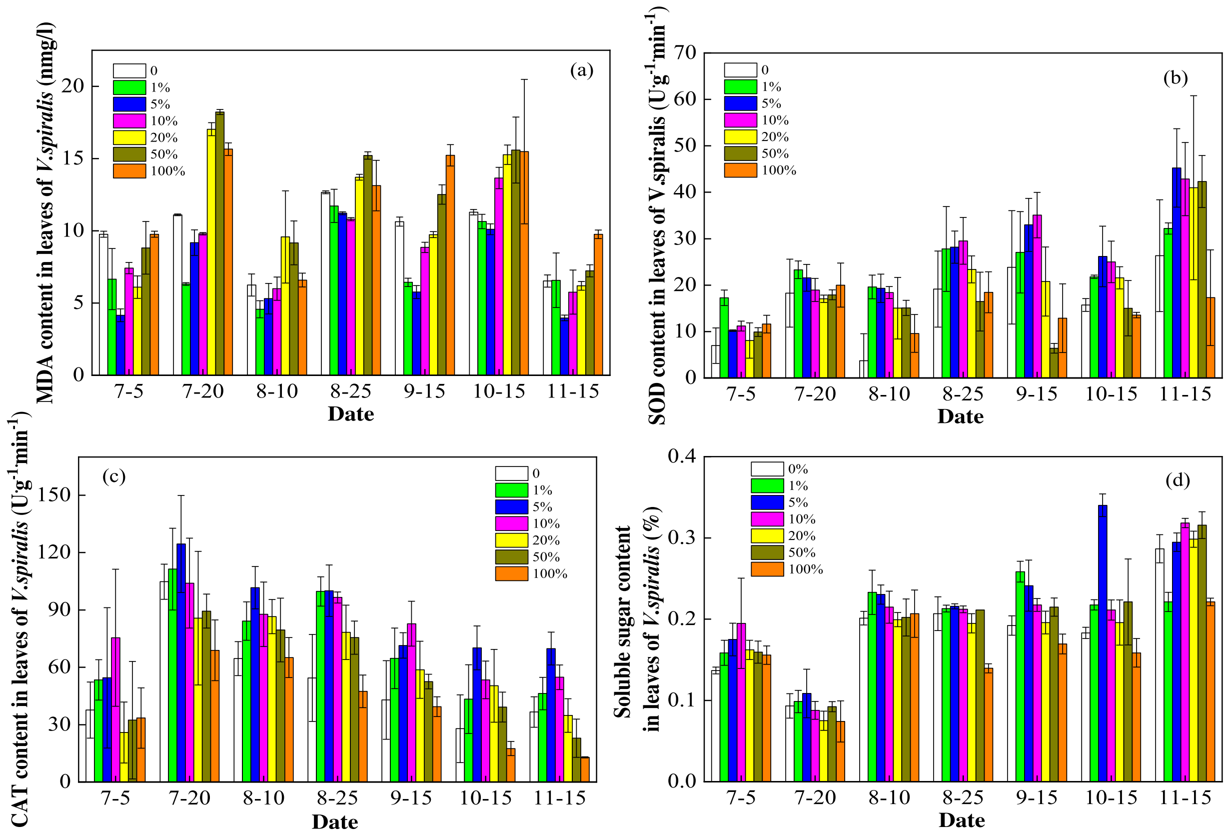

Fig. 3 – Effect of vermiculite on ecophysiological indexes of *Vallisneria spiralis* (Mean value ± SD, *n* = 3). a: MDA content, b: SOD activity, c: CAT activity, d: soluble sugar content. 1%, 5%, 10%, 20%, 50%: groups with different the mass proportion of vermiculite in the sediment; 0%: control group.

Table 1 – α diversity index of bacterial community in different vermiculite content groups.

Date	Sample	OTUs	Chao	ACE	Shannon	Simpson	Shannoneven	Coverage
2019.07.05	0%	1672.0	2135.01	2094.58	4.80	0.04	0.65	0.99
	5%	1701.0	2240.76	2208.57	4.77	0.06	0.64	0.99
	10%	1796.0	2158.65	2208.75	4.63	0.07	0.62	0.99
2019.12.15	0%	2138.0	2622.59	2623.11	5.22	0.03	0.68	0.99
	5%	2061.0	2509.92	2511.67	5.17	0.05	0.68	0.99
	10%	2574.0	2925.28	2910.29	5.31	0.04	0.68	0.99

2.5. *Influence of vermiculite on the substrate microecological environment*

2.5.1. *Influence of vermiculite on microbial diversity of substrate*

Through the above analysis, we can conclude that 5% and 10% vermiculite content have a certain promotion effect on the growth of *Vallisneria spiralis* compared with the control group. In order to further explore the related causes and mechanisms, the rhizosphere microorganisms at the initial and final period of the experiment were measured and analyzed.

The α diversity index of bacteria in different groups of vermiculite content in each stage is listed in Table 1. The microbial coverage rate of all the samples was 99%, which demonstrated that the obtained sequence library could adequately characterize the bacterial community. In 5th July, OTUs detected in 0%, 5%, and 10% groups were 1672, 1701, and 1796, respectively, the 5% and 10% groups were significantly higher than the control group. At the same time, Chao, and ACE indexes in 5% and 10% groups were also higher than that in the control group. In 15th December, the Chao, ACE, Shannon index in each group increased and the Simpson index decreased at the end of the experiment. The number of OTUs in each group increased to 2138, 2061, and 2574, respectively.

2.5.2. *Effects of vermiculite on microbial community structure in substrate*

Fig. 6 showed that the composition and abundance of dominant bacteria at the phylum level in the initial and final period of the experiment for the control group, 5% and 10% treatment groups. There are certain differences in the composition of bacteria between different groups in the same period,

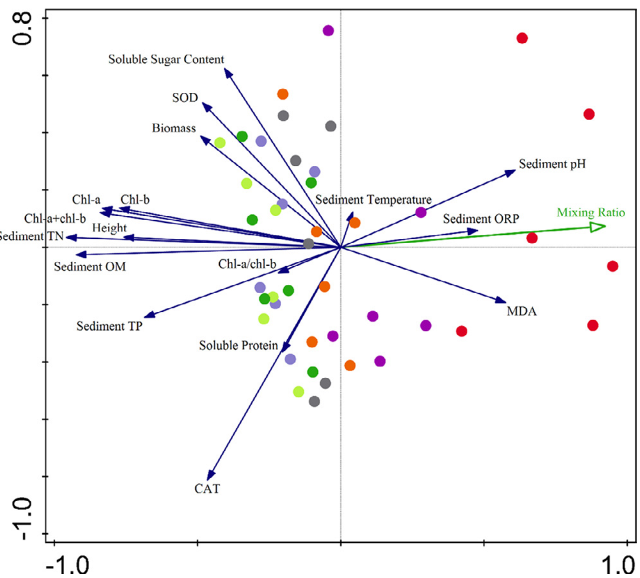

Fig. 4 – PCA results of the impact of vermiculite mixing ratio on *Vallisneria spiralis* ecophysiological indexes and main environmental variables. Gray, light blue, reseda, green, orange, purple and red represent 0%, 1%, 5%, 20%, 50%, and 100% mixing ration, respectively (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

and the same treatment also has significant changes in the two period of the experiment, indicating that the addition of vermiculite has a significant impact on the composition and structure of bacteria in the sediment. Fig. 6a exhibited that the phylum mainly has *Firmicutes, Proteobacteria, Bacteroidetes, Acidobacteria,* and *Actinobacteria,* these dominant species occupy 0.54%–62.28% of the samples, respectively. *Firmicutes* and *Proteobacteria* are the dominant community in all samples, accounting for 73.43%, 75.43%, and 75.33% of the total bacteria in 0%, 5%, and 10% group, as the content of vermiculite increases, the abundance of these two bacteria also increases.

3. Discussion

3.1. *Effect of vermiculite on the growth index of* Vallisneria spiralis

The growth rate results of plant indicates that a certain amount of vermiculite content can promote the growth of plants, but when it exceeds 20%, it has no promotion effect on the growth of plant. Addition of vermiculite with 5% and 10% mixing ratio also can delay the senescence of submerged plant.

3.2. *Effect of vermiculite on ecophysiological indexes of* Vallisneria spiralis

Chlorophyll is the most important pigment for photosynthesis in higher plants (Zhang et al., 2019), and its content reflects the

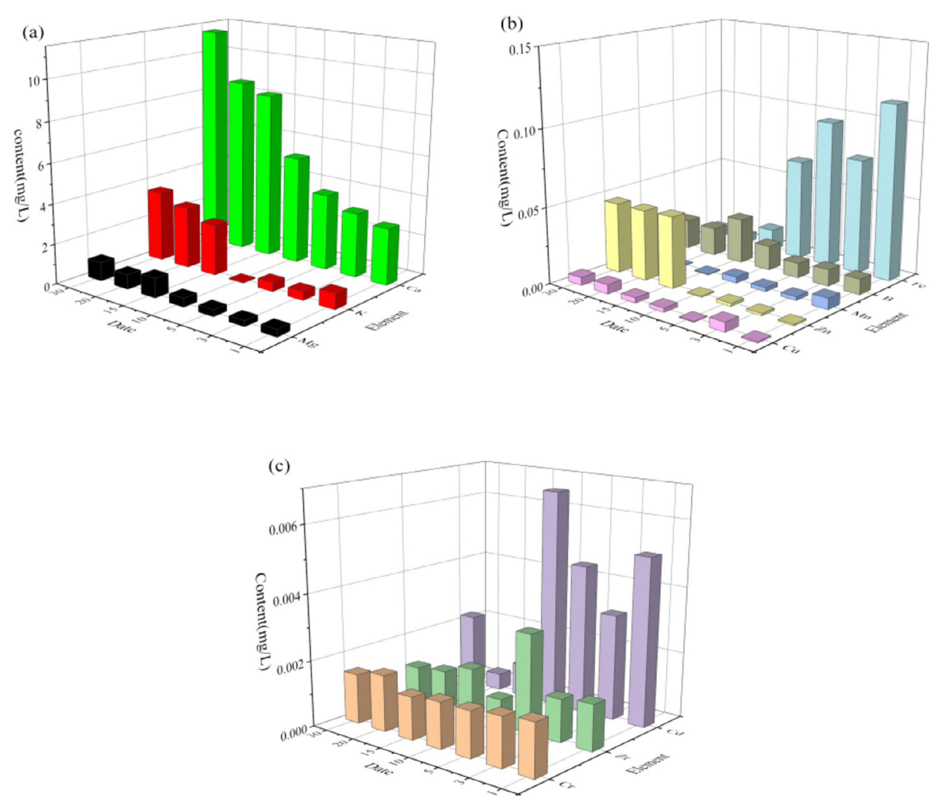

Fig. 5 – Results of dissolution of vermiculite along with time.

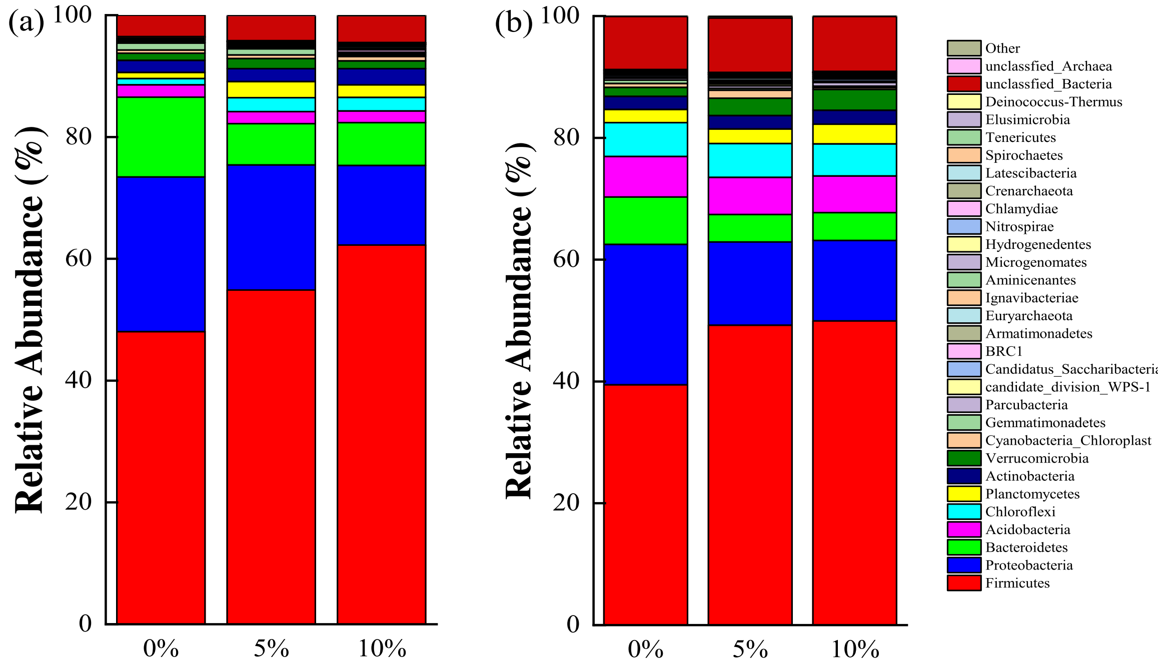

Fig. 6 – Distribution of bacterial 16S rRNA gene sequence in different vermiculite content groups at the phylum in different period. a: Sampling time on 2019.07.05; b: Sampling time on 2019.12.15.

photosynthetic capacity and growth status of plants to some extent (Bartwal et al., 2013). Excessive content of vermiculite is not conducive to promoting the growth of submerged plants, and 5% and 10% vermiculite content are the most suitable for plant growth.

Malondialdehyde (MDA) is one of the most important products of membrane lipid peroxidation (Pilarska et al., 2017). Its production may increase membrane damage. High content indicates that the environment is not suitable for the growth of the plant. The MDA results showed that the environment with less than 10% vermiculite mixing ratio is more suitable for plant growth. On the stage of senescence, the MDA mixing ratio of 5% group was lower than that of the control group, indicating that adding a certain amount of vermiculite could slow down the aging of plants.

Superoxide dismutase (SOD) and catalase (CAT) are antioxidant enzymes produced by plant leaves during senescence. They can remove excess oxygen radicals in plants and thus reduce the damage to plants (Singh et al., 2020). Furthermore, the soluble sugar content can reflect the carbon nutritional status of plants. When the content is higher, the osmotic potential and freezing point can be reduced, to better adapt to changes in external environmental conditions (Passaia and Margis-Pinheiro, 2015). The soluble sugar content of the 1%, 5%, and 10% groups were always higher than that of the control group during the whole experimental period, and the content of the 5% group increased significantly in October ($p < 0.05$), which demonstrated that *Vallisneria spiralis* in the group can better adapt to that environment and has the strongest resistance to cope with changes in the growth environment. The results of SOD and CAT of *Vallisneria spiralis* also support the conclusion that 5% and 10% vermiculite mixing ratio alleviate the stress from environment and its senescence of *Vallisneria spiralis*.

3.3. *Principal component analysis*

Vermiculite mainly affects the growth of plants by changing physical properties of the sediment. This result could be contributed to that vermiculite has a layered structure with water molecules and exchangeable cations between the layers, which affect the pH and ORP of the sediment to a certain extent. In addition, the sample sites have an obviously heterogeneous distribution among every treatment with different vermiculite mixing ratio, especially the treatment with 5% and 10% mixing ratio exhibited the higher improvement of substrate on *Vallisneria spiralis* ecophysiological indexes, and sediment chemical properties, next the treatment with 0%, 1%, and 20% mixing ratio stood transition period, but the treatment with 50% and 100% mixing ratio exhibited the opposite effect. The results are consistent with the preceding results in this study.

3.4. *Vermiculite dissolution analysis*

The main sources of nutrients needed by submerged macrophyte during the growth process are the overlying water and sediment, and the sediment plays a more important role. The addition of vermiculite not only increases the content of constant elements such as K, Ca, Mg in the sediment, but also dissolves trace elements such as B, Fe, and Zn needed for plant growth. Mg is a cofactor of many enzymes and an important part of plant chlorophyll and vitamins (Dalcorso et al., 2013). The addition of vermiculite can enhance the photosynthesis ability of plants, this is the main reason why the chlorophyll content of the 5% and 10% groups is higher than that of the control during the vigorous growth period. Fe directly participates in the processes of electron transport and hormone synthesis (Hamner et al., 2017), K and B can promote the growth of plant branches and roots, and Zn can promote the synthesis of protein, and improve the resistance of plants to high temperature, high salt, frost, and drought (Gupta et al., 2019), therefore, the stress resistance of plants in the 5% and 10% treatment groups were stronger. While the promotion effect is negligible as the mixing ratio exceeds 10%. This may be due to the fact that high concentrations of major and trace elements may not have a positive effect on plant growth.

3.5. *Influence of vermiculite on the substrate microecological environment*

α diversity analysis can reflect the abundance and diversity of the microbial community, including a series of statistical analysis indexes such as Chao, ACE, Shannon, and Simpson values to estimate the microbial diversity in the system. The higher values of Chao, ACE, and Shannon and the smaller the Simpson value, the more abundant the microbial species in the sample (Su et al., 2015). The microbial diversity of each group increased through the growth and reproduction of the plants, and the Chao, ACE, and Shannon indexes of the 10% group were higher than those of the control group, and the microbial species were more abundant.

Firmicutes and *Proteobacteria* are the dominant community in 0%, 5%, and 10% group, and the abundance of these two bacteria also increases as the content of vermiculite increases. Some researchers reported that *Proteobacteria* are widely distributed in sewage treatment systems. The genus of bacteria related to the nitrogen and sulfur cycle plays an important role in the biodegradation or conversion of pollutants (Guan et al., 2015). The result of Fig. 6b showed that the dominant species of bacteria did not change significantly at the end of the experiment. Although the abundance decreased, it was still the main community. However, we found that *Acidobacteria* increased in the 5% and 10% groups, while the abundance of bacteria did not increase in the control group. *Acidobacteria* plays an irreplaceable role in maintaining the stability of the ecosystem (Zhang et al., 2014). In the control group, certain phosphorus removal bacteria such as *Burkholderia,* GP6 and GP7 have lower levels, while in the 5% and 10% groups, they were more abundant. At the same time, the plant growth-promoting bacteria *Bacillus* was found in the 5% and 10% groups.

In aquatic ecosystems, microorganisms are not only an important part of the biomass in the system, but also affect the material circulation and nutrient transfer process (Cotner and Biddanda, 2002), and play an irreplaceable role in plant growth. Rhizosphere microorganisms can synthesize many kinds of auxin to promote plant growth. The function of microorganism enhances the degradation of organic matter, promotes the mineralization of plant nutrient elements, and enhances the supply of plant nutrients, some rhizosphere microorganisms can secrete antibiotic substances to enhance the resistance of plants (Ciurli et al., 2009). Table 1 and Fig. 5 demonstrated that a proper amount of vermiculite mixing ratio in sediment can promote the growth of rhizosphere microflora community and enhance the diversity of microorganisms. This may be due to vermiculite has a special surface structure that is scaly, the scales overlap, and the overall structure is loose and porous. This structure makes vermiculite has a larger specific surface area and more adsorption sites, thereby providing a better microbial growth environment, and providing a subterranean environment more suitable for the growth of submerged macrophyte. It can also be seen from the results of the microbial community structure, as the plant grows, the diversity of microorganisms increases. This is because of the growth of plant roots increases oxygen and adsorption sites for microorganisms. Therefore, 5% and 10% vermiculite mixing ratio improve the sediment physical conditions and create more ecological niche for microorganisms directly, which enhance the diversity and product of microorganisms that benefit to submerged plant growth.

4. Conclusion

The growth indicators and physiological and ecological indicators of the 5% and 10% groups were better than those of the control group. Meanwhile, the constant and trace elements dissolved by vermiculite promoted the growth of plants and improved the resistance of plants. In addition, the promotion of 5% and 10% vermiculite mixing ratio on microbial diversity in the substrate was obviously. These findings attribute to the fact that the porous structure of vermiculite provides more living space for rhizosphere microorganisms and improves the physical properties of sediments. This research demonstrated that 5% to 10% addition amount can promote the growth of submerged macrophyte *Vallisneria spiralis* for the sediment of Xiaonanhu Lake of West Lake in Hangzhou, Zhejiang Province. Therefore, vermiculite further applied to the ecological restoration project has a vital significance in eutrophic lakes.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (Nos. 51709254; 31830013), the Youth Innovation Promotion Association, Chinese Academy of Sciences (No. 2020335), and the Natural Science Foundation of Hubei Province (No. 2020CFB555). We thank Ms. Liping Zhang for the experimental help.

Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2021.08.038.

REFERENCES

Abell, J.M., Ozkundakci, D., Hamilton, D.P., Van, D.P., Mcdowell, R., 2019. Quantifying the extent of anthropogenic eutrophication of lakes at a national scale in New Zealand. Environ. Sci. Technol. 53, 9439–9452.

Bartwal, A., Mall, R., Lohani, P., Guru, S.K., Arora, S., 2013. Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. J. Plant Growth Regul. 32, 216–232.

Cotner, J.B., Biddanda, B.A., 2002. Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5, 105–121.

Ciurli, A., Zuccarini, P., Alpi, A., 2009. Growth and nutrient absorption of two submerged aquatic macrophytes in mesocosms, for reinsertion in a eutrophicated shallow lake. Wetl. Ecol. Manag. 17, 107–115.
Chen, M.S., Cui, J.Z., Lin, J., Ding, S.M., Gong, M.D., Ren, M.Y., et al., 2018. Successful control of internal phosphorus loading after sediment dredging for 6 years: a field assessment using high-resolution sampling techniques. Sci. Total Environ. 616, 927–936.
Da Silva, U.G., Melo, M.A.D., Da Silva, A.F., De Farias, R.F., 2003. Adsorption of crude oil on anhydrous and hydrophobized vermiculite. J. Colloid Interface Sci. 260, 302–304.
Devesa-Rey, R., Fernandez, N., Cruz, J.M., Moldes, A.B., 2011. Optimization of the dose of calcium lactate as a new coagulant for the coagulation-flocculation of suspended particles in water. Desalination 280, 63–71.
Dalcorso, G., Manara, A., Furini, A., 2013. An overview of heavy metal challengein plants: from roots to shoots. Metallomics 5, 1117–1132.
Egemose, S., Reitzel, K., Andersen, F.O., Flindt, M.R., 2010. Chemical lake restoration products: sediment stability and phosphorus dynamics. Environ. Sci. Technol. 44, 985–991.
Guan, W., Yin, M., He, T., Xie, S.G., 2015. Influence of substrate type on microbial community structure in vertical-flow constructed wetland treating polluted river water. Environ. Sci. Pollut. Res. 22, 16202–16209.
Gao, H.L., Shi, Q.Y., Qian, X., 2017. A multi-species modelling approach to select appropriate submerged macrophyte species for ecological restoration in Gonghu Bay, Lake Taihu, China. Ecol. Model. 360, 179–188.
Gupta, N., Yadav, K.K., Kumar, V., Kumar, S., Chadd, R.P., Kumar, A., et al., 2019. Trace elements in soil-vegetables interface: Translocation, bioaccumulation, toxicity and amelioration - a review. Sci. Total Environ. 615, 2927–2942.
Huang, L., Fang, H.W., Fazeli, M., Chen, Y.S., He, G.J., Chen, D.Y., 2015. Mobility of phosphorus induced by sediment resuspension in the three Gorges Reservoir by flume experiment. Chemosphere 134, 374–379.
Human, L.R.D., Snow, G.C., Adams, J.B., Bate, G.C., Yang, S.C., 2015. The role of submerged macrophytes and macroalgae in nutrient cycling: a budget approach. Estuar. Coast. Shelf Sci 154, 169–178.
Hamner, K., Weih, M., Eriksson, J., Kirchmann, H., 2017. Influence of nitrogen supply on macro- and micronutrient accumulation during growth of winter wheat. Field Crops Res. 213, 118–129.
Han, C., Ren, J.H., Wang, Z.D., Yang, S.K., Ke, F., Xu, D., et al., 2018. Characterization of phosphorus availability in response to radial oxygen losses in the rhizosphere of Vallisneria spiralis. Chemosphere 208, 740–748.
Kopacek, J., Borovec, J., Hejzlar, J., Ulrich, K.U, Norton, S.A., Amirbahman, A., 2005. Aluminum control of phosphorus sorption by lake sediments. Environ. Sci. Technol. 39, 8784–8789.
Liu, Z.S., Zhang, Y., Liu, B.Y., Zeng, L., Xu, D., He, F., et al., 2017. Adsorption performance of modified bentonite granular (MBG) on sediment phosphorus in all fractions in the West Lake, Hangzhou, China. Ecol. Eng. 106, 124–131.
Li, Q., Gu, P., Ji, X.Y., Li, H.M., Zhang, J.B., Zheng, Z., 2020. Response of submerged macrophytes and periphyton biofilm to water flow in eutrophic environment: plant structural, physicochemical and microbial properties. Ecotoxicol. Environ. Saf. 189, 109990.
Liu, Y.L., Zou, Y.L.Y., Kong, L.W., Bai, G.L., Luo, F., Liu, Z.S., et al., 2021. Effects of bentonite on the growth process of submerged macrophytes and sediment microenvironment. J. Environ. Manag. 287, 112308.
Moraes, D.S., Rodrigues, E.M.S., Lamarao, C.N., Marques, G.T., Rente, A.F.S., 2019. New sodium activated vermiculite process. Testing on Cu^{2+} removal from tailing dam waters. J. Hazard. Mater. 366, 34–38.
Passaia, G., Margis-Pinheiro, M., 2015. Glutathione peroxidases as redox sensor proteins in plant cells. Plant Sci. 234, 22–26.
Pilarska, M., Skowron, E., Pietras, R., Krupinska, K., Niewiadomska, E., 2017. Changes in lipid peroxidation in stay-green leaves of tobacco with senescence-induced synthesis of cytokinins. Plant Physiol. Biochem. 118, 161–167.
Rama, M., Laiho, T., Eklund, O., Warna, J., 2019. An evaluation of the capability of nanomodified vermiculite to *in situ* ammonium removal from landfill leachate. Environ. Technol. Innov. 14, 100340.
Su, J.Q., Wei, B., Ou-Yang, W.Y., Huang, F.Y., Zhao, Y., Xu, H.J., et al., 2015. Antibiotic resistome and its association with bacterial communities during sewage sludge composting. Environ. Sci. Technol. 49, 7356–7363.
Song, Y., Liew, J.H., Sim, D.Z.H., Mowe, M.A.D., Mitrovic, S.M., Tan, H.T.W., et al., 2018. Effects of macrophytes on lake-water quality across latitudes: a meta-analysis. Oikos 128, 468–481.
Singh, D.P., Singh, V., Shukla, R., Sahu, P., Prabha, R., Gupta, A., et al., 2020. Stage-dependent concomitant microbial fortification improves soil nutrient status, plant growth, antioxidative defense system and gene expression in rice. Microbiol. Res. 239, 126538.
Tang, X.Q., Wu, M., Dai, X.C., Chaia, P.H., 2014. Phosphorus storage dynamics and adsorption characteristics for sediment from a drinking water source reservoir and its relation with sediment compositions. Ecol. Eng. 64, 276–284.
Xie, Y.H., An, S.Q., Yao, X., Xiao, K.Y., Zhang, C., 2005. Short-time response in root morphology of Vallisneria natans to sediment type and water-column nutrient. Aquat. Bot. 81, 85–96.
Yang, X.S., Chen, G.X., Wei, X.D., Xie, K.B., 2011. Enhanced antioxidant protection at the early stages of leaf expansion in ginkgo under natural environmental conditions. Biol. Plant. 56, 181–186.
Yang, M.J., Lin, J.W., Zhan, Y.H., Zhang, H.H., 2014. Adsorption of phosphate from water on lake sediments amended with zirconium-modified zeolites in batch mode. Ecol. Eng. 71, 223–233.
Yin, H.B., Kong, M., 2015. Reduction of sediment internal P-loading from eutrophic lakes using thermally modified calcium-rich attapulgite-based thin-layer cap. J. Environ. Manag. 151, 178–185.
Zhong, J.C., You, B.S., Fan, C.X., Li, B., Zhang, L., Ding, S.M., 2008. Influence of sediment dredging on chemical forms and release of phosphorus. Pedosphere 18, 34–44.
Zhao, Y., Yang, Z.F., Xia, X.H., Wang, F., 2012. A shallow lake remediation regime with Phragmites australis: incorporating nutrient removal and water evapotranspiration. Water Res. 46, 5635–5644.
Zhang, Y.G., Cong, J., Lu, H., Li, G.L., Qu, Y.Y., Su, X.J., et al., 2014. Community structure and elevational diversity patterns of soil Acidobacteria. J. Environ. Sci. 26, 1717–1724.
Zhu, T.S., Cao, T., Ni, L.Y., Ni, L., He, L., Yi, C.L., et al., 2016. Improvement of water quality by sediment capping and re-vegetation with Vallisneria natans L.: a short-term investigation using an *in situ* enclosure experiment in Lake Erhai, China. Ecol. Eng. 86, 113–119.
Zhang, S.M., Yang, C., Chen, M.M., Chen, J., Pan, Y.H., Chen, Y.L., et al., 2019. Influence of nitrogen availability on Cd accumulation and acclimation strategy of Populus leaves under Cd exposure. Ecotoxicol. Environ. Saf. 180, 439–448.
Zhen, Z., Yan, C.Z., Zhao, Y., 2020. Influence of epiphytic bacteria on arsenic metabolism in Hydrilla verticillata. Environ. Pollut. 261, 114232.