RESEARCH ARTICLE

Effects of biochar on the growth of *Vallisneria natans* in surface flow constructed wetland

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Abstract

To improve the nitrogen and phosphorus removal efficiency of surface flow constructed wetlands (SFCWs), biochar was added to an SFCW matrix. The effects of adding different amounts of biochar on water purification, the growth of *Vallisneria natans* (*V. natans*), and microbial mechanisms were explored through SFCW simulation experiments. The results showed that through the joint action of biochar and *V. natans*, the concentrations of total nitrogen, total phosphorus, and ammonia nitrogen in the effluent significantly decreased. The total biomass, relative growth rate, and chlorophyll content of *V. natans* were significantly reduced by adding biochar ($\geq 20\%$, *v/v*), as the root activity and the root to leaf biomass ratio slightly increased at first and then decreased. The carbon and nitrogen contents of *V. natans* slightly increased with the addition of biochar ($\geq 10\%$, *v/v*), but the phosphorus content slightly decreased. Moreover, the nitrogen content of the matrices decreased significantly over time ($P<0.05$), and the phosphorus content in the matrix showed an increasing trend in the same period. In addition, the microbial 16S rDNA sequencing results indicated that the diversity and abundance of the microbial community in the matrix of the biochar-added SFCW tended to decrease. Nevertheless, the abundance of functional bacteria related to nitrogen and phosphorus removal (i.e., *Pseudomonas* and *Dechloromonas*) slightly increased, which would benefit denitrification and dephosphorization in the SFCW. Hence, the addition of biochar to the SFCW matrix facilitated the improvement of effluent water quality, while excessive biochar addition ($\geq 10\%$, *v/v*) restrained the growth of *V. natans* but did not cause death. This conclusion provides valid data support regarding the ability of biochar-added SFCW to purify lightly contaminated water.

Keywords *Vallisneria natans* · Biochar · Surface flow constructed wetland · Microbial community · Nitrogen and phosphorus uptake

Highlights
• Too much biochar added in surface flow constructed wetland (SFCW) was not conducive to the growth of *Vallisneria natans* (*V. natans*).
• High microbial activity led to decreasing bioavailability of phosphorus when excessive biochar was added in SFCW, and it would cause slow growth of *V. natans*.
• The addition of biochar promoted nitrogen and phosphorus removal in SFCW, and there was no obvious effect on *V. natans* if the addition of biochar was less than 10% (*v/v*) in SFCW.

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Introduction

Rapid urbanization and economic development have led to the continuous deterioration of urban rivers in developing countries (Ge et al. 2015; Wei et al. 2020; Xu et al. 2019b). Constructed wetlands (CWs) are engineered systems designed to utilize natural processes for wastewater treatment and have been in use since the 1960s. They have been widely used around the world for several decades due to their low external energy requirements, simple operation and maintenance, high pollutant removal efficiency, and effective recycling of water resources for domestic, industrial, and agricultural wastewater (Stefanakis 2020; Fu et al. 2019). Notably, constructing CWs inside effluent-fed urban rivers, which can enhance wastewater natural treatment efficiency, is a viable option for improving the rate of water recycling and minimizing the additional areal requirements of CWs (Vymazal 2011; Kadlec and Wallace 2008). Based on their hydrological characteristics

and flow forms, CWs can be divided into two broad categories: surface flow constructed wetlands (SFCWs) and subsurface flow constructed wetlands (SSFCWs) (Abedi and Mojiri 2019; Zheng et al. 2016). Compared with SSFCWs (Nuamah et al. 2020; Liu et al. 2019), SFCWs are simpler and easier to construct and operate but lack processing efficiency (Guo et al. 2017; Ding et al. 2018). Therefore, the approach of improving water purification by SFCWs should be explored.

Submerged plants, which are not only the main producers in aquatic ecosystems but also the basis for the structural complexity, spatial heterogeneity, and functional stability of aquatic ecosystems, can be regarded as an effective means for maintaining the stability of the clear-water state in CWs (Gao et al. 2017; Nixon 2009). Through a series of biotic interactions and physicochemical reactions with water and the CW matrix, submerged plants remove nutrients (especially nitrogen and phosphorus) to improve the effluent water quality (Ciurli et al. 2009; Dai et al. 2014). Biochar, a carbon-rich multifunctional material that has high physical stability, high porosity, a large surface area, and a high cation exchange capacity (Beesley et al. 2011; Mohan et al. 2014), has been widely applied for pollutant removal, soil quality improvement, and carbon sequestration (Dai et al. 2017a; Dai et al. 2017b; Zhang et al. 2018). For instance, biochar can affect water quality directly via the strong adsorption/release of compounds (Li et al. 2020; Laird et al. 2010) and indirectly by modifying the oxygen conditions, soil enzymes, and microbial activities (Song et al. 2020; Tian et al. 2016). Consequently, biochar incorporation into the substrate of CWs can serve as an efficient way to improve the reduction of ammonia nitrogen, total nitrogen, chemical oxygen demand (COD), and organic compounds in wastewater (Wang et al. 2020; Feng et al. 2020; Nguyen et al. 2020). However, there are still few studies on the application of biochar to aquatic plants in CWs. Therefore, it is of great significance to explore the effects of adding biochar on the purification of lightly contaminated water and the effects of submerged plants in CW.

Carbon, nitrogen, and phosphorus, the most important and essential elements for plants (Elser et al. 2007; Vitousek and Howarth 1991), have strongly connected biochemical processes. Moreover, as a key component in CWs, microbes play a core role in purifying wastewater and thus affect the growth of plants (Fu et al. 2019; Song et al. 2019). Likewise, plants can affect the activity and composition of microbial communities by altering the physical environment during root growth, modifying nutrient availability through nutrient uptake, and affecting substrate availability through root exudation (Jiang and Guo 2019; Sun et al. 2012). In addition, there may be some functional bacteria in the matrix used for potential bioremediation. Therefore, it is necessary to explore how biochar could influence microbial communities in the CW matrix, in order to analyse its influences on submerged plants whose growth process and nutrient cycles interact with microbial communities in the matrix.

In this study, a SFCW supplemented with biochar was set up to evaluate its long-term performance in treating lightly contaminated water. An artificial substrate constructed through the addition of different amounts of biochar was set up to investigate the treatment effect of the SFCW on lightly contaminated water and the growth response of *Vallisneria natans* (*V. natans*), a typically submerged plant, to the substrate conditions as well as to explore the prospect of applying biochar in CWs to treat lightly contaminated water.

Materials and methods

SFCW system design

A customized rectangular frame was made of polyvinyl chloride plexiglass, with a length, width, and height of 30, 15, and 30 cm, respectively. As shown in Fig. 1, five plant-culture bottles (a diameter of 7.5 cm and a height of 8 cm) filled with a matrix mixed with different proportions of biochar (Suichang County, Zhejiang Province) were placed at the bottom of the structure. The main component of the matrix was a sandy soil substrate taken from the lightly contaminated river on the Xianlin Campus of Nanjing University (Nanjing City, Jiangsu Province, China, 118° 56′ E, 32° 07′ N). The matrix was collected from 0 to 20 cm depth and air-dried. The biochar had a diameter ranging from 1 to 3 mm and was made from bamboo poles (*Phyllostachys heterocycla (Carr.) Mitford cv. Pubescens*) that were heated under controlled oxygen conditions for 3 days and then heated at 800 °C for 48 h (Gao et al. 2018). Correspondingly, five biochar proportions, ranging from 0 to 20% (*v/v*) with a 5% interval, were established in the bottles; the experimental setup was designed by referring to the literature (Li et al. 2018; Rozari et al. 2016; Rozari et al. 2018; Liang et al. 2020). Then, the bottles were divided into five groups and labelled B0 (control), B1 (5%), B2 (10%), B3 (15%), and B4 (20%), with three duplicates in each group.

The seeds of *V. natans* used in the experiment were provided by Nanjing Gucheng Lake Farm. They were cultivated in the laboratory for approximately 2 months, and then *V. natans* plants of approximately 15 cm with 3 to 4 leaves were selected for the experiment. They were planted at a density of 6 rhizomes per bottle. The lightly contaminated water added to the test device was collected from the same river used to obtain the matrix material. The influent concentration is shown in Table 1. Then the equipment was placed in a glass greenhouse with a daily temperature of 16 to 23 °C under natural light. In addition, the positions of the different groups in the structure were rotated frequently.
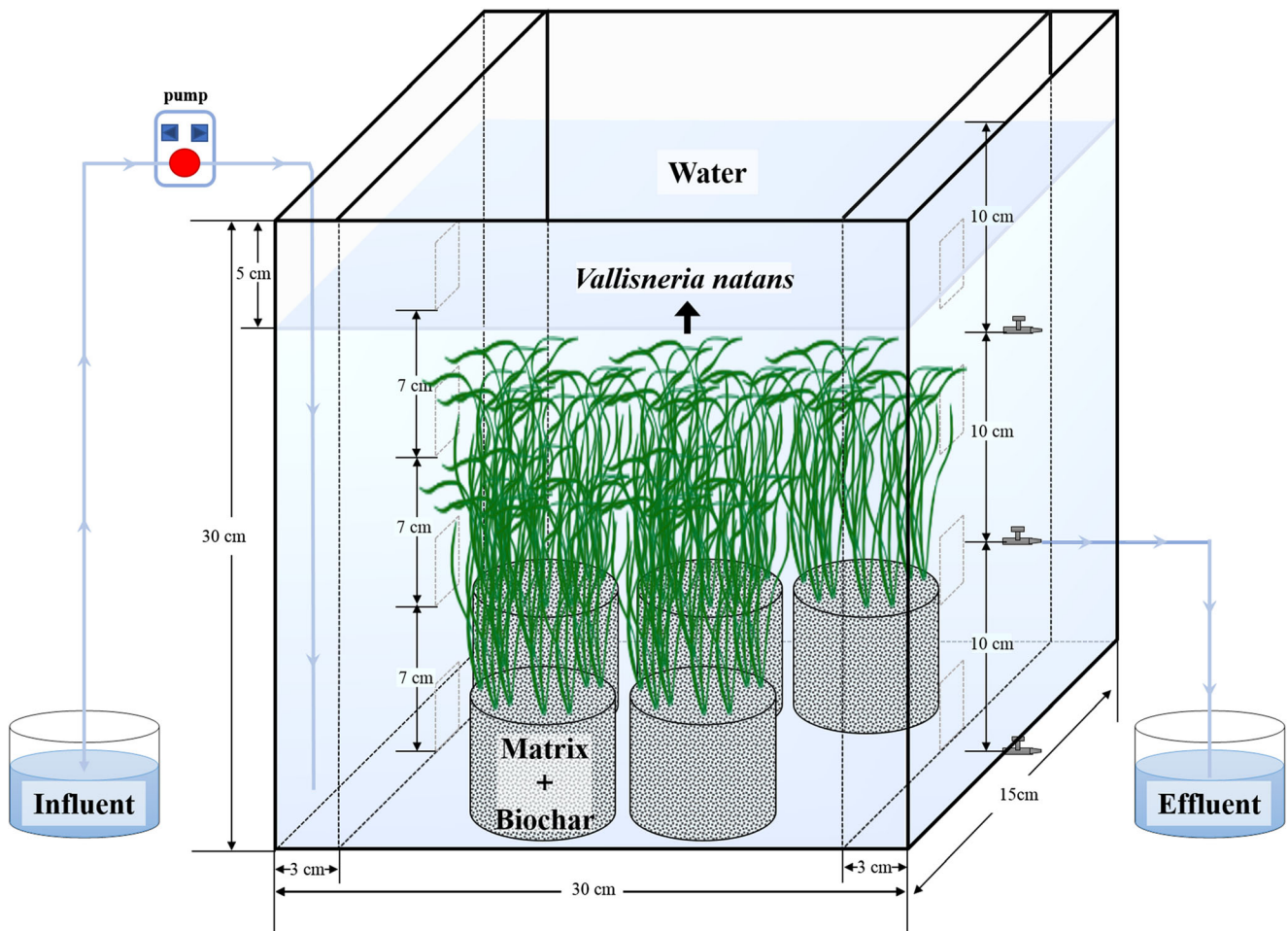
Fig. 1 Schematic diagram of the surface flow constructed wetland

Measurements of water chemicals and plant growth parameters

The concentrations of total nitrogen, total phosphorus, ammonia nitrogen, and COD were measured every 3 days, and all the tests were performed according to the criteria of the national standard method formulated by China's State Environmental Protection Administration (SEPA 2002). In addition, the water in each device was set to a 54-day experimental period with a hydraulic retention time of 3 days.

Table 1. Influent water quality parameters. Present as the mean ± SE (n=18).

Parameters	Values (mg/L)
Total nitrogen	2.81±0.06
Total phosphorus	0.098±0.007
Ammonia nitrogen	0.85±0.04
Chemical oxygen demand	8.67±1.07

Simultaneously, the total biomass, leaf biomass, root biomass, root to leaf biomass ratio, relative growth rate (RGR), and root activity were selected as the growth indicators of *V. natans*, while the total chlorophyll was measured as a subsidiary physiological index. The biomass of *V. natans* was measured as follows: the plants were divided into leaves and roots and oven-dried at 60 °C to constant weight, and then the dried weight of each part was measured to record the biomass. The root to leaf biomass ratio and RGR were calculated according to the following formulas:

$$\text{Root to leaf ratio} = W_1/W_2 \tag{1}$$

$$\text{RGR} = \ln(W_3/W_4)/\text{dt} \tag{2}$$

where W_1 is the root dry weight (g), W_2 is the leaf dry weight (g), W_3 is the final dry weight (g), W_4 is the initial dry weight (g), and dt is the growth time (d).

Root activity was determined by the triphenyl tetrazolium chloride (TTC) method (Liu et al. 2014), and calculated according to the following formula:

$$\text{Rootactivity} = \left(\text{Root activity}[\mu gTTF/(g \cdot h)] = (C*m)/(W_5*h) \right) \quad (3)$$

where C is the reduction of tetrazolium in the root of
V. natans (g), W_5 is the root weight (g), h is the chromogenic
time (h), m is the dilution ratio of the extraction solution, and
triphenylformazan (TTF) is a red insoluble compound obtain-
ed by reduction of TTC.

Furthermore, the ethanol extraction method (Peng et al.
2013) was adopted to measure the chlorophyll content in the
leaves of *V. natans*. In short, 0.5 g fresh leaves were immersed
in an incubator at 37 °C for 24 h with 95% ethanol and shaken
several times during the soaking process. Then, when the
leaves were completely whitened, the supernatants were mea-
sured on a spectrophotometer at 665 nm and 649 nm. Finally,
the chlorophyll content in leaves was expressed in mg
pigment/g fresh weight.

Carbon, nitrogen, and phosphorus in the matrix and *V. natans*

The concentrations of carbon, nitrogen, and phosphorus in the
matrix were measured every 14 days. According to the nation-
al standard method formulated by the Ministry of
Environmental Protection, nitrogen, phosphorus, and carbon
were measured with the Kjeldahl method (Bremner 2009),
molybdenum antimony method (Dick and Tabatabai 1977),
and potassium dichromate oxidation spectrophotometry
(Schumacher 2002), respectively. The carbon and nitrogen
contents of *V. natans* were determined through an elemental
analyser (CHN-O-Rapid, Heraeus Inc., Germany), and the
phosphorus content was H_2SO_4-H_2O_2 digested first and then
analysed by ammonium molybdate ascorbic acid methods
(Chen et al. 2007).

DNA extraction and high-throughput sequencing of bacteria in the matrix

Matrix samples were collected at the end of the experiment
and stored at −80 °C for microbial community analysis. High-
throughput sequencing of microbial 16S rDNA in the matrix
was performed by Sangon Biotech, Inc. (Shanghai, China).
Genomic DNA was extracted by using an E.Z.N.A. Soil
DNA Kit (Omega, USA) following the manufacturer's in-
structions. PCR was started immediately after the DNA ex-
traction. The 16S rRNA V3-V4 amplicons were amplified
using KAPA HiFi Hot Start Ready Mix (2×) (TaKaRa Bio
Inc., Japan). Then, high-throughput sequencing was per-
formed using the Illumina MiSeq system (Illumina MiSeq,
USA) according to the manufacturer's instructions.

Subsequently, the data were collected as follows: the
MiSeq sequences included the barcode sequence, as well as
the primer and linker sequences added during sequencing.
First, the primer linker sequence was removed, and then the
pairs of reads were merged into a sequence according to the
overlapping relationship between PE reads. Next, the samples
were identified and differentiated according to the barcode
label sequence. Finally, quality control filtration was per-
formed on each sample to obtain valid data for the sample.

Statistical analysis

All data were presented as the mean ± SE. Data analysis was
performed using GraphPad Prism 7 software (GraphPad
Software, Inc., La Jolla, CA, USA) by Student's paired t-test
(Zhu et al. 2015) and one-way analysis of variance (ANOVA)
(Gao et al. 2017). The significance level for the statistical
analysis was $\alpha=0.05$.

Results and discussion

Pollutant removal in SFCW with biochar and *V. natans*

SFCWs are engineered wastewater treatment systems that
have been designed and constructed to utilize natural process-
es including biological, chemical, and physical processes,
within a controlled environment. The results showed that
SFCW amended with biochar and *V. natans* had a positive
impact on the water purification effect with time. High remov-
al efficiencies were observed for total nitrogen and ammonia
nitrogen (Fig. 2a, c). Especially, after 24 days, the total nitro-
gen removal rate was in the range of 20.0–33.8%, which is
generally consistent with the results of earlier studies (Wei
et al. 2020; Gunes et al. 2012). In addition, the removal rate
of ammonia nitrogen was as high as 92.8± 4.2%. The ammo-
nia nitrogen purification capacity attained in this study was
higher than the results in Rozari et al.'s (2018) report, which
demonstrated a reduction of 65–79%. The plants used in that
study were paperbark trees (*Melaleuca quinquenervia*) and
lemongrass (*Cymbopogon citratus*) in wetlands. These results
suggest that *V. natans* played a positive role in the nitrogen
removal process of the SFCW. Nitrogen in SFCWs is mainly
removed through plant uptake, nitrification-denitrification ac-
tivities, sediment storage, and volatilization (Sawaittayothin
and Polprasert 2007). Fig. 2 b shows that the removal efficien-
cy of total phosphorus was in the range of 56.5–83.3%. This
could be explained by the potential of biochar to absorb and
deposit phosphorous, while plants and microorganisms can
also promote phosphorus removal in CWs (Huett et al.
2005; Du et al. 2017). The effluent water quality was obvi-
ously better than that of the influent, which corroborates mul-
tiple studies that have reported that adding biochar is extreme-
ly effective for total nitrogen and total phosphorus removal (Li
et al. 2020; Dai et al. 2014; Jia et al. 2020). This is due to the
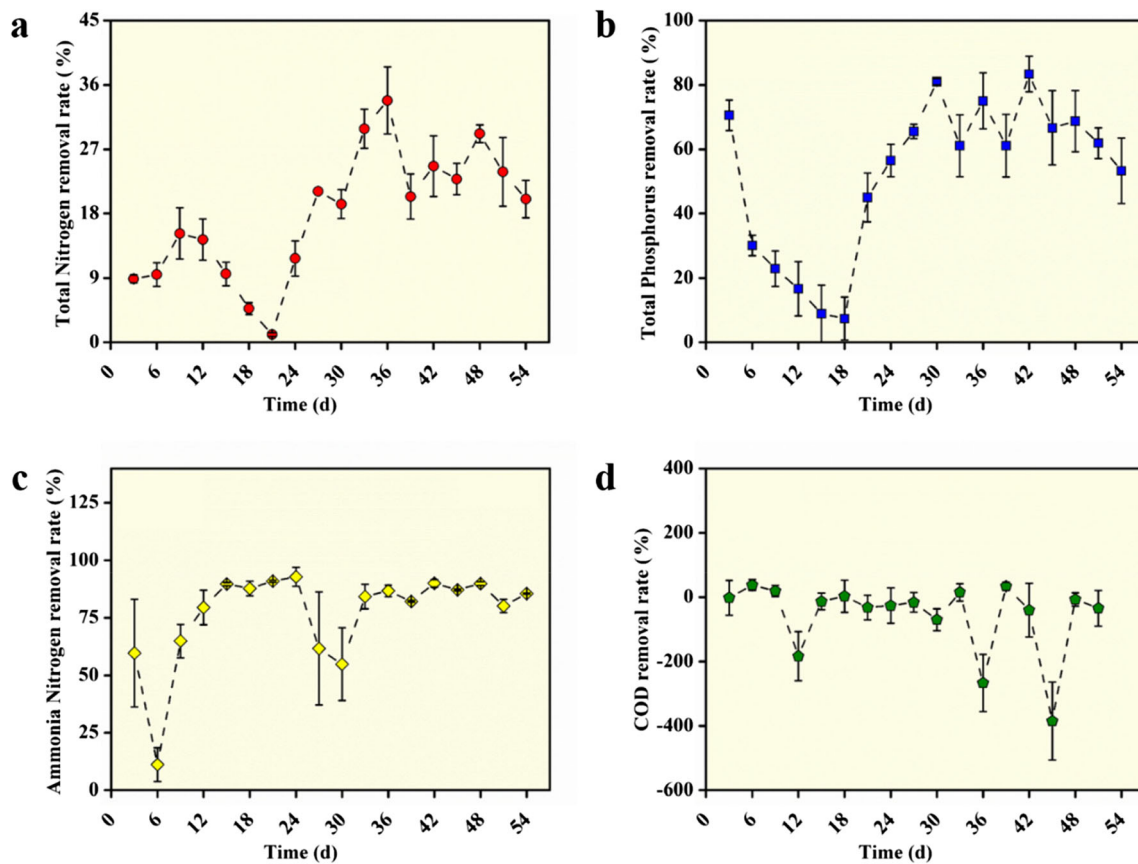strong adsorption affinity of biochar for soluble nutrients such

Fig. 2 Effects of the matrix with added biochar on the characteristics of the influent and effluent: total nitrogen (**a**), total phosphorus (**b**), ammonia nitrogen (**c**), and COD (**d**) removal rate. Bars represent the mean ± SE (*n*=3)

as ammonium, nitrate, phosphate, and other solutes (Yuan et al. 2019). However, the removal efficiency for COD was low or even negative during the entire test period (Fig. 2d). This is because the low concentration of COD, ranging from 8 to 11 mg/L, the low concentration of influent organic matter, and the insufficient bioavailable organic carbon were not conducive to the removal of organic pollutants (Huang et al. 2012). The results of this study were consistent with those of previous studies. For example, Li et al. (2008) and Wang et al. (2016) also found that the organic matter removal performance was undesirable when the influent COD concentrations were 7.63 mg/L and 5.99 mg/L, respectively. In addition, the organic root exudates from *V. natans* increase the effluent COD.

Growth of *V. natans* in SFCW with biochar

Nutrient acquisition by roots and leaves are two dominant pathways for the growth of submerged macrophytes (Rattray et al. 1991; Xu et al. 2019a), and lower nitrogen and phosphorus contents in water may lead to slower growth of *V. natans*. To explore the optimal amount of biochar to add during the joint remediation process, the effects of the biochar content of the matrix on the growth of *V. natans* were further discussed.

Here, total biomass, RGR, root activity, and the biomass allocation ratio of leaves and roots (leaf mass ratio, root mass ratio, and the ratio of roots to leaves) were used as indicators to evaluate the growth status of *V. natans*. According to the measurement results for the total biomass in the different experimental groups (Fig. 3a), the total biomass of *V. natans* in the B2 (10%), B3 (15%), and B4 (20%) groups was significantly lower than that in the B0 (control) and B1 (5%) groups ($P<0.05$). At the same time, the RGR of *V. natans* had a decreasing trend with increasing biochar content; in particular, the RGR of the B4 (20%) group was significantly lower than that of the control group (Fig. 3b, $P<0.05$). These results were accord with those of a study on the growth of the seedlings, shoots, and roots of *Robinia pseudoacacia* L. by Bu et al. (2020). However, Li et al. (2018) found that the development of the macrophyte *Arundo donax* was significantly promoted in SFCWs with 20% (*v/v*) biochar addition. Since the roots of emergent plants are submerged, they can absorb nutrients from water directly and without interacting with the biochar and microorganisms in the matrix.

To determine why biochar inhibited the growth of *V. natans* leaves, the root activity, chlorophyll content, leaf mass ratio, root mass ratio, and the root to leaf biomass ratio were further studied. The root activity and the ratio of roots to
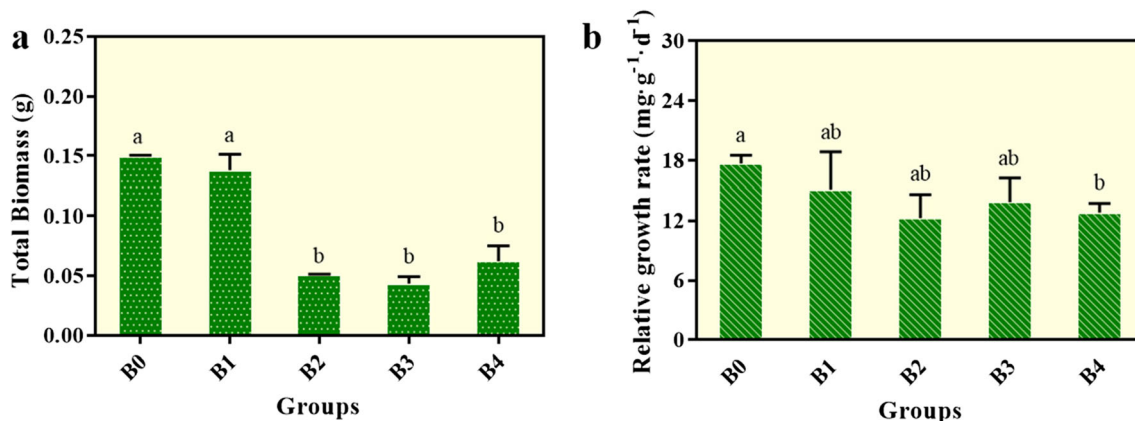
Fig. 3 Effects of sediment with different levels of biochar addition on the biomass of *Vallisneria natans* (*V. natans*). **a** Total biomass. **b** Relative growth rate. Five biochar proportions, from 0 to 20% (v/v) with 5% intervals, are labelled B0, B1, B2, B3, and B4. Bars represent the mean ± SE (*n*=3). Different letters indicate statistically significant differences (*P*<0.05).

leaves slightly increased and then decreased as the biochar content increased, although the difference was not significant (Fig. 4a, Table 2). An increase in the proportion of roots to the total mass can increase the exposed absorptive surface area in nutrient-insufficient sediment, which is advantageous for nutrient acquisition in adverse environments (Chen et al. 2017). The results of this study revealed that root activity was stressed by biochar, which led to a higher root to leaf biomass ratio and a lower RGR. Additionally, chlorophyll content is an important parameter for evaluating the photosynthetic activity of plants, and these measured values can be used as indicators of pollutant-induced plant stress (Zeng et al. 2019). Therefore, chlorophyll was used as an auxiliary physiological index to evaluate the effect of biochar on the growth of *V. natans*. As shown in Fig. 4b, with the increase in biochar content in the matrix, the chlorophyll content in the leaves of *V. natans* tended to decrease. In particular, the chlorophyll content in group B4 (20%) was significantly lower than that in group B0 (*P*<0.05), with a difference of 0.20 mg/g.

The carbon, nitrogen, and phosphorus contents of *V. natans* and the matrix

The carbon, nitrogen, and phosphorus contents of *V. natans* were used to determine the limiting nutrients in the soil. Here, changes in the carbon, nitrogen, and phosphorus contents of *V. natans* and the matrix are further discussed. Fig. 5 a–c show the effects of different amounts of biochar on the contents of carbon, nitrogen, and phosphorus in *V. natans*. When compared with the carbon and nitrogen contents of *V. natans* in the B0 (control) group, those in the three experimental groups (with 10%, 15%, and 20% biochar) all increased (carbon increased 29.17–34.80 mg/g and nitrogen increased 0.27–3.27 mg/g). Many studies have shown that adding low concentrations of biochar is beneficial to plant growth (Liang et al. 2019; Li et al. 2021) because biochar can not only absorb nutrients and provide nutrients for plants but can also reduce the mechanical resistance of plant root growth and directly or indirectly promote the growth of plant roots (Backer et al. 2017). This study also found that the addition of biochar somewhat increased the nitrogen content in *V. natans* (Fig.
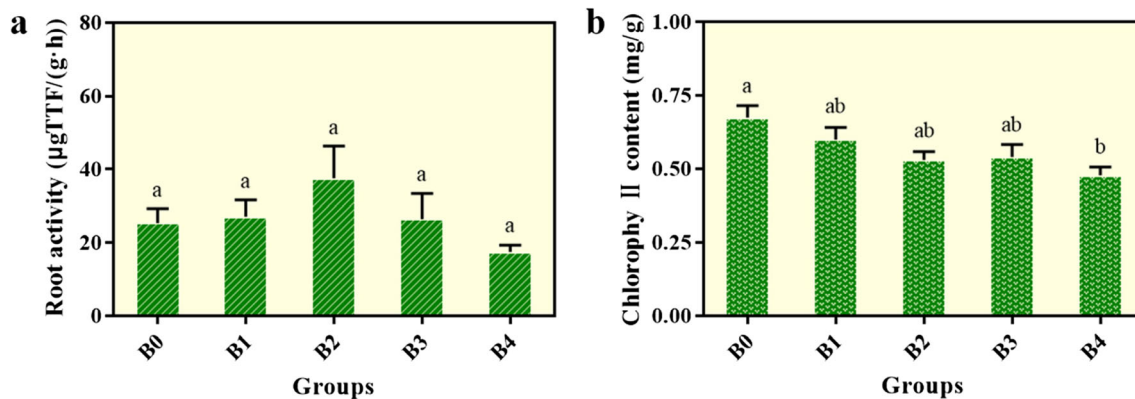

Fig. 4 Effects of matrices with different biochar addition levels on the root activity (**a**) and chlorophyll content (**b**) of *V. natans*. Five biochar proportions, from 0 to 20% (v/v) with 5% intervals, are labelled B0, B1, B2, B3, and B4. Bars represent the mean ± SE (*n*=6). Different letters indicate statistically significant differences (*P*<0.05).

Table 2 Effects of matrices with different biochar addition levels on the leaf and root biomass allocation ratio of *Vallisneria natans* (*V. natans*). Five biochar proportions, from 0 to 20% (*v/v*) with 5% intervals, are labelled B0, B1, B2, B3, and B4. The values are shown as the mean ± SE (*n*=6).

Treatment groups	Leaf mass ratio (%)	Root mass ratio (%)	Ratio of root to leaf (%)
B0 (control)	91.41±2.29	8.59±2.29	9.60±2.82
B1 (5%)	88.81±4.05	11.19±4.05	13.27±4.92
B2 (10%)	87.64±2.44	12.36±2.44	14.37±3.11
B3 (15%)	84.57±7.02	15.43±7.02	21.03±11.19
B4 (20%)	85.84±2.84	14.16±2.84	16.89±4.03

5b), which demonstrated that biochar can promote plant nitrogen absorption; this result is consistent with previous studies (Liang et al. 2019; Li et al. 2021). However, compared with
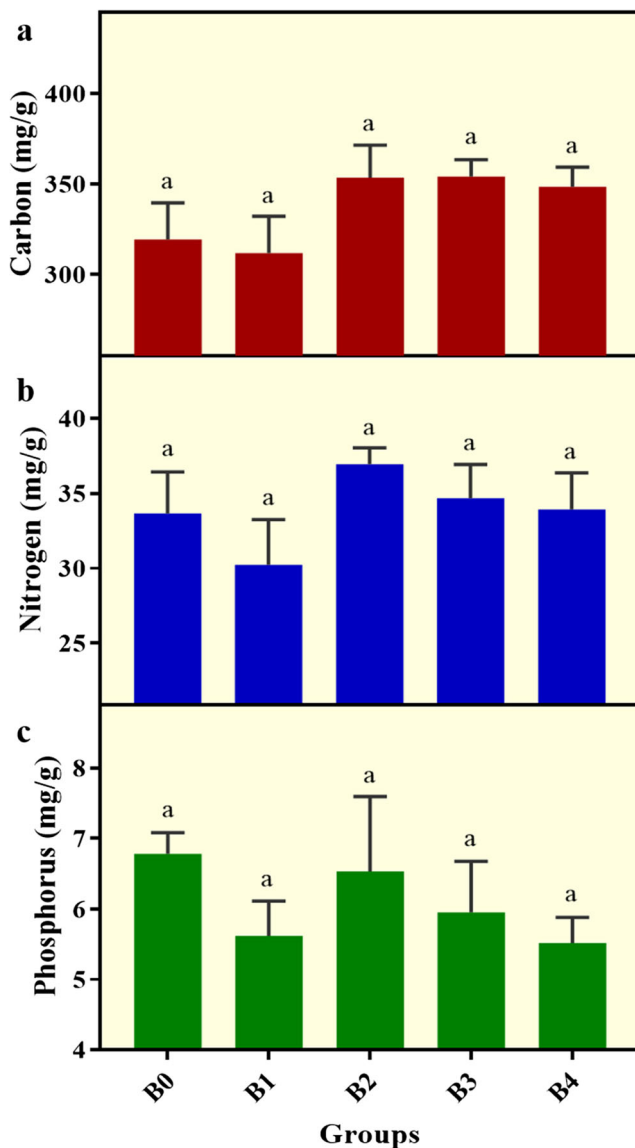

Fig. 5 Effects of matrices with different biochar addition levels on the carbon (**a**), nitrogen (**b**), and phosphorus (**c**) contents of *V. natans*. Five biochar proportions, from 0 to 20% (*v/v*) with 5% intervals, are labelled B0, B1, B2, B3, and B4. Bars represent the mean ± SE (*n*=3). Different letters indicate statistically significant differences (*P*<0.05).

the phosphorus content in the B0 (control) group, those in the three experimental groups (B2–B4) decreased by 0.25–1.26 mg/g (Fig. 5c). At the same time, Wu et al. (2021) found that magnetic biochar reduced plant biomass and photosynthetic capacity and inhibited the absorption of phosphorus, which is similar to our findings (Fig. 3; Fig. 4b; Fig 5c).

The carbon, nitrogen, and phosphorus contents in the matrix of the SFCW were further discussed. In this study, matrix samples were collected every 14 days, and the contents of total organic carbon, total nitrogen, and total phosphorus were measured. As shown in Fig. 6a, the carbon content apparently increased in the matrix of the SFCW, but it did not change much over time. According to the measurement results for nitrogen in the different groups, there was no obvious change in the nitrogen content of the matrices in the different groups in the same period; however, over time, the nitrogen content of the matrices decreased significantly (Fig. 6b, *P*<0.05). In addition, the phosphorus content in the matrix showed an increasing trend in the same period (Fig. 6c) because biochar can absorb phosphorus from water (Qiu et al. 2019). Which means, with more biochar added in the matrix, phosphorus concentration, especially available phosphorus for *V. natans* utilization, would decrease and thus caused *V. natans* to grow slowly.

Microbial communities in the matrices of SFCW

PCR cloning of the 16S rRNA gene was applied to detect the microbial community structures in the different biochar groups in order to understand the microbial community composition and its potential effects on the SFCW. The statistical results for the diversity indexes of the microbial community structure in the matrix are presented in Table 3. The Shannon index of the B0–B3 group decreased as the amount of added biochar increased. The Simpson index showed the opposite trend. These results indicate that the more biochar was added, the less diverse the microbial communities were, and the fewer species there were in the matrix, even though the differences were not significant (*P*<0.05). Similarly, the community richness indexes (ACE and Chao) (Table 3) indicated the same phenomenon. Microbial communities are important for the health and function of ecosystems (Liang et al. 2018), and the results demonstrate that the addition of biochar can

Fig. 6 Carbon (**a**), nitrogen (**b**), and phosphorus (**c**) contents in matrices with different amounts of biochar. Five biochar proportions, from 0 to 20% (*v/v*) with 5% intervals, are labelled B0, B1, B2, B3, and B4. Bars represent the mean ± SE (*n*=3). Different letters indicate statistically significant differences: the uppercase letters show the comparison of the same dose at different times, and the lowercase letters show the comparison of different doses at the same time (*P*<0.05).
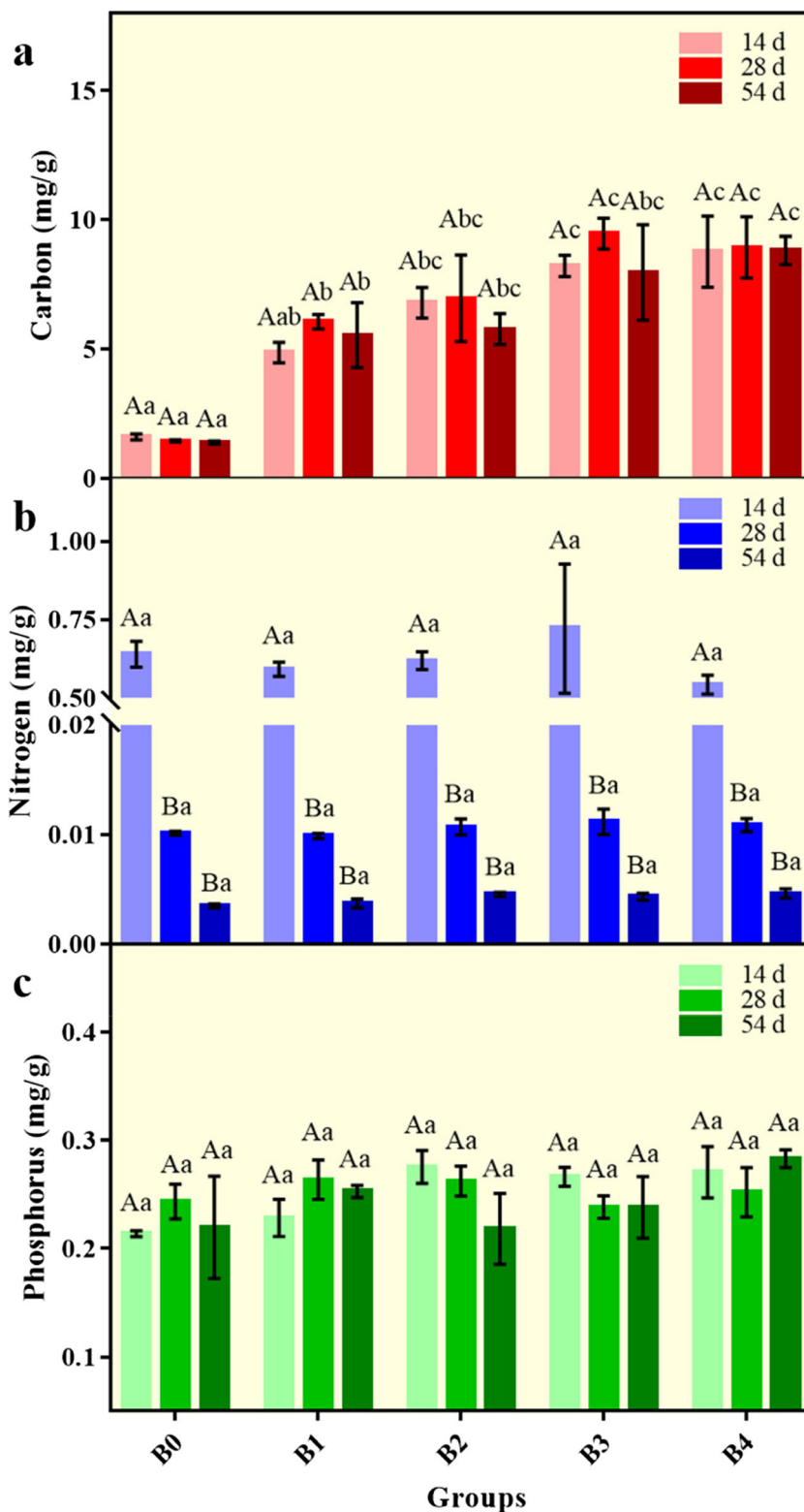
partially reduce the microbial biomass and reshape the microbial community structure in the substrate (Table 3; Fig. 7).

The microbial community structure was further analysed, and it was found that *Acidobacteria Gp6* was the most abundant genus in every group, followed by *Sphingomonas*, *Gemmatimonas*, *Thiobacillus*, and *Dechloromonas* (Fig. 7a). These five genera accounted for 16.03 to 18.33% of the total microbial communities. *Gp6* is an acidophilic bacterium that is widely used in the field of sewage treatment because of its prolific enzymatic system and strong metabolic capacity

Table 3. The community diversity indexes of the bacteria in the matrix. Five biochar proportions, from 0 to 20% (*v/v*) with 5% intervals, are labelled B0, B1, B2, B3, and B4. Values are shown as the mean ± SE (*n*=3). Different letters indicate statistically significant differences (*P*<0.05).

Sample	Seq_num[I]	OTU_num[II]	ACE_index	Chao1_index	Coverage (%)	Shannon	Simpson ($\times 10^{-3}$)
B0 (control)	49132±5866	7754±412	15990±379	12319±410	92.53±0.76	7.49±0.05[a]	2.98±0.46 [a]
B1 (5%)	40008±4258	6833±422	15093±594	11480±441	91.61±0.58	7.37±0.07 [a]	3.80±0.86 [a]
B2 (10%)	38330±2954	6407±163	14197±296	10805±220	91.81±0.52	7.30±0.02 [a]	4.13±0.26 [a]
B3 (15%)	33564±3407	5830±305	13327±669	10012±490	91.26±0.63	7.19±0.07 [a]	4.21±0.69 [a]
B4 (20%)	44353±5689	7047±557	15013±444	11525±565	92.32±0.74	7.35±0.09 [a]	3.68±0.62 [a]

[I] Seq_num: number of quality reads of samples

[II] OTU_num: number of OTUs obtained by sample clustering

(Boopathy et al. 2015). Moreover, the abundances of the dominant microbial taxa of denitrifying bacteria (i.e., *Pseudomonas*, *Nitrosospira*, Fig. 7b) and polyphosphate-accumulating bacteria (i.e., *Dechloromonas*, *Pseudomonas*,

Fig. 7c) increased in the matrices with higher biochar content. As the abundance of the dominant denitrification and dephosphorization bacteria increased, the microbial community diversity decreased. Among them, *Dechloromonas* is a
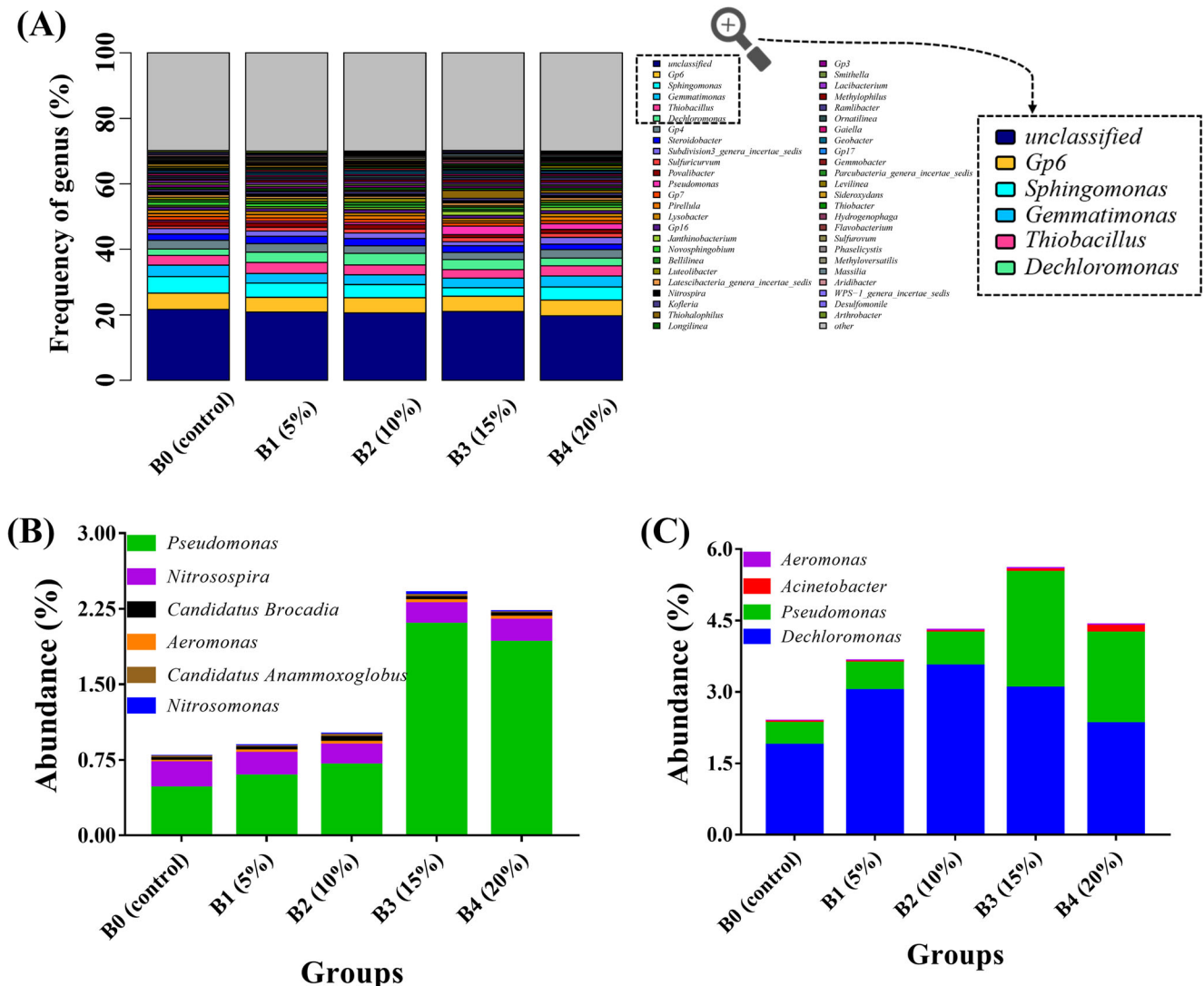

Fig. 7 Distribution of the microbial community in the matrix at the genus level (**a**) and distribution of dominant bacteria related to nitrogen transformation (**b**) and phosphorus removal (**c**) in the matrix at the genus level. Five biochar proportions, from 0 to 20% (*v/v*) with 5% intervals, are labelled B0, B1, B2, B3, and B4.

facultative anaerobic bacterium capable of accumulating polyphosphate within cells that plays an important role in the degradation of organic compounds and nitrogen and phosphorus removal (Terashima et al. 2016). *Pseudomonas* is not only beneficial for nitrogen removal but also good for phosphorus removal (Tan et al. 2021). These results confirmed that the increase in functional bacteria was conducive to the treatment of lightly contaminated water and was in line with previous results showing that the combined effect of biochar and *V. natans* contributed to the purification of water quality.

As shown in Fig. 8, *V. natans* plants acquire nutrients such as nitrogen and phosphorus through their leaves and roots. Biochar promoted the adsorption of nitrogen and phosphorus in water by the matrix, which caused the nitrogen and phosphorus contents in the matrix to increase with the increase in the amount of biochar. Dissolved organic matter released from biochar has great potential to provide essential carbon sources for denitrification (Li et al. 2018). Furthermore, biochar was beneficial to the growth of the nitrogen and phosphorus removal microbial community, and there was a cooperative and competitive relationship between microorganisms and

V. natans in the substrate. Plants can not only assimilate nitrogen and phosphorus to promote their growth but can also offer attachment sites for microorganisms and affect microbial nitrification and denitrification by releasing oxygen from roots (Huang et al. 2013). Plants and microbial interactions play a major role in CWs (Li et al. 2019). Nitrifying bacteria convert ammonia ions into nitrite ions and nitrate ions through nitrification. Denitrifying bacteria convert nitrate ions into nitrite ions, nitrous oxide, and nitrogen oxide (Kuypers et al. 2018), which results in a decrease in the available nitrogen content in the matrix and leads to a decrease in nitrogen uptake by the root system, further causing the nitrogen content in *V. natans* to decrease with the increase in biochar. Additionally, major phosphorus removal processes include sorption, precipitation, plant uptake, and microorganismal absorption and utilization (Vymazal 2007; Xu et al. 2020). The absorption of phosphorus by phosphorus-accumulating bacteria reduced the amount of phosphorus in the matrix that could be used by *V. natans*. This caused the phosphorus content of *V. natans* to decrease with the increase in biochar content, which damaged the photosynthetic system of *V. natans*;
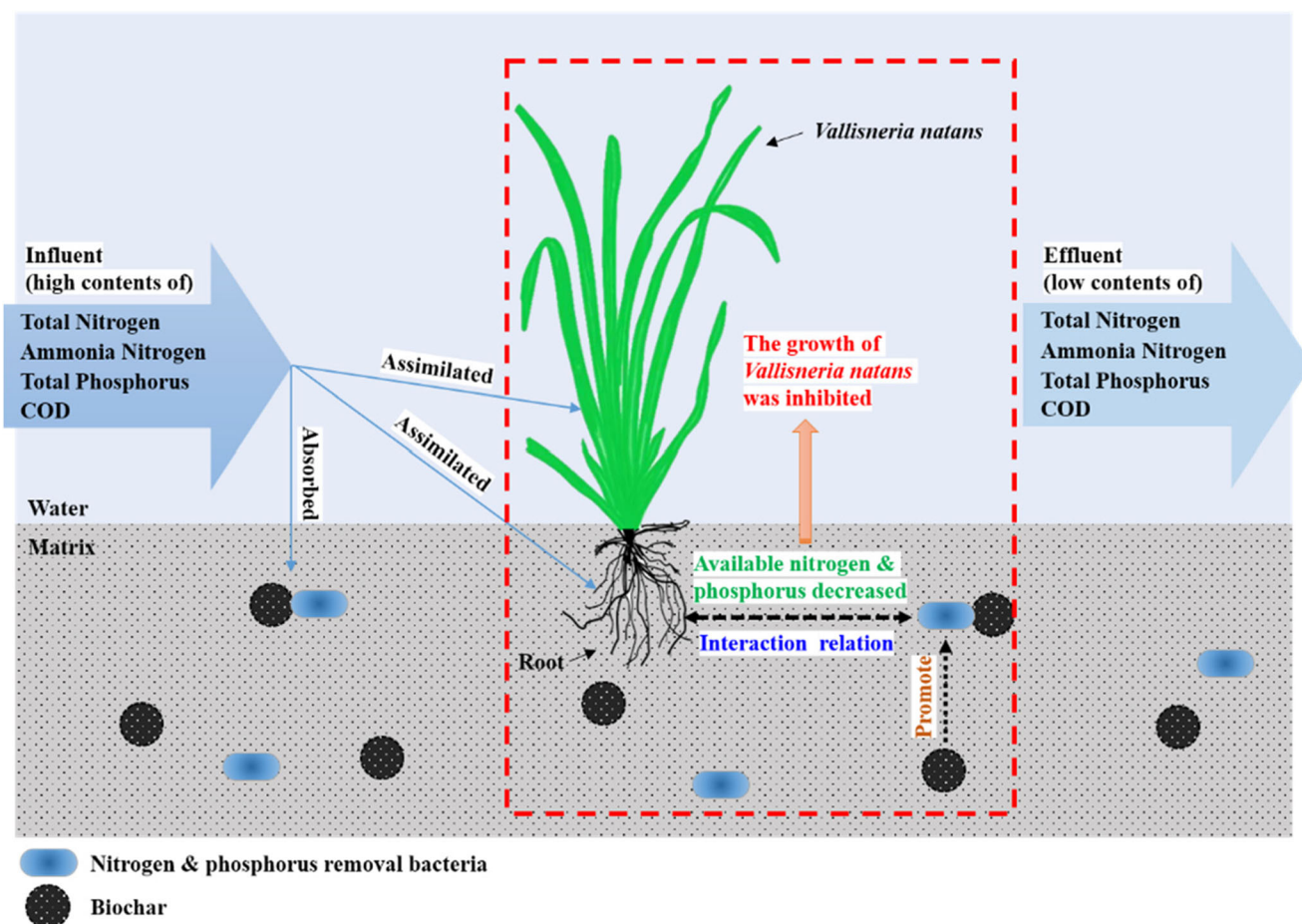

Fig. 8 Potential mechanism of the effects of surface flow constructed wetlands with biochar on *V. natans* and lightly contaminated water. Biochar promoted the removal of nitrogen from the matrixes of the surface flow constructed wetland by microbes, reduced the content of available nutrients, and restricted the growth of *V. natans*.

eventually, the biomass of *V. natans* decreased significantly, i.e., its growth was inhibited. In a word, from the perspective of nutrients, due to the effective removal of nitrogen and phosphorus from water by biochar, the absorption of nitrogen and phosphorus from water by *V. natans* may be reduced, and the growth of *V. natans* is inhibited to a certain extent. However, there are still other factors which can influence the growth of *V. natans*, such as allelochemical, inserted algae, and hazardous substance. Further research will be determined.

Conclusion

By simulating an SFCW, the authors investigated the effects of adding biochar on water purification and *V. natans* growth. The growth, physiological and biochemical indexes of *V. natans*, water quality parameters, and microbial community structure in the matrix were measured. The results showed that adding different amounts of biochar could positively affect the growth of nitrogen- and phosphorus-removing bacteria. The addition of biochar and *V. natans* to the SFCW provided well effect of water purification over time. Although adding biochar slightly restrained the growth of *V. natans*, the tested biochar concentrations did not cause plant death. In summary, the combination of biochar with *V. natans* has strong application potential for improving the purification capacity of SFCWs.

Declarations

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