

Article

Added Biochars Promoted Nitrogen and Phosphorus Removal from Ecological Ditches at Low Temperature

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Abstract: The global issue of ecological ditches being poor in removing nitrogen and phosphorus under cold winter temperatures has been identified. This study introduced three types of biochar (reed, rice, and corn) into ecological ditch sediments via two application methods: rhizosphere and mixed addition. The purpose was to explore how these methods affect the removal of nitrogen and phosphorus, as well as their influences on microbial communities in sediments. The results indicated that the addition of biochar to ecological ditches significantly enhanced the removal of nitrogen and phosphorus. Among the three types of biochar, the mixed addition of corn biochar yielded the greatest results, achieving removal rates of 77.1% for total nitrogen (TN), 93.3% for $\text{NH}_4^+\text{-N}$, and 90.3% for total phosphorus (TP). The growth of *Vallisneria natans* was greatly improved by the mixed addition method, resulting in an average increase of 154%. This improvement was superior to the rhizosphere addition group, which led to a growth increase of 125%. In comparison, the control group (CK) showed a decrease of 4.8% in growth. Different methods of biochar addition resulted in changes in the physicochemical properties and stoichiometry of the plants. Microbial analyses showed that the addition of biochar reduced the diversity and abundance of the substrate microbial community.

Keywords: ecological ditch; biochar; *Vallisneria natans*; microbial community; nitrogen and phosphorus removal



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1. Introduction

The excessive discharge of nitrogen (N) and phosphorus (P) from agricultural non-point source pollution is a global environmental issue that causes water eutrophication, resulting in a decline in the stability of aquatic ecosystems and a loss of ecosystem services [1–3]. Ecological engineering systems including constructed wetlands, ecological ditches, floating islands, retention ponds, riparian buffer plants, and hard grass hedges were commonly used to intercept agricultural non-point source pollution. Ecological ditches were regarded as a best pollutant mitigation practice due to their small footprint, low costs, and high efficiency [4]. However, the effective operation of ecological ditches at low temperature has always been a difficult problem [5]. As integrated ecosystems, N and P were removed by the combined processes of plant absorption, microbial assimilation, and sediment adsorption in ecological ditches. However, these processes were significantly influenced by low temperature conditions [6,7]. Low temperature not only led to slow a growth rate and metabolism, but also damaged plant tissues, and further

affected the uptake and utilization of N and P nutrients by plants [8,9]. On the one hand, the decomposition of plant matter throughout autumn and winter resulted in the release of nutrients, which contributed to the eutrophication in water. On the other hand, the activity and metabolism of micro-organisms were seriously inhibited at low temperature [10]. For example, the growth rate of phosphorus-polymerizing bacteria was hindered at low temperatures, so nitrification and denitrification were significantly hampered below 15 °C [11].

Biochar is a carbon-rich material utilized as a soil amendment to enhance the physical and chemical characteristics of soil, owing to its inherent stability, high adsorption capacity, ample pores, and extensive specific surface area [12,13]. In recent years, biochars were widely used in aquatic ecological restoration for the high adsorption capacity for ammonia nitrogen ($\text{NH}_4^+\text{-N}$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$), which alleviated the stress of pollutants on aquatic organisms. On the one hand, the internal pore size in biochars provided favorable conditions for the growth of microbial communities. On the other hand, biochars were organic carbon in the low-carbon-ratio influent, improving denitrification efficiency [14–16]. A recent work of research indicated that the biochar raw materials were divided into four categories: wood, agricultural waste, sludge, and food waste [17]. The pollutant removal of different biochar materials were different: Guo et al. found that biochars made from different wetland plants had varied denitrification efficiencies [18], while Zheng et al. discovered that the TN removal rates in constructed wetlands supplemented with sludge-derived biochar and cattail-derived biochar increased by 24% and 14%, respectively [19]. Commonly used biochars, such as those made of reeds, rice, and corn, were widely applied in constructed wetlands, effectively [17,20–22]. Additionally, the method of biochar addition also caused variations in efficiencies. Liang et al.'s study indicated that rhizosphere addition was more beneficial for reed growth [23]; hence, exploring the methods of biochar addition in ecological ditch sediments is worthwhile. At present, the application of biochar is mainly concentrated in river restoration and constructed wetlands [24,25] instead of ecological ditches, and the research time was almost always chosen in the warm season [22,26]. Therefore, it is of great significance to investigate the effects of biochar on nitrogen and phosphorus removal in ecological ditch systems under low temperature conditions.

Vallisneria natans (*V. natans*) is a prevalent evergreen submerged plant, which has been proven to have a good pollution removal effect and is widely used in ecological ditches [27]. In this study, *V. natans* was chosen as the experimental plant, and three different types of biochar were added to sediments, which were combined in two different ways. The objectives of this study were: (1) to investigate the effects of different biochar additions on the removal of N and P from the ecological ditch at low temperatures for non-point source ammonia pollution; (2) to investigate the effects of different biochar addition methods on the physicochemical properties of plants and sediments in an ecological ditch; and (3) to explore the correlation between the effectiveness of nitrogen removal from ecological ditches and microbial communities in sediments.

2. Materials and Methods

2.1. Experimental Setup

This experiment was carried out in a sunlight room at the Zhuanghang Experimental Station of the Shanghai Academy of Agricultural Sciences (121°23'15" E, 30°53'24" N), Shanghai, China. The regional climate exhibits characteristics of a subtropical oceanic monsoon. The local climate is subtropical oceanic monsoon, with an average temperature of 16.1 °C over 10 years and an annual precipitation of 1191.5 mm. A total of 21 plastic buckets with a volume of 160 L were used in this study; the dimensions of the plastic buckets are as follows: it measures 62 cm in length and 42 cm in width at the bottom of bucket, and 74 cm in length and 54 cm in width at the top of bucket, and the height of every bucket is 49 cm.

The sediments and *V. natans* were collected from the ecological pond at Zhuanghang Experimental Station of Shanghai Academy of Agricultural Sciences. After removing stones and plant limbs, sediments were thoroughly mixed. The physicochemical parameters of the substrate were as follows: N: 3.12 mg/g, P: 7.62 mg/g. Three kinds of biochar (made by reed straw, rice straw, and corn straw, respectively) were purchased from Zhengzhou Jinbang Environmental Protection Technology Co., Ltd. (Zhengzhou, China). The three kinds of biochar were produced by pyrolysis at 500 °C for 2 h, and the particle size was 50 mesh.

Seven groups of experimental setups were established, and three parallel experiments were set up in each group: control group without any biochar (CK), reed biochar mixed with the sediment (K1), rice biochar mixed with the sediment (K2), corn biochar mixed with the sediment (K3), reed biochar buried under the sediment (S1), rice biochar buried under the sediment (S2), and corn biochar buried under the sediment (S3). The C and N contents of reed biochar are, respectively, 67.2% and 0.92%; for rice biochar, they are 83% and 1.41%; and, for corn biochar, they are 84.9% and 1.5%. S1, S2, and S3 were first spread with 2 cm of biochar, and then 10 cm of sediment; the K1, K2, and K3 treatments mixed 480 g biochar with 40 kg of sediment and evenly spread it into the plastic bucket, to maintain a 12 cm depth of substrate in each plastic bucket. The biochar content accounted for three percent of the dry weight of the substrate.

V. natans were initially cultivated for 7 days to acclimate to the new environment. Individuals with comparable biomass were chosen for transplantation at a density of 54 plants per device. The *V. natans* were then stabilized in devices for 14 days, replenishing pond water every 3 days during the stabilization period.

Synthetic wastewater was prepared with KNO₃, KH₂PO₄, and C₆H₁₂O₆ to make the water quality similar to peripheral domestic sewage [28]. Ammonia synthetic wastewater was as follows: the concentrations of TN, TP, chemical oxygen demand (COD), NO₃⁻-N, NO₂⁻-N, and NH₄⁺-N were 9.64 ± 0.23, 0.96 ± 0.02, 60, 0.94 ± 0.01, 0.00 ± 0.00, and 8.70 ± 0.12 mg/L, respectively.

2.2. Experimental Operation and Sampling

Constructed ecological ditches were operated for 42 days in a batch mode with a hydraulic retention time of seven days for six batches from 31 January to 14 March 2023. Synthetic wastewater was added into the plastic bucket device by siphoning to avoid disturbance [29], maintaining a water depth of 30 cm, and the working volume for each bucket was approximately 100 L. Water samples were collected every 2 days with a vacuum syringe.

V. natans were sampled at the beginning and the end of this study to measure the biomass. Both above and underground biomass of *V. natans* were harvested in each group for the analysis of plant biomass, TN, and TP content. The fresh plants were cleaned with Phosphate-Buffered Saline (PBS) before measuring. Then, the leaves and roots of fresh *V. natans* were subjected to a temperature of 105 °C in an oven for thirty minutes. Subsequently, the samples were oven-dried at 70 °C until they attained a stable weight.

The sediments were stored in 50 mL sterilized centrifuge tubes; a portion of these samples was stored at −80 °C for microbial community analysis, while another portion was air-dried and sieved at room temperature for TN and TP analysis.

Water temperature, dissolved oxygen (DO), and pH were assessed utilizing HI9829 (Hanna, Padova, Italy). TN and TP were carried out according to Standard Methods of the State Environmental Protection Administration of China (2002). The concentrations of NO₂⁻-N, NO₃⁻-N, and NH₄⁺-N in water were measured using a flow injection autoanalyzer (Seal, AA3, Frankfurt, Germany). TN concentrations of *V. natans* and sediments were determined by using the elemental analyzer (Vario EL III, Elementar, Hanau, Germany).

2.3. Data Analysis

High-throughput sequencing of the microbial communities was undertaken in Shanghai Personal Biotechnology Co., Ltd., Shanghai, China. Data processing and analyses were completed using SPSS software (SPSS V19.0, SPSS Inc., Chicago, IL, USA) and Microsoft Excel (Office 2010, Microsoft Corporation, Washington, DC, USA), plotting was completed using Origin 2022 software. Results are expressed as mean \pm standard deviation and were tested by two-way ANOVA, with $p < 0.05$ considered statistically significant.

3. Results and Discussion

3.1. Physical and Chemical Properties of Water

The changes in the physicochemical properties of synthetic wastewater during the period of feeding with NH_4^+ -N-dominated wastewater were shown in Figure 1. The average temperature varied from 11.8 °C to 14.7 °C throughout the experimental period. On the initial day, the DO levels in each treatment group experienced a substantial decrease, followed by a gradual increase. This can be attributed to the consumption of oxygen by the nitrification reaction: as the experiment proceeding the photosynthesis of *V. natans* made the DO gradually recover, the DO concentrations of K1, K2, and K3 were significantly higher than that of S1, S2, S3, and CK ($p < 0.05$), which was conducive to the nitrification reaction. The DO concentrations of S1, S2, and S3 were also higher than that of CK. The pH values of K1, K2, and K3 were significantly higher than that of S1, S2, and S3 ($p < 0.05$), with an average variation in the range of 7.7~7.9, which is more suitable for the survival of the nitrifying bacteria [2,30].

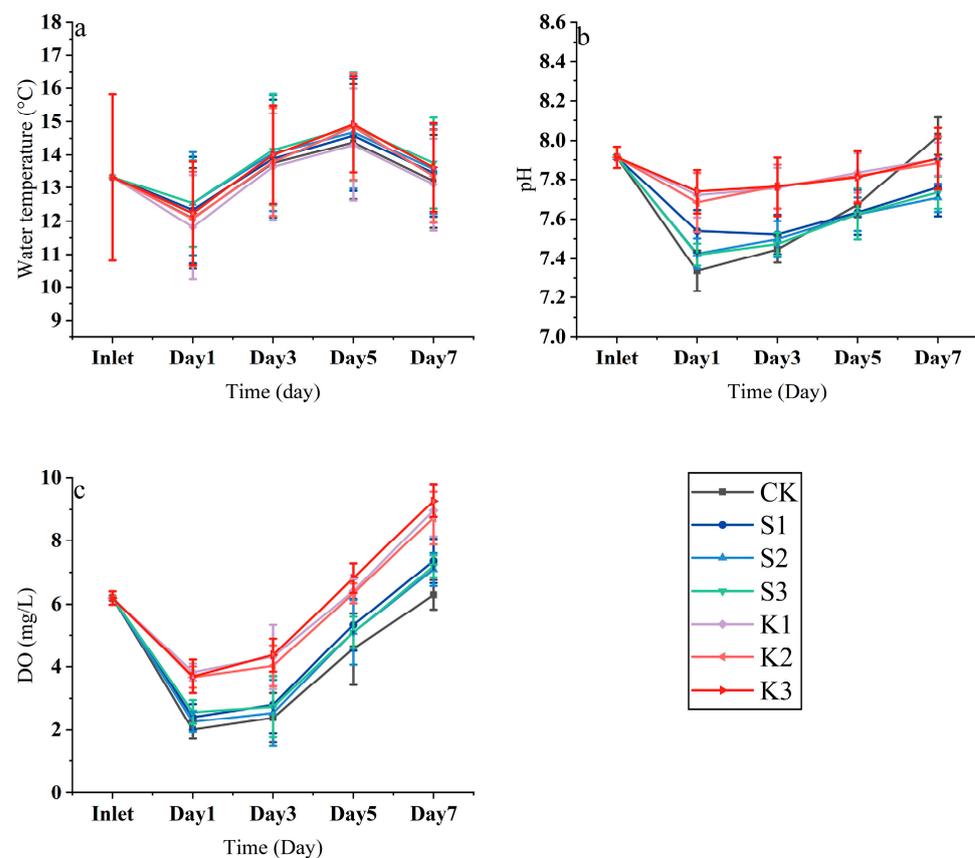


Figure 1. Temporal variations of water temperature (a), pH (b), and DO (c) in mixed addition of biochar treatment group (K1, K2, and K3), with rhizosphere addition of biochar treatment group (S1, S2, and S3), and without biochar (CK).

3.2. N and P Removal by Each Group

The removal of TN was $K3 > K1 > K2 > S3 > S1 > S2 > CK$ (Figure 2a). The average removal of CK was 55.08%, and the TN removals in K1, K2, K3, S1, S2, and S3 were significantly higher than that of CK ($p < 0.01$). Among them, the highest TN removal was 77.12% in K3. Comparing to S1, S2, and S3, the TN removals of K1, K2, and K3 were improved by 11.07%, 8.26%, and 11.4%, respectively. The study by Zhou et al. showed that the TN removal in the biochar treatment group was 72%, which is similar to the findings of this experiment. This suggested that the addition of biochar was effective in enhancing nitrification in ecological ditches [20]. Further, the mixed addition method was more effective than rhizosphere addition, and the TN removal of corn biochar was better than reed biochar and rice biochar.

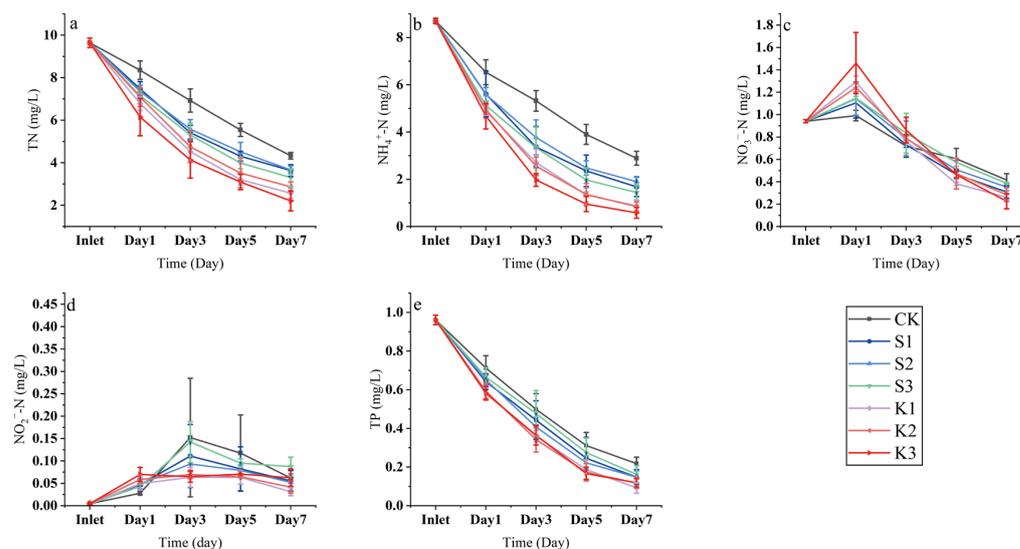


Figure 2. Temporal variations of TN (a), NO₃⁻-N (b), NH₄⁺-N (c), NO₂⁻-N (d), and TP (e) in mixed addition of biochar treatment group (K1, K2, and K3), with rhizosphere addition of biochar treatment group (S1, S2, and S3), and without biochar (CK).

NH₄⁺-N, as the main component of TN, showed a similar purification curve to that of TN (Figure 2b). At Day 7, the average removals of NH₄⁺-N in K1, K2, and K3 were 89.94%, 90.26%, and 93.31%, respectively, and the average removals of NH₄⁺-N in S1, S2, and S3 were 80.69%, 78.15%, and 83.4%, respectively. The NH₄⁺-N removals of K3 and S3 were promoted by 26.53% and 16.61% compared to CK, respectively. These results were consistent with the findings of a summer study [31], indicating that the addition of biochar to the ecological ditch at low temperature also had a significant improvement on NH₄⁺-N removal. It is widely recognized that DO is the key factor affecting NH₄⁺-N removal; studies have shown that aeration increased DO to improve the NH₄⁺-N removal in constructed wetlands [32,33]. The addition of biochar greatly improved the DO level in this study, which provided advantages for nitrification. Additionally, biochar involved electrostatic attraction, ion exchange, and hydrogen bonding [34]. The surface of biochar carried more negative charge, which can combine with NH₄⁺ through the electrostatic effect, resulting in better adsorption on NH₄⁺-N [35]. However, the efficiency of the rhizosphere addition was not as good as that of the mixed addition, which indicated that surface sediments delayed the interaction between biochar and NH₄⁺-N in wastewater.

As shown in Figure 2c, NO₃⁻-N concentrations in each treatment group reached the maximum on the first day, and then decreased with the duration until Day 7. During denitrification, the DO and carbon source are the main factors affecting nitrogen removal [36]. Attributed to relatively lower DO concentration, the NO₃⁻-N concentration in K3 decreased to 0.24 mg/L at Day 7, which was the lowest concentration compared to other groups. Further, the TN and NH₄⁺-N concentration in K3 also showed the best degradation efficiency,

indicating that corn biochar in a mixed addition was the best choice for promoting the nitrogen removal in an ecological ditch at low temperature.

The disparity in NO_2^- -N levels was not statistically significant, while it exhibited an upward trend in all groups, followed by a subsequent decline (Figure 2d). Except for the first day, the concentrations in all groups of mixed additions were smaller than the concentrations in the treatment groups corresponding to the CK and rhizosphere addition groups (S1, S2, and S3). The accumulation of NO_2^- -N was mainly due to the lack of an organic carbon source, but FU et al. proposed that the nitrification rate was greater than the denitrification rate in a high NH_4^+ -N concentration, which was due to the DO limitation [31]. Due to the photosynthesis of *V. natans*, the treatment groups in this experiment maintained a satisfactory DO level. In this study, NH_4^+ -N was the main form and the concentration of NO_3^- -N was low, so it did not produce too much nitrite nitrogen salt reductase in the competition with nitrate nitrogen salt reductase for the electron acceptor, so the experimental process did not accumulate too much NO_2^- -N.

Figure 2e showed a decrease in TP concentration in each treatment group as the experimental period increased. Similar to the trend observed in TN removal, the TP removals of K1, K2, and K3 were significantly higher than that of S1, S2, S3, and CK ($p < 0.01$). The average removal rates for group K ranged from 87.6% to 90.3%. It showed that the addition of biochar could effectively enhance the TP removal at low temperature, and the mixed addition method was significantly better than the rhizosphere addition. This may be attributed to the strong adsorption capacity of biochar. When the input P concentration was higher than 0.5 mg/L, adsorption was the main removal mechanism, while, when the input P concentration was lower than 0.25 mg/L, the biomass became more important [37,38]. In this study, the intra-group difference between mixed addition and rhizosphere addition was not significant, indicating that different biochar types did not affect TP removal. However, the TP removal of CK also reached 77.3%, which was similar to the results measured in summer [39]. Submerged plants mitigated the effects of low temperatures by improving the rhizosphere environment; therefore, temperature is not the primary factor influencing TP removal [40,41].

3.3. Changes in Plant Physicochemical Properties

As shown in Figure 3a, the difference between the treatment groups with biochar and CK was significant ($p < 0.05$), and the leaf and root biomass of K1, K2, K3, S1, S2, and S3 were significantly higher than that of CK. Compared with the initial value, the wet weight biomass of CK remained nearly constant, while the leaf biomass decreased by 22.49%, which was attributed to the fact that the low temperature decelerated the metabolism of the plant and the enzyme activity of the plant, resulting in the damage of the leaf and the reduction in the biomass.

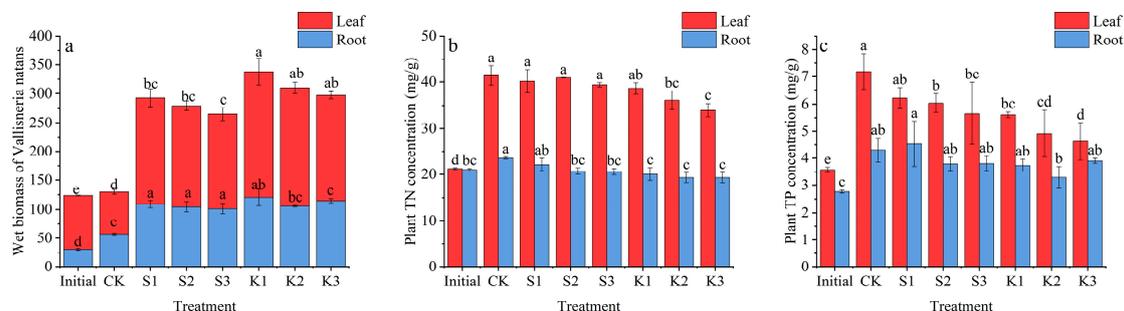


Figure 3. Wet biomass (a), nitrogen (b), and phosphorus (c) concentrations of plant tissue in mixed addition of biochar treatment group (K1, K2, and K3), with rhizosphere addition of biochar treatment group (S1, S2, and S3), and without biochar (CK). Different lowercase letters indicates significant differences ($p < 0.05$).

Additionally, the high concentration of $\text{NH}_4^+\text{-N}$ caused stress to the growth of *V. natans*, further inhibiting its growth [42]. The biomass of S1, S2, S3, K1, K2, and K3 increased by 137%, 126%, 114%, 173%, 150%, and 140%, respectively. The wet biomasses of leaves and roots in K1, K2, and K3 were significantly higher than those of S1, S2, and S3 ($p < 0.05$). It indicated that the addition of biochar effectively promoted the growth of *V. natans* at low temperature, while the mixed addition method was better than the rhizosphere addition method. The incorporation of biochar alleviated the stress of high concentrations of $\text{NH}_4^+\text{-N}$ on the leaves of *V. natans* and reduced the damage of low temperatures to the leaves of *V. natans*.

As shown in Figure 3b, both the TN and TP contents of leaves in S1, S2, S3, K1, K2, and K3 were significantly lower than that of CK ($p < 0.01$); further, the TN and TP contents of leaves in K1, K2, and K3 were significantly lower than that of S1, S2, and S3 ($p < 0.01$). These results indicated that the biochar successfully decreased the levels of N and P in the water body, resulting in a reduction in the amount of nutrients that can be taken up by plants. Both the substrate and wastewater significantly affected the stoichiometric characteristics of the submerged plant, and the N and P contents of leaves of submerged plants increased with the aggravating of the eutrophication degree of water [43]; research shows that, as the TN content and N:P ratio in the water body increase, the nitrogen content in submerged plants also increases [44]. Compared with the initial values [44], The TP and TN in the roots increased in all samples (Figure 3c), and the TN contents of the root in K1, K2, and K3 were significantly lower than that of CK and the corresponding S1, S2, and S3 ($p < 0.05$). However, the difference in TP content between mixed biochar and bottom biochar was not significant. It has been shown that submerged plants can obtain nutrients from roots through sediments, and the roots of submerged plants are closely related to the N and P contents of sediments [45].

As indicated in Table 1, the minimum N/P of the leaf in CK was 5.8, which closely resembled the beginning value, while the variation in N/P ratios between the treatment groups did not show statistical significance. It was observed that the N/P ratio was higher in the biochar addition groups compared to CK. This suggested that the addition of biochar considerably increased the N/P ratio of *V. natans* leaves. This study demonstrated a notable inverse relationship between the N/P ratio of submerged plant leaves and the TP concentration in sediment (Table 2). However, there was no significant link between the N/P ratio and TN content in sediment, which aligns with the findings of a previous study [44]. Compared to the initial values, the C/P ratio decreased significantly in all samples, with the leaf C/P ratio being significantly higher ($p < 0.05$) in K1, K2, and K3 than that in CK, S1, S2, and S3 ($p < 0.05$). The lowest C/P in CK was only 46.4. It has been shown that C/P in submerged plants was inversely related to the degree of water eutrophication, which is consistent with the results of this study [46].

Table 1. Physico-chemical properties of plants in each treatment group.

	Original Value	CK	S1	S2	S3	K1	K2	K3
N/P (leaf)	5.95 ± 0.21 ^{bc}	5.83 ± 0.76 ^c	6.48 ± 0.36 ^{abc}	6.8 ± 0.36 ^{abc}	7.22 ± 1.26 ^{abc}	6.87 ± 0.14 ^{abc}	7.63 ± 1.65 ^a	7.53 ± 1.25 ^{ab}
N/P (root)	7.59 ± 0.27 ^b	5.53 ± 0.49 ^a	5.02 ± 0.8 ^a	5.5 ± 0.54 ^a	5.44 ± 0.46 ^a	5.4 ± 0.55 ^a	5.95 ± 0.67 ^a	4.95 ± 0.39 ^a
C/P (leaf)	96.48 ± 3.35 ^d	46.41 ± 4.63 ^b	53.41 ± 3.06 ^b	54.69 ± 2.48 ^{ab}	59.40 ± 10.49 ^{ab}	59.88 ± 1.12 ^{ab}	68.23 ± 15.47 ^a	68.40 ± 12.5 ^a
C/P (root)	135.13 ± 2.85 ^d	74.55 ± 7.19 ^{ab}	68.87 ± 14.33 ^b	81.28 ± 7.55 ^{ab}	80.26 ± 5.55 ^{ab}	78.82 ± 5.89 ^{ab}	90.76 ± 9.64 ^a	76.47 ± 3.95 ^{ab}
C/N (leaf)	16.22 ± 0.02 ^c	8 ± 0.28 ^a	8.25 ± 0.2 ^a	8.05 ± 0.09 ^a	8.22 ± 0.18 ^a	8.71 ± 0.01 ^b	8.92 ± 0.17 ^b	9.06 ± 0.35 ^b
C/N (root)	17.81 ± 0.27 ^d	13.46 ± 0.24 ^c	13.64 ± 0.93 ^{bc}	14.8 ± 0.18 ^a	14.77 ± 0.45 ^a	14.63 ± 0.4 ^{ab}	15.31 ± 1.03 ^a	15.49 ± 0.46 ^a

Note: Different lowercases indicate $p < 0.05$.

Table 2. Physical and chemical properties of sediments.

	Original Value	CK	S1	S2	S3	K1	K2	K3
TN (mg/kg)	3.12 ± 0.29 ^a	3.55 ± 0.62 ^a	3.58 ± 0.29 ^a	3.46 ± 0.36 ^a	3.76 ± 0.03 ^a	3.47 ± 0.16 ^a	3.15 ± 0.18 ^a	3.52 ± 0.03 ^a
TP (mg/kg)	7.62 ± 0.33 ^c	11.92 ± 1.25 ^d	15.2 ± 0.49 ^{abc}	14 ± 0.88 ^c	14.83 ± 0.83 ^{bc}	16.51 ± 1.29 ^{ab}	17.29 ± 0.79 ^a	16.82 ± 0.07 ^{ab}
C/N	8.56 ± 0.28 ^c	8.46 ± 0.21 ^c	8.32 ± 0.15 ^c	8.25 ± 0.26 ^c	8.19 ± 0.05 ^c	10.91 ± 0.4 ^a	9.67 ± 0.93 ^b	10.15 ± 0.43 ^{ab}

Note: Different lowercases indicate $p < 0.05$.

The C/N ratio of both the leaf and root in experimental groups were much lower than the initial value, and the C/N ratios in K1, K2, and K3 were significantly higher than S1, S2, S3, and CK ($p < 0.05$). On the one hand, the excess $\text{NH}_4^+\text{-N}$ inhibited the uptake and transport of Mg^{2+} , which, in turn, suppressed photosynthesis and further disrupted the photosynthesis system of the plants, leading to the inhibition of carbohydrate formation in the plants [47]. On the other hand, high concentrations of N and P pollutants in wastewater lead to water eutrophication, which reduced the water clarity and inhibited the photosynthesis of *V. natans* leaves. These two factors accounted for the decrease in the C/N ratio in *V. natans* [48]. Therefore, it is further shown that the addition of biochar to the ecological ditch can reduce the stressful effects of high salt concentration on submerged plants, reducing the degree of water eutrophication, enhancing the transparency of water to submerged plants. In addition, the mixed addition biochar was more effective than the rhizosphere addition biochar.

3.4. Changes in Physical and Chemical Properties of Sediments

Compared with the initial value, the sediment N content increased in all treatment groups (Table 2), which proved that sediments in this study played a certain adsorption effect. The TP contents in the sediment with biochar were significantly higher than that in CK ($p < 0.01$), which indicated that the addition of biochar to sediments effectively enhanced the adsorption effect of P in sediments. Further, the sediment TP contents in K1, K2, and K3 were significantly higher than that in S1, S2, and S3 ($p < 0.01$), giving that the mixed method of addition was better than the rhizosphere addition. However, the difference in sediment N content in this study was insignificant, indicating that the addition of biochar did not have much effect on the N content in sediments, which was similar to the results of Li et al. [2]. In view of the fact that the C/N ratio of K1, K2, and K3 was significantly higher than that of CK with the corresponding S1, S2, and S3 ($p < 0.01$), and there was no significant difference between CK and the rhizosphere addition group, hence, the addition of bottom biochar had a lesser influence on the topsoil.

3.5. Microbial Community Analysis

The microbial diversity and community structure of sediments in ecological ditches were analyzed using a 16S rRNA sequence analysis, and the abundance and variation of functional micro-organisms were compared.

3.5.1. Diversity Analysis

The petal plot more visually counts the number of OTUs shared and unique to each sample to understand their compositional similarity and overlap (Figure S4). The number of detected OTUs ranges from 1752 (K1) to 2425 (S3), with 989 shared OTUs, where the number of OTUs in K1, K2, and K3 are smaller than the corresponding S1, S2, and S3 with CK. Figure 4 showed that the chao1 indices of the seven sediment samples were not significantly different; however, the values of the Simpson index and Pielou index of sediment samples in K1, K2, and K3 were significantly smaller than those of S1, S2, S3, and CK ($p < 0.05$). The Simpson index combines species richness and diversity, with larger values indicating higher species diversity. The Pielou index indicates homogeneity, with larger values indicating greater homogeneity [49].

In view of the fact that the microbial diversity of sediments in K1, K2, and K3 were smaller than that of S1, S2, S3, and CK, the addition of biochar reduced the diversity of microbial communities and the mixed addition approach suppressed microbial communities more significantly than the rhizosphere addition. In contrast with this study, it has been found that biochar increased the abundance of microbial communities [26,50]. The effect of biochar on sediment microbial abundance and community structure was complex and depended mainly on the physicochemical properties of biochar and the sediment physicochemical properties, as well as sediment enzyme activities, so further research is needed to investigate the interaction mechanism between biochar and micro-organisms.

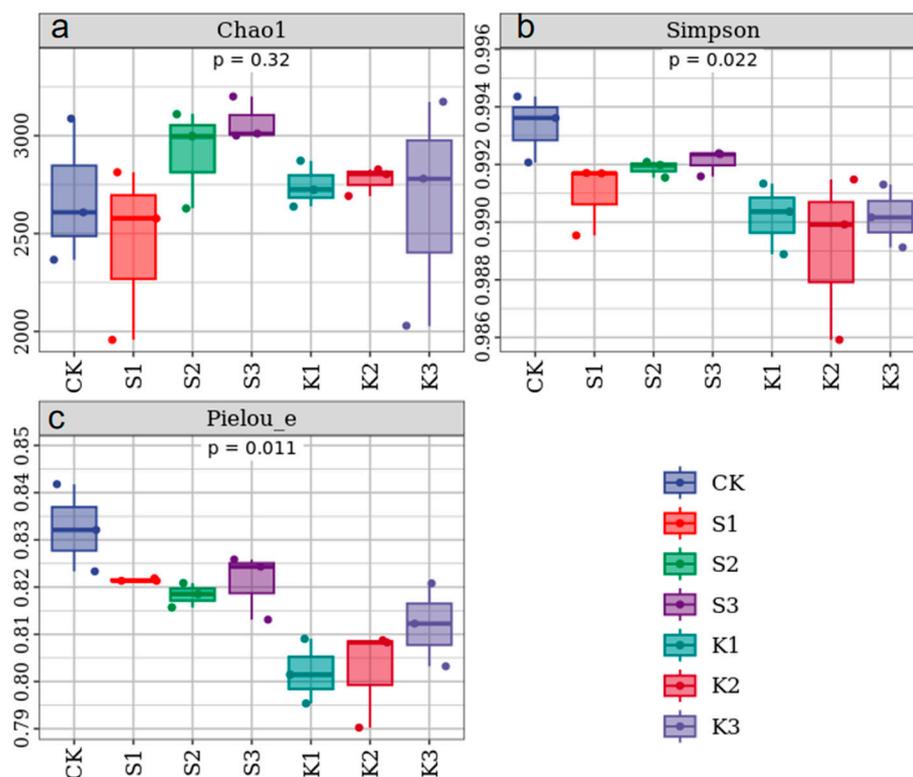


Figure 4. Alpha diversity of the sediments taken from each ecological ditch revealed by Illumina high-throughput sequencing analysis, Chao1 Index (a), Simpson Index (b) and Pielou_e Index (c).

The investigation of microbial community diversity was conducted using a PCoA analysis. As can be seen in Figure S5, the PCoA1 and PCoA2 axes represented the 35.6% and 13.4% of the variation among the sample positions, respectively. The mixed addition treatment groups (K1, K2, and K3) were tightly clustered in quadrants 1 and 4, and the rhizosphere addition treatment groups (S1, S2, and S3) were tightly clustered in quadrants 2 and 3. This result pointed out that the total diversity of the bacterial community was mostly caused by the adding method of biochar rather than by the kinds of biochar.

3.5.2. Bacterial Community Composition

In order to better understand the effects of different additions of biochar on sediments, the microbial community composition was analyzed from the phylum (Figure 5). Taxonomic assignments showed that the phylum of Proteobacteria (55.24–59.43%) was basically the major community in all samples. Other dominant phyla were Acidobacteriota (8.31–9.96%), Desulfobacterotas (6.37–8.9%), Bacteroidotas (5.55–6.36%), Chloroflexis (2.64–4.52%), and Firmicutes (2.75–4.44%).

These phyla were discovered to have significant ecological roles in the reduction in nitrate and nitrite. Acidobacteria played a crucial role in the denitrification process, and some denitrifying bacteria as well as most of the nitrogen-fixing bacteria in nature basically

belong to this phylum. Acidobacteria was reported to break down plant leftovers and played a crucial role in the decomposition of single-carbon molecules. The presence of Chloroflexis favored the removal of large molecules that are difficult to degrade. Firmicutes played a role in nitrogen metabolism, which could degrade COD and lactic acid [51,52]. The investigation revealed that the seven treatment groups had comparable species composition, with very minor variations in the frequency of bacterial communities. Combined with the differences in the performance of the nitrogen removal effect in the water of each treatment group, it can be inferred that the change in sediment bacteria is not the main factor affecting the removal effect of nitrogen and phosphorus pollutants in the ecological ditch, and the microbial community of the water body and leaf biofilm should be further explored.

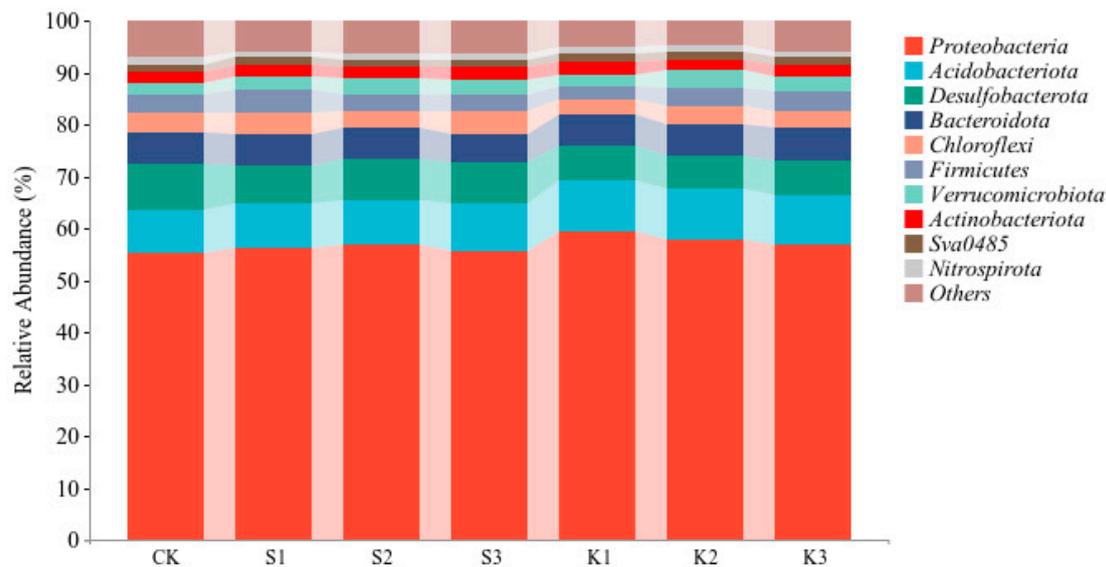


Figure 5. Distribution of top 10 phyla in all samples.

4. Conclusions

The results in this study demonstrated that incorporating biochar during winter at low temperatures can greatly enhance the efficiency of removing N and P pollutants in ecological ditches. Moreover, the mixed addition method outperformed the rhizosphere addition method. The addition of biochar significantly promoted the growth of *V. natans* under low temperature conditions, changing the physiological structure of *V. natans*, and reducing the stress of high nitrate nitrogen and ammonia nitrogen on *V. natans*. However, the impact of biochar on sediments was not significant. The addition of biochar reduced the diversity and richness of microbial communities in sediments. The differences in microbial communities were caused by the different addition methods of biochar, rather than the types of biochar. In summary, added biochar effectively enhanced the performance of ecological ditches at low temperatures. The influence of different addition methods was greater than that of biochar types. Changes in microbial communities in sediments were not the main factors affecting the removal of nitrogen and phosphorus in ecological ditches. Future research on the microbial communities of water and epiphytic biofilms is worth exploring.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w16081191/s1>, Figure S1. Schematic diagram of simulated ecological ditches. Figure S2. Biochar scanning electron microscope. Figure S3. Two types of biochar Fourier transform spectra (YM: Corn biochar; SD: Rice biochar). Figure S4. The number of OTUs in all samples. Figure S5. Principal co-ordinates analysis (PCoA) of all samples at phylum level. Figure S6. NH₄⁺-N removal (a) and TP removal (b) in mixed addition of biochar treatment group (K1, K2, K3), with rhizosphere addition of biochar treatment group (S1, S2, S3) and without biochar (CK).

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