Journal of Environmental Management 287 (2021) 112308

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: http://www.elsevier.com/locate/jenvman

Effects of bentonite on the growth process of submerged macrophytes and sediment microenvironment

Yunli Liu [a,b], Yilingyun Zou [a,b], Lingwei Kong [c], Guoliang Bai [d], Feng Luo [e], Zisen Liu [a], Chuan Wang [f], Zimao Ding [e], Feng He [a], Zhenbin Wu [a], Yi Zhang [a,b,*]

[a] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China
[b] University of Chinese Academy of Sciences, Beijing, 100049, China
[c] School of Engineering, Westlake University, Hangzhou, Zhejiang, 310024, China
[d] School of Environmental Studies, China University of Geosciences, Wuhan, 430074, China
[e] School of Resources and Environmental Engineering, Wuhan University of Technology, Wuhan, 430070, China
[f] Faculty of Resources and Environmental Science, Hubei University, Wuhan, 430062, China

ARTICLE INFO

Keywords:
Modified bentonite
V. spiralis
Growth process
Sediment microenvironment
Rhizosphere microorganisms

ABSTRACT

The effects of clay mineral bentonite on the growth process of submerged macrophyte *V. spiralis* and sediment microenvironment were investigated in the study for the first time, aiming to determine whether it is suitable for application in the field of ecological restoration. The growth index, and physiological and biochemical index of *V. spiralis* in the experiments were measured once a month, and the changes of rhizosphere microorganisms and physicochemical properties of sediments were also studied at the same time. The results demonstrated that bentonite can effectively promote the growth of *V. spiralis*. The treatment groups of RB1/1 and MB1/5 (the mass ratios of bentonite to sediment were 1/1 and 1/5, respectively.) showed the best *V. spiralis* growth promotion rates which were 18.78%, and 11.79%, respectively. The highest microbial diversity and abundance existed in group of RB10 (the mass ratio of sediment to bentonite was 10/1), in which the OTUs, Shannon, Chao and Ace were 1521.0, 5.20, 1712.26, and 1686.31, respectively. Bentonite was conducive to the propagation of rhizosphere microorganisms, and further changed the physical and chemical properties of the sediment microenvironment. The nutrient elements dissolved from bentonite may be one of the main reasons that promoted the growth of *V. spiralis*. The purpose of this result is to prove that bentonite can be further applied as sediment improver and growing media in ecological restoration projects in eutrophic shallow lakes.

1. Introduction

Eutrophication has posed a serious threat to the sustainability of aquatic ecosystems (Zhang et al., 2015; Wu et al., 2020; Morelli et al., 2018). Bioremediation technology has gradually become the research hotspot of ecological remediation technology due to its low cost, environmental friendliness and lasting effect. Submerged macrophyte plays an important role in bioremediation for improving productivity, stabilizing sediment, and reducing N and P nutrients loads (Lynette et al., 2010).

Sediment can provide nutrients for submerged macrophyte, the condition of lake sediment is directly related to the propagation, root taking and growth of plants (Barko et al., 1991; Xie et al., 2005). However, many eutrophicated and special sediment contain a high level of organic matter, nitrogen and phosphorus, which are not conducive to the growth of submerged plants (Jeppesen et al., 2007; Zhu et al., 2016). Thus, improving the physical and chemical conditions of sediment is a necessary measure for water ecological restoration.

The sediment remediation technologies include sediment dredging, biological ventilation and aeration, and in-situ sediment improvement, etc. Sediment dredging and biological aeration often have problems such as large amounts of work, occupied land, and waste of resources (Choi et al., 2016; Pourabadehei et al., 2016). Therefore, more and more scholars have begun to pay attention to the research and development of in-situ sediment improvement technology. The sediment improver includes biochar (Chi et al., 2016), Phoslock (Meis et al., 2012) and

* Corresponding author. State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China.

E-mail address: zhangyi@ihb.ac.cn (Y. Zhang).

https://doi.org/10.1016/j.jenvman.2021.112308
Received 23 November 2020; Received in revised form 22 February 2021; Accepted 1 March 2021
Available online 8 March 2021
0301-4797/

mineral materials (Liu et al., 2020). Biochar is usually prepared by pyrolysis of rice husk, straw, reed, peanut shell, bamboo, etc., which has a complicated process and is easy to inhibit the root growth of submerged plants (Li et al., 2020). Although compound preparations such as phosphorus lockers are beneficial to the growth of plant roots to a certain extent, they often inhibit the growth of plant aboveground parts (Dong et al., 2011).

Bentonite is a type of clay with a high adsorption capability of anionic, cationic and organic pollutants (Karaca et al., 2016; Shanableh et al., 2015). Raw bentonite (RB) has been widely studied as adsorbing material in environmental remediation due to the characteristics of inexpensive, environment friendly, high cation exchange capacities and strong sorption properties (Karaca et al., 2016; Reitzel et al., 2013). Researchers have found modified bentonite (MB) may improve the sorption properties (Dithmer et al., 2015; Oveissi et al., 2014; Liu et al. (2017)). In order to effectively apply bentonite in ecological remediation, it is necessary to investigate its effect on the growth process of submerged plants and sediment microenvironment.

In order to promote the effective application of bentonite in the field of water ecological restoration, this research was carried out from the following aspects: (1) investigate the effects of RB and MB on the growth of *V. spiralis*. (2) determine the changes of rhizosphere microorganisms during the experiment. (3) analyze the variation of physical and chemical properties of substrate in the growth process of the plant. (4) explore the mechanism of plant growth promoted by bentonite.

2. Materials and methods

2.1. Study site and sediment collection

Surface sediments (0–10 cm) from Xiaonan Lake (30°23′16″N, 120°13′18″E), one sub-lake of West Lake, located in Hangzhou, China, were collected using a Peterson grab sampler. The Eh 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 h, and then sieved with a stand 100-mesh sieve for further testing (Liu et al., 2017). Sediment organic materials content was measured using an ignition method. Contents of TP and IP were tested based on the Standards of Measurements and Testing (SMT method) (Ruban et al., 2001). The values of Eh, pH, TN, TP and TOC of sediment in West Lake were 52.6 mv, 6.86, 7750 mg/kg, 1313 mg/kg and 13.1%, respectively. The dried sediment was filtered with a sieve to remove large dinas and residues of plants and animals.

2.2. Materials

The RB with the diameter of 3–5 mm was purchased from Qingdao Sheng Pulin Environmental Technology Co., Ltd. RB were mixed with Na_2CO_3 (10%) solution at liquid/solid of 3 mL/g at 70 °C for 2 h in the thermostatic water bath, and then washed with distilled water until the pH value reached 7.0, dried at 150 °C for 24 h. Finally, the samples were calcined in a muffle furnace (450 °C, 2 h), named as MB (Wang et al., 2018; Yang et al., 2011). The typical chemical compositions and physical properties of RB and MB were directly measured using an X-ray fluorescence spectrometer (Zetium-Ultimate edition, Netherlands), whose detection limit is 1.0–50 mg/kg, and the results were shown in Table .1. Liu et al., 2017 have observed the comparison of morphology and microstructure between RB and MB (modified by nitrification and calcination), the result showed that RB displayed a loose and porous microstructure, and the microstructure of MB was microporous and rough. The *V. spiralis* seedings, a common submerged macrophytes in shallow lakes which has the characteristics of strong adaptability and grow fast were purchased from Hangzhou Lixin Landscaping Co., Ltd. After incubating in the phytotron for 7 d, the healthy and equal-sized submerged plants were selected and pruned to a uniform length of 15 cm, biomass of 1 g and root length of 4 cm before they were transplanted to aquariums.

Table 1
Physical properties and chemical compositions of RB (a) and MB (b) (wt%).

Properties		A	b
Grain size	SiO_2	3–5 mm	2–4 mm
Major element (wt.%)	Al_2O_3	58.36	55.94
pH	MgO	19.55	19.07
Loss on ignition	Fe_2O_3	4.04	4.04
	CaO	4.39	4.65
	Na_2O	2.93	3.50
	K_2O	0.40	3.95
	ZnO	0.58	0.41
		0.02	0.02
		6.5–7.5	6.5–7.5
		1.5	1.9

2.3. Batch experiments

2.3.1. Effect of bentonite on the growth of V. Spiralis

The experiments were carried out in aquariums, all experimental apparatus were placed in the standard laboratory located in Wuhan, China. The indoor temperature was about 25 °C, indoor atmospheric pressure was about 101.325 Pa. 20 plants *V. spiralis* were transplanted in a series of tanks. Different mass ratio of bentonite to sediment 1/10, 1/5, 1/1, 5/1, and 10/1 were named as RB1/10, RB1/5, RB1/1, RB5/1, and RB10/1, respectively, the nomenclature was the same as in the modified bentonite groups. Aquariums contained a single component named RB100, MB100 and control group (sediment). The samples were carried out monthly.

During the experiment, three plants were sampled at random. After washing, the height and root length of the plant were measured with a tape measure, used a scale to weigh the biomass, recorded the leaf number of *V. spiralis*. 2 g leaves were collected every month to measure physiological and biochemical indicators, the chlorophyll content of leaves was detected by the Lichtenthaler-Arnon method, malondialdehyde (MDA) content and peroxidase (POD) activity were respectively determined by the thiobarbituric acid and guaiacol method (Yang et al., 2011).

2.3.2. Sediment sampling and analysis

The redox potential (Eh), organic materials, inorganic phosphorus (IP), and total phosphorus (TP) of the sediment can reflect the influence of bentonite on the physical and chemical properties of the sediment, and the sediment samples were carried out once a month. The Eh of sediments were directly measured using a portable instrument Thermo Orion-230 m (Shanghai, China) during sediment sampling. Sediments were centrifuged at 2000 rpm for 10 min by a centrifuge (Sigma 3–30 KS, Germany), and precipitates were air-dried and screened for sample determination (Lin et al., 2019).

Sediment organic materials content was measured using ignition method. Contents of total phosphorus (TP) and inorganic phosphorus (IP) were tested based on the Standards of Measurements and Testing (SMT method) (Ruban et al., 2001).

2.3.3. Material dissolution test

200 g of RB and MB 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 the 1 d, 3 d, 5 d, 10 d, 15 d, 20 d, and 30 d, 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%.

2.3.4. Microcosm experiments

The sediment samples (10 g) 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 sediment microcosm samples were rapidly transferred to centrifuge tube (10 mL) and all samples were stored at −80 °C frozen refrigerator for further analysis.

The high-throughput sequencing technology was used to detect the microbial community structure of all samples. Omega E.Z.N.A™ Mag-Bind DNA kit was used to extract DNA from sediment samples at various stages, and the V3–V4 region of 16Rrna gene was amplified with forward primer 341 F(CCTACGGGNGGCWGCAG) and reverse primer 805 R (GGACTACHVGGGTATCTAATCC). After a series of operations, such as purification, quantification, primer sequence removing, PE reads splicing, and quality control, the effective data was obtained. The composition and abundance of microbial communities at different levels were used for further analysis. All carriers used in the experiment were sterilized in autoclave (sterilization at 121 °C for 30 min) before sampling.

2.3.5. Data analyses

Using the SPSS program (SPSS Inc., Chicago, IL, USA; Version 23.0) to analyze all the test data, and the acceptable statistical significance level is $\alpha = 0.05$. The two-way ANOVA was implemented to compare the differences between the mean values of the index between the treatments. The data was expressed as mean ± SD, and the number of parallel samples for each treatment was three.

The Alpha diversity and richness of the microbial communities in the sediments of each treatment group were analyzed on the basis of the microbial data. At the same time, the taxonomic composition of the rhizosphere microflora community was statistically analyzed on the genus level. The calculation formulas used in the analysis were as follows:

$$C = 1 - \frac{n_1}{N}$$

$$H_{shannon} = -\sum_{i=1}^{S_{obs}} \frac{n_i}{N} \ln \frac{n_i}{N}$$

$$S_{chao1} = S_{obs} + \frac{n_1(n_1 - 1)}{2(n_2 + 1)}$$

where, $S_{chao1}$is the number of OTU estimated; Sobs is the number of OTU actually observed; n_i is the number of sequences contained in the OTU; n_1 is the OTU number of singletons; n_1 is the OTU number of doubletons; N is the number of all individuals, in this case the total number of sequences.

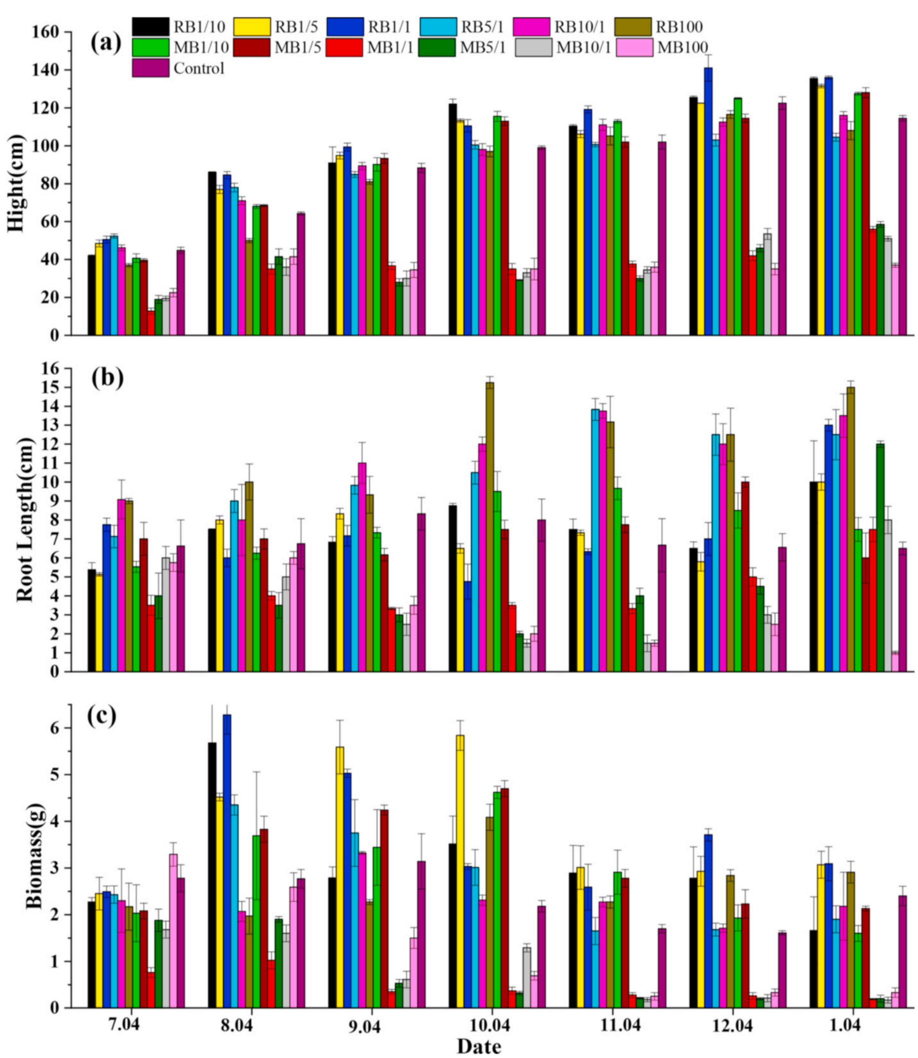

Fig. 1. Effect of bentonite on growth index of *V. spiralis* (a: height, b: root length, c: biomass). Mean value ± SD, n = 3. MB1/10, MB1/5, MB1/1, MB5/1, MB10/1: groups with different mass ratio of modified bentonite to sediment (1/10, 1/5, 1/1, 5/1,10/1); RB1/10, RB1/5, RB1/1, RB5/1, RB10/1: groups with different mass ratio of raw bentonite to sediment (1/10, 1/5, 1/1, 5/1,10/1); RB100, MB100: Aquariums contained a single component; control group: sediment group. Mean value ± SD, n = 3.

3. Results and discussions

3.1. Effect of bentonite on the growth index of V. Spiralis

The plant growth index can directly reflect the growth status, so the height, biomass and root length of *V. spiralis* were measured during the test. Fig. 1 showed the plant growth rate slowed down and gradually reached a stable level after 4 months. Compared with the control group, groups with high proportion of the material inhibited plant growth, while low proportion of the material promoted plant growth. It can be seen from Fig. 1(a) and Table.S1, the promoting rates of plants in the test group of RB1/10, RB1/5, RB1/1, RB5/1, RB10/1, RB100, MB1/10, MB1/5, MB1/1, MB5/1, MB10/1, MB100 and control were 18.34%, 14.85%, 18.78%, −8.73%, 1.31%, −5.68%, 11.35%, 11.79%, −51.09%, −48.91%, −55.46% and −67.69%, respectively. Fig. 1(b) and Table.S1 showed the plant root promoting rates by the materials in the groups of RB1/10, RB1/5, RB1/1, RB5/1, RB10/1, RB100, MB1/10, MB1/5, MB1/1, MB5/1, MB10/1, MB100 and control were 53.85%, 53.85%, 100%, 92.31%, 107.69%, 130.77%, 15.38%, −7.69%, 15.38%, 84.62%, 23.08%, and −84.62%, respectively. Fig. 1(c) and Table.S1 indicated the plant biomass promoting rates by the materials in groups were −31.12%, 27.39%, 28.22%, −21.16%, −9.54%, 20.75%, −33.61%, −11.62%, −92.12%, −91.70%, −92.95%, and −86.31%, correspondingly.

To sum up, according to the changes of plant height, root length and biomass in each treatment group, RB was more conducive to plant growth than the MB, and the substrate soil of RB1/1, MB1/5, and MB1/10 had better growth-promoting effect on plants than other groups.

3.2. Effect of bentonite on physiological and phytochemical indexes of V. Spiralis

3.2.1. Chlorophyll content of V. Spiralis

The chlorophyll content is related to the photosynthetic capacity of plants, and to some extent can reflect the metabolic rate of plants (Bartwal et al., 2013). There are many factors affecting chlorophyll synthesis, such as light, mineral elements, temperature and oxygen. The changes of chlorophyll content in *V. spiralis* were shown in Fig. 2. Fig. 2 (c) and Table.S2 indicated the average total chlorophyll of *V. spiralis* in the groups of RB1/10, RB1/5, RB1/1, RB5/1, RB10/1, RB100, MB1/10, MB1/5, MB1/1, MB5/1, MB10/1, MB100, and control were 0.827, 0.901, 0.885, 0.800, 0.739, 0.436, 0.815, 0.806, 0.626, 0.563, 0.591, 0.597, and 0.926 mg/kg, respectively. In the course of the experiment, there were some decreases in synthesis of plant chlorophyll in all the treatments compared with the control group, which may be related to the Mg and N leached from the substrates is lower than the control

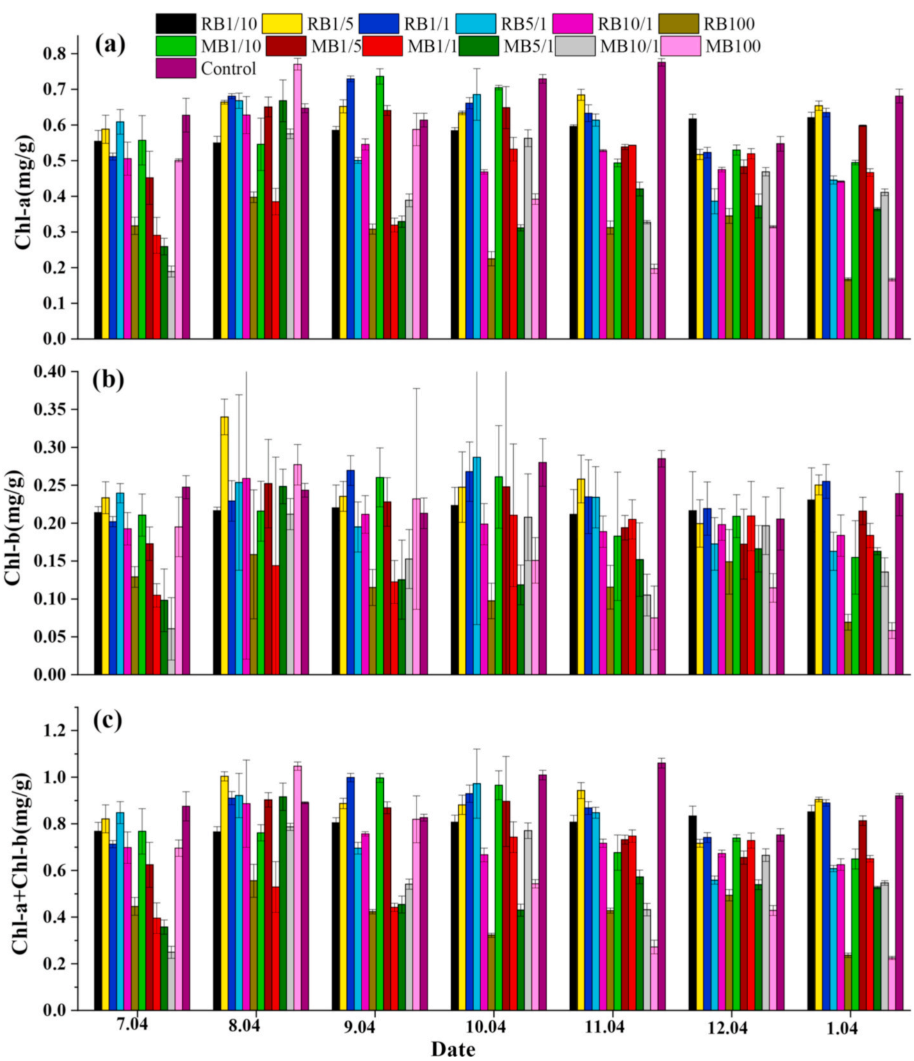

Fig. 2. Effect of bentonite on chlorophyll content of *V. spiralis*. (a: Chl-a, b: Chl-b, c: Chl-a+Chl-b). MB1/10, MB1/5, MB1/1, MB5/1, MB10/1: groups with different mass ratio of modified bentonite to sediment (1/10, 1/5, 1/1, 5/1,10/1); RB1/10, RB1/5, RB1/1, RB5/1, RB10/1: groups with different mass ratio of raw bentonite to sediment (1/10, 1/5, 1/1, 5/1,10/1); RB100, MB100: Aquariums contained a single component; control group: sediment group. Mean value ± SD, n = 3.

group, and affects the plant chlorophyll synthesis. In addition, luxuriant growth of leaves in RB1/10, RB1/5, RB1/1, MB1/10, and MB1/5 weaken the illumination intensity.

3.2.2. Effects of bentonite on the enzymatic system of V. Spiralis

In the process of plant growth, reactive oxygen species will be continuously produced, and the enzymatic systems will be formed to remove reactive oxygen species, so as to maintain a dynamic balance between the production and removal of reactive oxygen species in plants. The reactive oxygen scavenging system gradually decreases over time, resulting in the accumulation of reactive oxygen radicals. Finally, the membrane peroxidation of plants is induced. MDA level can reflect the degree of plant membrane lipid peroxidation, the high content of MDA indicates a high harm degree occurred in plants (Pamplona et al., 2011). Peroxidase (POD) is closely related to respiration and photosynthesis, it can eliminate reactive oxygen species and membrane lipid peroxidation, thus reducing the damage to plants (Chen et al., 2020).

Fig. 3 showed the change of the POD activity and MDA content in leaves of *V. spiralis* throughout this experiment. In the early stage of the experiment, the activity of POD enzyme was the highest due to the new substrate circumstances, and it maintained at relatively stable values at other times (Fig. 1(a)). The POD activity of plants was negatively correlated with the content of bentonite, that may be due to the plants in high proportion mixture groups were damaged, which was the result of limited defensive ability, meanwhile, the result of MDA content showed that the degrees of membrane lipid peroxidation have decreased in low proportion mixture groups compared with the control group.

In conclusion, the *V. spiralis* in low mixture proportion groups (the mass ratio is less than 1:1) showed a good enzymatic defense system compared with the sediment group, and the damage degree of the plant could be alleviated to some extent.

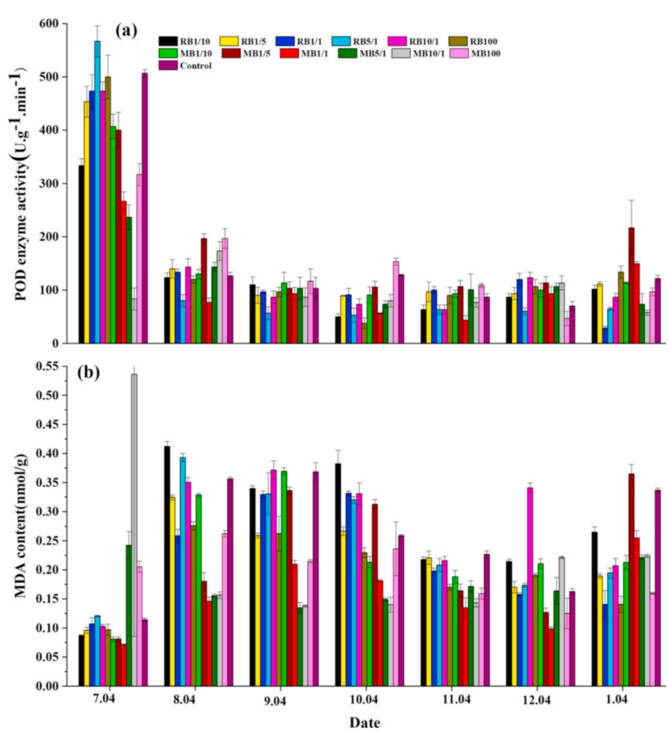

Fig. 3. Effect of bentonite on enzymes of *V. spiralis*. (a: POD activity, b: MDA content). MB1/10, MB1/5, MB1/1, MB5/1, MB10/1: groups with different mass ratio of modified bentonite to sediment (1/10, 1/5, 1/1, 5/1,10/1); RB1/10, RB1/5, RB1/1, RB5/1, RB10/1: groups with different mass ratio of raw bentonite to sediment (1/10, 1/5, 1/1, 5/1,10/1); RB100, MB100: Aquariums contained a single component; control group: sediment group. Mean value ± SD, n = 3.

3.3. Physical and chemical properties variation in sediment

As shown in Fig. 4(a) and Fig. 4(b), both the redox potential (Eh) and organic matter (OM) content of the RB group were higher than those of the MB group, which indicating more plant roots exuded oxygen in the RB group and the plant roots were more active. The change of organic matter content was consistent with the Eh variation, which was related to the aerobic degradation of organic matter by microorganisms. At the end of the experiment, the Eh decreased compared with that of the early stage, which might be caused by the decreased rhizosphere oxygen secretion capacity of aging plants, and the consumption of a large amount of oxygen in the organic matter degradation by microorganisms.

The variation of TP and IP in the substrate of all treatment groups were similar, and the content of TP in the sediment of all treatment groups decreased during the experiments due to the absorption of IP in the sediments by *V. spiralis* and microbial mineralization. The content of sediment was directly proportional to TP content in the substrate (Fig. 4(c)). The high-proportion mixed materials were not conducive to P degradation due to the lack of phosphorus-degrading microorganisms and the poor growth of plant roots. When substrates mixed with the same proportion of materials, the contents of TP in RB groups were lower than that of MB groups for more beneficial growth environment to microorganisms and roots in RB groups (Fig. 6(c) and Fig. 6(d)).

In conclusion, bentonite is beneficial to the degradation of phosphorus in the sediments and the increase of dissolved oxygen in the rhizosphere, thus providing a better substrate environment for the growth of submerged plants.

3.4. Redundancy analysis

Redundancy analysis was performed on the test results to explain the relationship between submerged macrophytes characters and substrate conditions. The first and second ordination axes of the RDA eigenvalues were 0.3322 and 0.2700, respectively. The correlation of the first axis between plant characters and substrate conditions was 0.7488 and accounted for 33.22%, the values of the second axis were 0.8139 and 60.22%, respectively. The major submerged macrophytes characters contributed by substrate were plant height, root length and POD (Fig. 5).

It can be seen from Fig. 5 that the biomass, height, chlorophyll content, and sediment organic matter were negatively affected by the mixing ratio or modification, which indicate that RB groups with low mixture ratio are slightly better groups in this study. The results showed root length and sediment P content were positively correlated with mixing ratio and modification, respectively. Fig. 5 displayed that MB1/1, MB5/1, MB10/1, and MB100 groups with low mixture bentonite were beneficial to the growth of *V. spiralis.* RB with mass ratio of 5/1, 10/1, and 100 can mostly promote root growth.

3.5. Elements dissolution of the bentonite

As can be seen from Fig. 6, bentonite can dissolve some major and trace elements conducive to plant growth. The dissolved elements include Cd, Pb, Cr, As and Fe, among which the content of Fe and As in MB groups were higher than that in RB, and a large amount of dissolution of Fe and As might be the reason for *V. spiralis* growth inhibition under the high mixture proportion of materials. The dissolution concentration of other heavy metals, except elements of Fe and As, is basically close to zero that far below the harmful concentration. According to the dissolution results, both the RB and MB have dissolved some nutrient elements needed for plant growth. The concentration of dissolution of B, Mg, Ca, K, Cu, Mn and Zn in RB were 0.1739, 11.1084, 101.5037, 9.7179, 0.015, 0.0072 and 0.0433 mg/L, respectively, and the corresponding proportions of the MB were 0.5824, 12.8339, 2.489, 5.8728, 0.0086, 0.0207 and 0.0338 mg/L. The elements of K and B are related to the development of plant branches and roots, and Mg is related to the chlorophyll synthesis in plants.

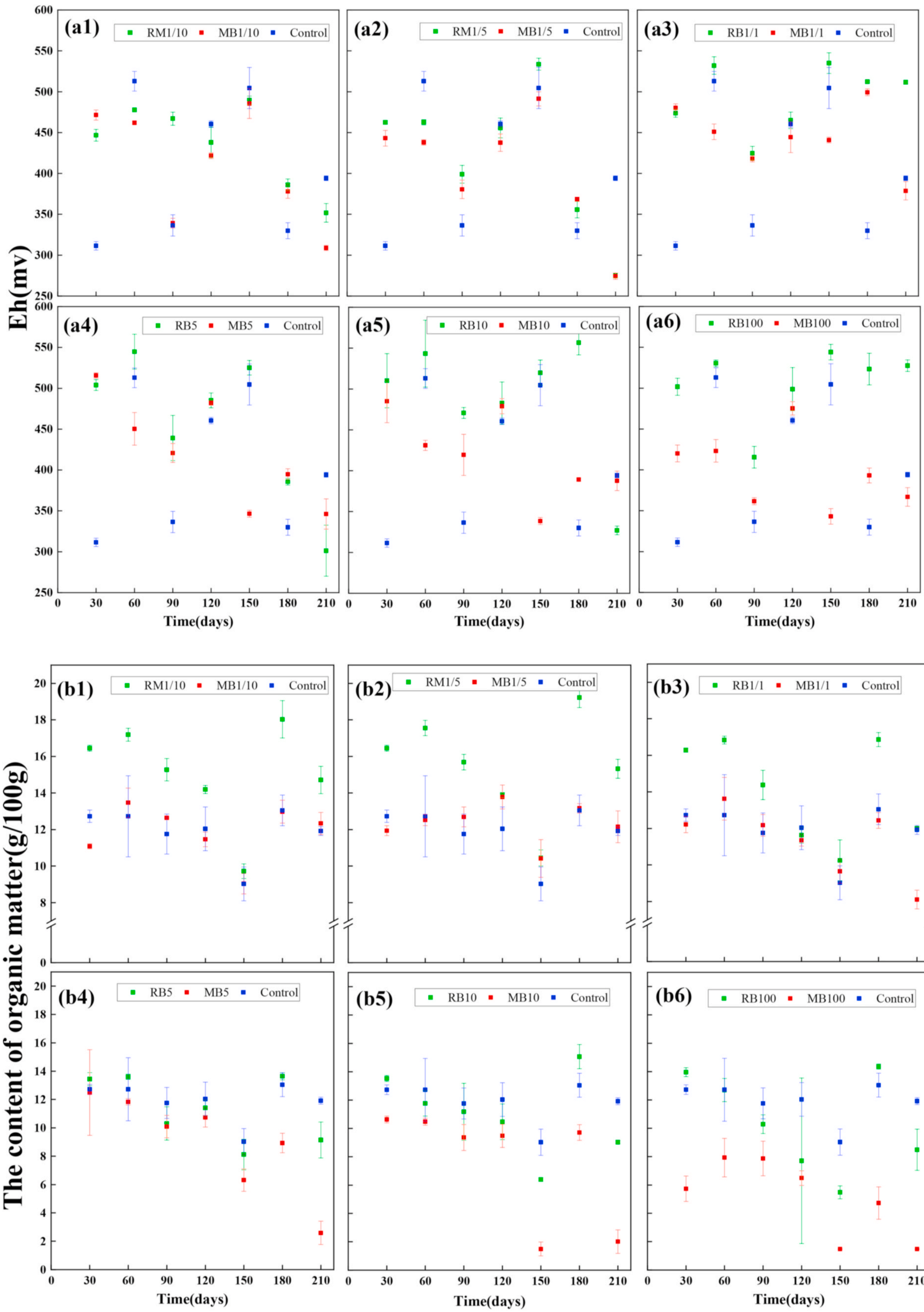

Fig. 4. The Eh (a1-a6) and concentration of organic matter (b1-b6), TP (c1-c6) and IP (d1-d6) in sediments. Data represent means ± SD. n = 3. MB1/10, MB1/5, MB1/1, MB5/1, MB10/1: groups with different mass ratio of modified bentonite to sediment (1/10, 1/5, 1/1, 5/1,10/1); RB1/10, RB1/5, RB1/1, RB5/1, RB10/1: groups with different mass ratio of raw bentonite to sediment (1/10, 1/5, 1/1, 5/1,10/1); RB100, MB100: Aquariums contained a single component; control group: sediment group. Mean value ± SD, n = 3.

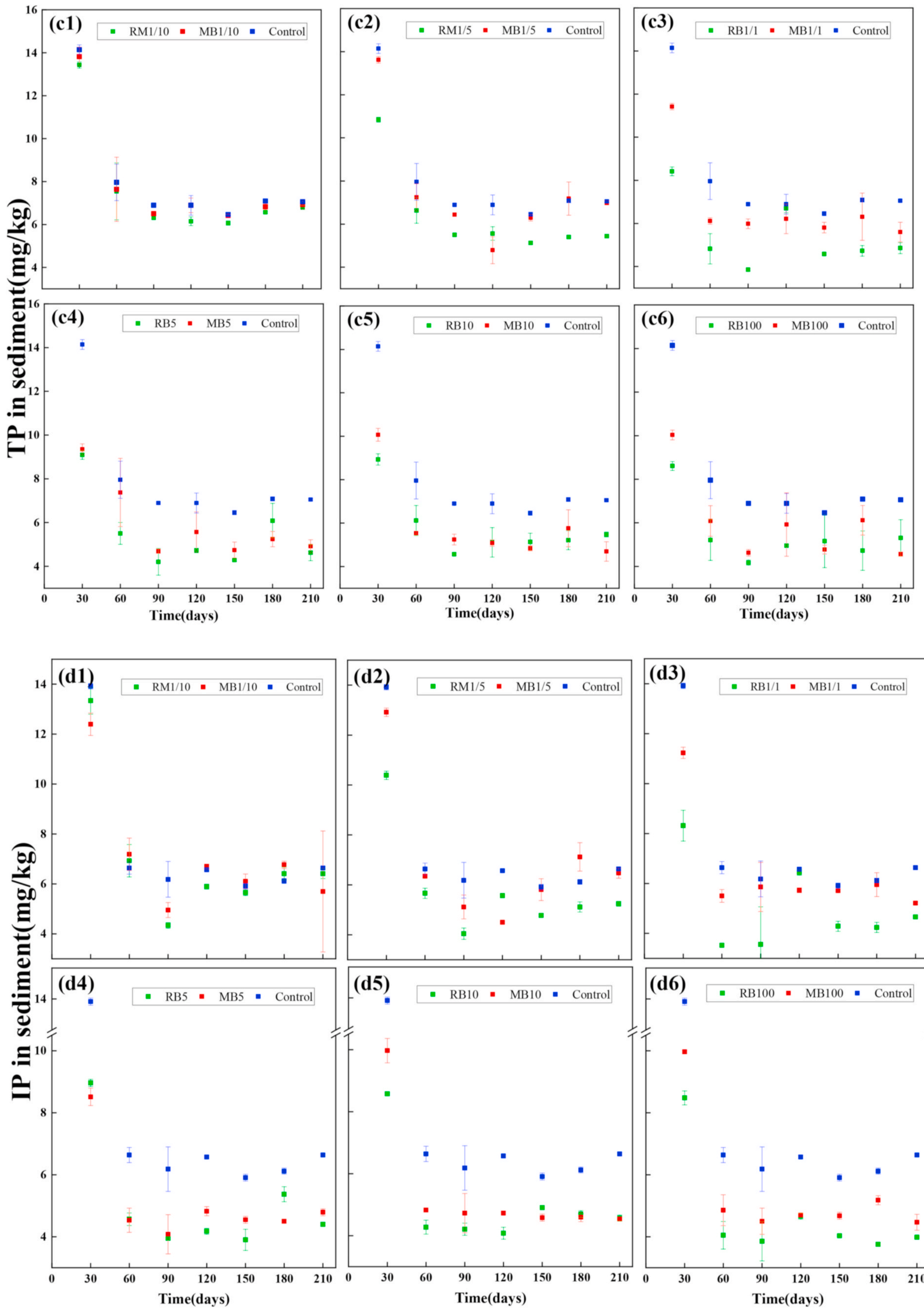

Fig. 4. (*continued*).

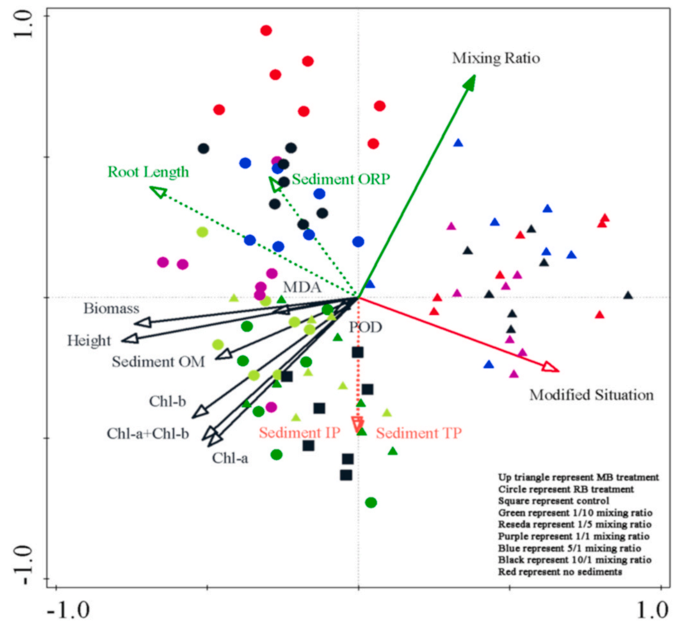

Fig. 5. RDA result of submerged macrophytes characters correlated with the substance.

3.6. Microbial ecology analysis

Some rhizosphere microorganisms, such as *Azotobacter* (Kraepiel et al., 2009), *Azospirillum* (Steenhoudt and Vanderleyden, 2000), *Azorhizobium* and *Rhizobium* (Cocking, 2003), were proved to promote plant root growth and development by mechanism of biofertilizer, biostimulator or biocontrol (Chauhan et al., 2015; Gupta et al., 2018; Urakawa et al., 2017).

Alpha diversity, a great statistical tool to estimate microbial diversity and abundance, was calculated using metrics observed species (OTU), Chao1, Ace, Simpson and Shannon (Rodrigues et al., 2015; Su et al., 2015). Table .2 displayed the alpha diversity and richness of the microbial communities in the sediments of each treatment group. At the end of the experiment, OTUs, Shannon, Chao and Ace of RB10 group were the highest among all treatment groups (1521.0, 5.20, 1712.26 and 1686.31, respectively), followed by MB10 group (1406.0, 4.58, 1667.45 and 1614.70, respectively). Therefore, the low proportion of materials was conducive to the reproduction of rhizosphere microorganisms. The number of rhizosphere microbes in the RB group was slightly higher than that in the MB group, which may be because the root growth of plants in the bentonite group provided more oxygen and adsorption sites for the microorganisms.

Fig. 7 depicted the taxonomic composition of sediment microflora community at genus level. During the experiment, there was no significant difference in the composition of rhizosphere microbial community but significant difference in the relative abundance of dominant genus.

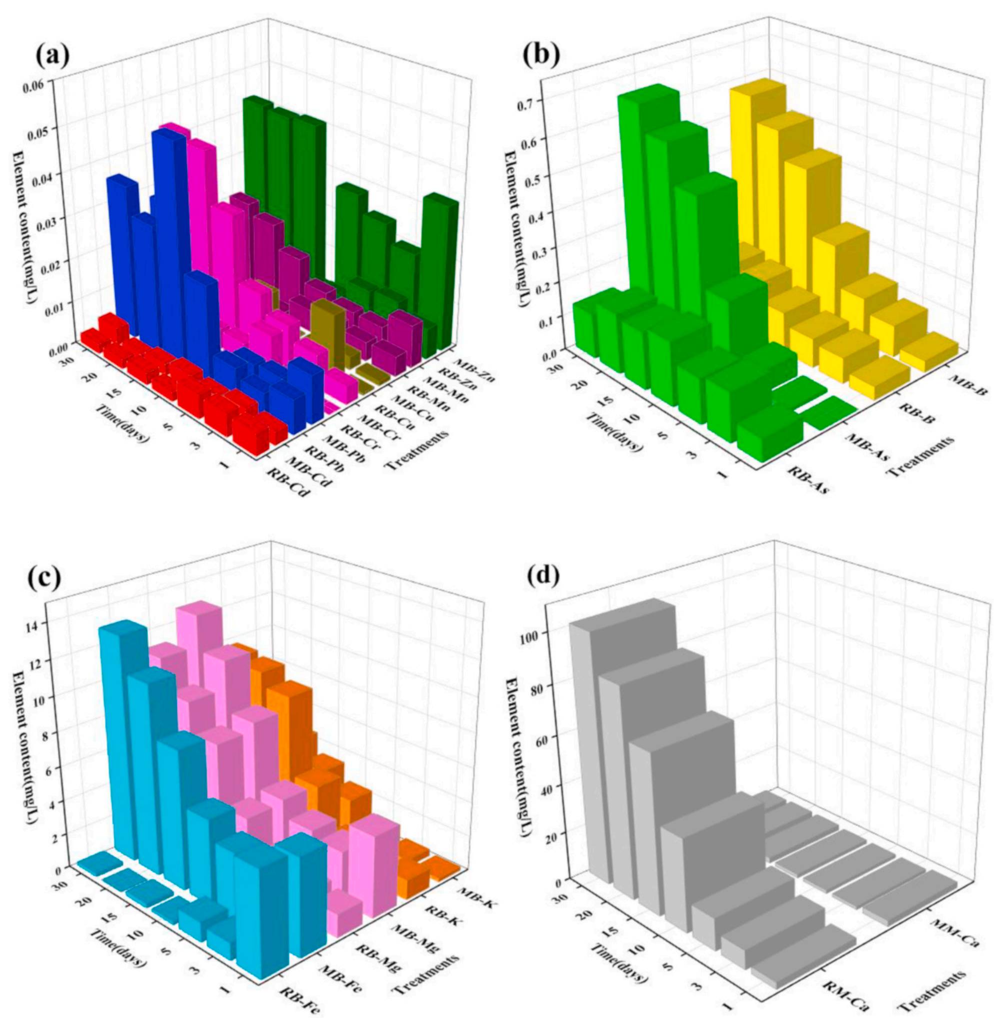

Fig. 6. Elements dissolution from RB and MB.

Table 2
Alpha diversity and abundance of sediment microbial community in each group. i: Initial period of the experiment; f: Final period of the experiment. MB10, RB10: the mass ratio of sediment to modified or raw bentonite is 10/1. samples contained a single component named RB, MB and sediment.

Sample	OTUs	Shannon	Simpson	Chao	Ace
iMB	788.0	4.46	0.04	1002.34	978.84
iMB10	1071.0	4.49	0.03	1382.64	1353.58
iRB	88.0	2.13	0.35	88.0	88.56
iRB10	1268.0	4.75	0.03	1512.84	1485.48
iSediment	1300.0	4.35	0.05	1517.44	1482.36
fMB	460.0	3.58	0.14	461.88	461.84
fRB	1406.0	4.58	0.06	1667.45	1614.70
fRB10	97.0	1.64	0.47	97.0	97.53
fSediment	1521.0	5.20	0.02	1712.26	1686.31
fMB10	1410.0	4.94	0.03	1586.97	1575.15

At genus level, the most dominant genius in groups of MB and RB was *Phenylobacterium* with the relative abundance ranging from 20% to 70%, the *Clostridium*, *Tumebacillus* and *Pedobacter* were primary genuses in sediments of RB10 and MB10. Genus *Bacillus* in the phylum *Firmicutes*, concerning to plant growth promotion characteristics, the production of ACCD, IAA, GA, ammonia and the capacity to solubilize inorganic phosphates by different *Bacillus* has been reported (Carlo et al., 2019). The number of genus bacillus in MB10 was the most, however, there were no presences of genus *Bacillus* in groups of RB and MB.

In addition, genus *Genera*, *Geobacter*, *Pseudomonas* and *Desulfitobacterium* were the major contributors involved in the Fe cycle, especially *Desulfitobacterium* can promote precipitation of metal sulfides by using simple organic compounds, which contain the most in MB10 group. Some P-removing bacteria, such as *Burkholderia*, *GP6* and *GP7*, were less in high-proportion materials mixture groups, but the most in RB10.

Therefore, it can be concluded that bentonite can promote the propagation of rhizosphere microorganisms, especially some growth-promoting bacteria and phosphorus-degrading bacteria in the rhizosphere, which plays an important role in plant growth and substrate improvement.

4. The mechanism of the plant growth promoted by bentonite

Some nutrient elements, such as Zn, Mg, Ca and Mn, can participate in the synthesis of enzymes, growth hormones and proteins. The submerged plants directly absorbed nutrients from sediment or overlying water. Therefore, the concentration of nutrients in the water and sediment directly affects the growth of submerged plants (Liu et al., 2018; Annica., 2018). In this study, it can be found that bentonite can dissolve some nutrient elements, like B, Mg, Ca, K, in which Ca and K played an important role in the growth of *V. spiralis* roots. In addition, the tensile strength, bulk density and porosity of bentonite are more suitable for the growth and extension of plant roots and promote the absorption of nutrients by plants. However, the plant roots in groups of high dose materials were slender, which was related to an adaptation mechanism of plants to infertile substrate conditions (Wang and Yu, 2007). The results of scanning electron microscope analysis showed that the surface of MB was rougher and looser than that of RB, and its spongy structure may be more conducive to nutrient attachment and microbial reproduction (Liu et al., 2017).

It was found that some plant growth promoting bacteria often existed in the rhizosphere or attached to the root surface (Basu et al., 2017). These bacteria live and interact with plants and improve plant growth by mechanism of biofertilizer, biostimulator or biocontrol (Chauhan et al., 2015; Gupta et al., 2018). Bacteria are an essential part of this ecosystem and part of them are helpful for plant growth (Bhattacharyya and Jha, 2012; Chauhan et al., 2015). Fig. 7 demonstrated that a proper amount of bentonite mixed in sediment can promote the growth of rhizosphere microflora community. Additionally, the OTU numbers in bentonite were higher than the control group, it may be related to the fact that bentonite is rich in microporous structure, providing shelter for microorganisms. Meanwhile, the growth of plant roots provides certain oxygen and adsorption points for microorganisms, which can also promote the growth of microbial community.

The MB showed higher cation exchange capacities and stronger

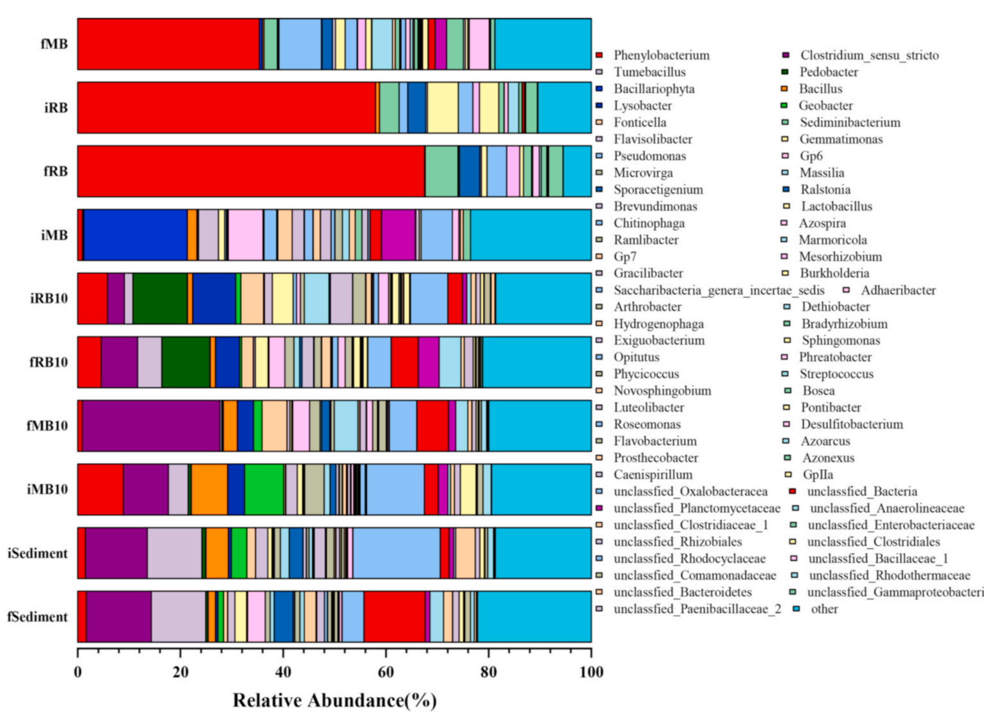

Fig. 7. Taxonomic composition and cluster trees of rhizosphere microflora community of *V. spiralis* at genus level in different groups. i: Initial period of the experiment; f: Final period of the experiment. MB10, RB10: the mass ratio of sediment to modified or raw bentonite is 10/1. samples contained a single component named RB, MB and sediment.

sorption properties than RB. The MB provide more surface area for the P adsorption than RB, and the minerals in bentonite can stimulate the growth of *V. spiralis*, promote the reproduction of rhizosphere microorganisms, which metabolize and decompose phosphorus in sediments (Wang et al., 2018). In this study, some bacteria, such as *Burkholderia*, *GP6*, *GP7* and *Flavobacterium*, were the major contributors involved in N and P absorption of *V. spiralis* (Fig. 7).

5. Conclusion

This study provided a practical method for submerged plants restoration in eutrophic lakes with serious internal pollution. This research demonstrated that the bentonite, an inexpensive and environment friendly clay mineral, could significantly promote the growth of *V. spiralis* by measuring the growth index, and physiological and biochemical index of *V. spiralis*. The redundancy analysis results showed that root length and sediment P content were positively correlated with mixing ratio or modification, and the biomass, height, chlorophyll content, and sediment organic matter were negatively affected by the mixing ratio or modification. Bentonite was conducive to the propagation of rhizosphere microorganisms (especially the OTU value of RB10 group was 7.87% higher than that of sediment group), and further improved P degradation and increased dissolved oxygen. The nutrient elements like K, B and Mg dissolved from bentonite may be one of the main reasons that promoted the growth of *V. spiralis*. The physicochemical interaction between dissolved elements of bentonite and sediment components should be further investigated to promote the application of bentonite in ecological restoration projects in eutrophic shallow lakes.

Credit author statement

Yunli Liu: Conceptualization, Methodology, Investigation, Writing – original draft, Yilingyun Zou: Conceptualization, Methodology, Investigation, Writing – review & editing, Lingwei Kong: Methodology, Validation, Guoliang Bai: Methodology, Data curation, Feng Luo: Methodology, Data curation, Zisen Liu: Investigation, Validation, Chuan Wang: Data curation, Zimao Ding: Methodology, Writing – review & editing, Feng He: Methodology, Project administration, Zhenbin Wu: Supervision, Funding acquisition, Yi Zhang: Supervision, Methodology, Funding acquisition, Writing – review & editing.

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.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 51709254; 31830013), Youth Innovation Promotion Association, Chinese Academy of Sciences (No. 2020335), and the Major Science and Technology Program for Water Pollution Control and Treatment of China 13 th Five-Year Plan (No. 2017ZX07602-002-004). We thank Ms. Liping Zhang for the experimental help.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2021.112308.

References

Annica, O., Jensen, S., Baisner, A., Baattrup-Pedersen, A., Lauridsen, T., Sorrell, B., Riis, T., 2018. Nutrient kinetics in submerged plant beds: a mesocosm study simulating constructed drainage wetlands. Ecol. Eng. 122, 263–270.
Barko, J., Gunnison, D., Carpenter, S., 1991. Sediment interactions with submerged macrophytes growth and community dynamics. Aquat. Bot. 41, 41–65.
Bartwal, A., Mall, R., Lohani, P., 2013. Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. J. Plant Growth Regul. 32, 216–232.
Basu, S., Rabara, R., Negi, S., 2017. Towards a better greener future-an alternative strategy using biofertilizers. I: plant growth promoting bacteria. Plant Gene 12, 43–49.
Bhattacharyya, P., Jha, D., 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J. Microbiol. Biotechnol. 28, 1327–1350.
Carlos, M., Helena, M., Eduardo, V., 2019. Promising bacterial genera for agricultural practices: an insight on plant growth-promoting properties and microbial safety aspects. Sci. Total Environ. 682, 779–799.
Chauhan, H., Bagyaraj, D., Selvakumar, G., Sundaram, S., 2015. Novel plant growth promoting rhizobacteria-prospects and potential. Appl. Soil Ecol. 95, 38–53.
Chen, Q., Man, H., Zhu, L., Guo, Z., Wang, X., Tu, J., Jin, G., Lou, J., Zhang, L., Ci, L., 2020. Enhanced plant antioxidant capacity and biodegradation of phenol by immobilizing peroxidase on amphoteric nitrogen-doped carbon dots. Catal. Commun. 134, 105847.
Chen, L., Tsui, M., Lam, J., Wang, Q., Hu, C., Wai, O., Zhou, B., Lam, P., 2019. Contamination by perfluoroalkyl substances and microbial community structure in Pearl River Delta sediments. Environ. Pollut. 245, 218–225.
Chi, J., Liu, H., 2016. Effects of biochars derived from different pyrolysis temperatures on growth of *vallisneria spiralis* and dissipation of polycyclic aromatic hydrocarbons in sediments. Ecol. Eng. 93, 199–206.
Choi, Y., Thompson, J.M., Lin, D., Cho, Y.M., Ismail, N.S., Hsieh, C.H., Luthy, R.G., 2016. Secondary environmental impacts of remedial alternatives for sediment contaminated with hydrophobic organic contaminants. J. Hazard Mater. 304, 352–359.
Cocking, E., 2003. Endophytic colonization of plant roots by nitrogen-fixing bacteria. Plant Soil 252, 169–175.
Dithmer, L., Lipton, A., Reitzel, K., Warner, T., Lundberg, D., Nielsen, U., 2015. Characterization of phosphate sequestration by a lanthanum modified bentonite clay: a solid-state NMR, EXAFS, and PXRD study. Environ. Sci. Technol. 49, 4559–4566.
Dong, B., Qin, B., Gong, Z., Wang, Y., 2011. Effects of sediment amendment on *Vallisneria natans* growth. Chinese Journal of Ecology 30, 2726–2731.
Gupta, S., Kaushal, R., Sood, G., 2018. Impact of plant growth-promoting Rhizobacteria on vegetable crop production. Int. J. Veg. Sci. 24, 289–300.
Jeppesen, E., Søndergaard, M., Meerhoff, M., Lauridsen, T., Jensen, J., 2007. Shallow lake restoration by nutrient loading reduction-some recent findings and challenges ahead. Hydrobiologia 584, 239–252.
Karaca, G., Baskaya, H., Tasdemir, Y., 2016. Removal of polycyclic aromatic hydrocarbons (PAHs) from inorganic clay mineral: bentonite. Environ. Sci. Pollut. Res. 23, 242–252.
Kraepiel, A., Bellenger, J., Wichard, T., Morel, F., 2009. Multiple roles of siderophores in free-living nitrogen-fixing bacteria. Biometals 22, 573–581.
Li, W., Zhou, J., Ding, H., Fu, H., Liu, J., Chen, Y., Dai, T., Lou, Q., Zhong, X., Fan, H., Zhong, J., 2020. Low-dose biochar added to sediment improves water quality and promoted the growth of submerged macrophytes. Sci. Total Environ. 742, 140602.
Lin, Q., Peng, X., Liu, B., Min, F., Zhang, Y., Zhou, Q., Ma, J., Wu, Z., 2019. Aluminum distribution heterogeneity and relationship with nitrogen, phosphorus and humic acid content in the eutrophic lake sediment. Environ. Pollut. 253, 516–524.
Liu, Y., Han, F., Bai, G., Kong, L., Liu, Z., Wang, C., Liu, B., He, F., Wu, Z., Zhang, Y., 2020. The promotion effects of silicate mineral maifanite on the growth of submerged macrophytes *Hydrilla verticillata*. Environ. Pollut. 267, 1–9.
Liu, Z., Zhang, Y., Han, F., Yan, P., Liu, B., Zhou, Q., Min, F., He, F., Wu, Z., 2018. Investigation on the adsorption of phosphorus in all fractions from sediment by modified maifanite. Sci. Rep. 8, 1–13.
Liu, Z., Zhang, Y., Liu, B., Zeng, L., Xu, D., He, F., Kong, L., Zhou, Q., Wu, Z., 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.
Lynette, M., John, R., Hans, B., 2010. Alum application to improve water quality in a municipal wastewater treatment wetland: effects on macrophyte growth and nutrient uptake. Chemosphere 79, 186–192.
Meis, S., Spears, B., Maberly, S., O'Malley, B., Perkins, R., 2012. Sediment amendment with Phoslock in Clatto Reservoir (Dundee, UK): investigating changes in sediment elemental composition and phosphorus fractionation. J. Environ. Manag. 93, 185–193.
Morelli, B., Hawking, T., Niblick, B., Henderson, A., Golden, H., Compton, J., Cooter, E., Bare, J., 2018. Critical review of eutrophication models for life cycle assessment. Environ. Sci. Technol. 52, 9562–9578.
Oveissi, F., Fatehi, P., 2014. Production of modified bentonite via adsorbing lignocelluloses from spent liquor of NSSC process. Bioresour. Technol. 174, 152–158.
Pamplona, R., 2011. Advanced lipoxidation end-products. Chem. Biol. Interact. 192, 14–20.
Pourabadehei, M., Mulligan, C.N., 2016. Resuspension of sediment, a new approach for remediation of contaminated sediment. Environ. Pollut. 213, 63–75.

Reitzel, k., Andersen, F., Egemose, S., Jensen, H., 2013. Phosphate adsorption by lanthanum modified bentonite clay in fresh and brackish water. Water Res. 47 (8), 2787–2796.

Rodrigues, R., Pineda, R., Barney, J., Nilsen, E., Barrett, J., Williams, M., 2015. Plant invasions associated with change in root-zone microbial community structure and diversity. PloS One 10, 1–19.

Ruban, V., Lopez-Sanchez, J., Pardo, P., Rauret, G., Muntau, H., Quevauviller, P., 2001. Harmonized protocol and certified reference material for the determination of extractable contents of phosphorus in freshwater sediments-a synthesis of recent works. Fresenius' J. Anal. Chem. 370, 224–228.

Shanableh, a., Enshasi, G., Elsergany, M., 2015. Phosphorous adsorption using Al3+/Fe3+-modified bentonite adsorbents-effect of Al3+ and Fe3+ combinations. Desalin. Water Treat. 3994, 1–7.

Steenhoudt, O., Vanderleyden, J., 2000. Azospirillum, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol. Rev. 24, 487–506.

Su, J., Wei, B., Ou-Yang, W., Huang, F., Zhao, Y., Xu, H., Zhu, Y., 2015. Antibiotic resistome and its association with bacterial communities during sewage sludge composting. Environ. Sci. Technol. 49 (12), 7356–7363.

Urakawa, H., Dettmar, D., Thomas, S., 2017. The uniqueness and biogeochemical cycling of plant root microbial communities in a floating treatment wetland. Ecol. Eng. 108, 573–580.

Wang, C., Liu, Z., Zhang, Y., Liu, B., Zhou, Q., Zeng, L., He, F., Wu, Z., 2018. Synergistic removal effect of P in sediment of all fractions by combining the modified bentonite granules and submerged macrophyte. Sci. Total Environ. 626, 458–467.

Wang, J., Yu, D., 2007. Influence of sediment fertility on morphological variability of *Vallisneria spiralis*. L. Aquat. Bot. 87, 127–133.

Wu, B., Wan, J., Zhang, Y., Pan, B., Lo, I., 2020. Selective phosphate removal from water and wastewater using sorption: process fundamentals and removal mechanisms. Environ. Sci. Technol. 54 (1), 50–66.

Xie, Y.H., An, S.Q., Yao, X., 2005. Short-time response in root morphology of Vallisnerianatans to sediment type and water column nutrient. Aquat. Bot. 81, 85–96.

Yang, X., Chen, G., Wang, X., 2011. Enhanced antioxidant protection at the early stages of leaf expansion in ginkgo under natural environmental conditions. Biol. Plant. (Prague) 56, 181–186.

Zhang, Y., He, F., Xia, S., Zhou, Q., Wu, Z., 2015. Studies on the treatment efficiency of sediment phosphorus with a combined technology of PCFM and submerged macrophytes. Environ. Pollut. 206, 705–711.

Zhu, T., Cao, T., Ni, L., He, L., Yi, C., Yuan, C., Xie, P., 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.