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Research article

Unveiling the nitrogen and phosphorus removal potential: Comparative analysis of three coastal wetland plant species in lab-scale constructed wetlands

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ABSTRACT

It is well accepted that tidal wetland vegetation performs a significant amount of water filtration for wetlands. However, there is currently little information on how various wetland plants remove nitrogen (N) and phosphorus (P) and how they differ in their denitrification processes. This study compared and investigated the denitrification and phosphorus removal effects of three typical wetland plants in the Yangtze River estuary wetland (*Phragmites australis*, *Spartina alterniflora*, and *Scirpus mariqueter*), as well as their relevant mechanisms, using an experimental laboratory-scale horizontal subsurface flow constructed wetland (CW). The results showed that all treatment groups with plants significantly reduced N pollutants as compared to the control group without plants. In comparison to *S. mariqueter* (77.2–83.2%), *S. alterniflora* and *P. australis* had a similar total nitrogen (TN)removal effectiveness of nearly 95%. With a removal effectiveness of over 99% for ammonium nitrogen (NH_4^+-N), *P. australis* outperformed *S. alterniflora* (95.6–96.8%) and *S. mariqueter* (94.6–96.5%). The removal of nitrite nitrogen (NO_2^--N)and nitrate nitrogen (NO_3^--N)from wastewater was significantly enhanced by *S. alterniflora* compared to the other treatment groups. Across all treatment groups, the removal rate of PO_4^{3-}-P was greater than 95%. *P. australis* and *S. alterniflora* considerably enriched more ^{15}N than *S. mariqueter*, according to the results of the ^{15}N isotope labeling experiment. While the rhizosphere and bulk sediments of *S. alterniflora* were enriched with more simultaneous desulfurization-denitrification bacterial genera (such as *Paracoccus*, *Sulfurovum*, and *Sulfurimonas*), which have denitrification functions, the rhizosphere and bulk sediments of *P. australis* were enriched with more ammonia-oxidizing archaea and ammonia-oxidizing bacteria. As a result, compared to the other plants, *P. australis* and *S. alterniflora* demonstrate substantially more significant ability to remove NH_4^+-N and NO_2^--N/NO_3^--N from simulated domestic wastewater.

1. Introduction

The acceleration of urbanization has led to an increasingly prominent water pollution problem on a global scale (Strokal et al., 2021). Excessive nitrogen (N) and phosphorus (P) from urban domestic wastewater are being discharged arbitrarily into rivers and lakes, causing serious eutrophication issues (Duan et al., 2017). Currently, constructed wetlands (CWs) are considered a sustainable wastewater treatment solution (Knight et al., 2000; Sharma et al., 2022; Vymazal, 2010). CWs are a wastewater treatment technology based on natural processes, and they are widely used for the treatment and purification of pollutants such as N and P due to their economic, environmental, efficient, and sustainable advantages (Vystavna et al., 2017; Yang et al., 2022; Zhang et al., 2021). According to the flow direction of water, CWs can be classified into two types: surface flow CW and subsurface flow CW, the latter of which can be further divided into horizontal subsurface flow CW and vertical subsurface flow CW (Dotro et al., 2017). The purification method of CWs is highly complicated, including the synergistic action of plants, substrates, and microbes, and employs physical, chemical, and biological processes to completely remove pollutants (Jayalakshmi et al., 2023). Plants are important in the purifying process (Ohore et al., 2022). They not only directly absorb nutrients like N and P

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from wastewater for their own growth, but they also oxygenate the root zone, increasing the quantity and altering the types of microorganisms in the CW system, thereby improving the overall purification capacity of the system (Jesus et al., 2018; Kulshreshtha et al., 2022). As a result, selecting suitable plants can considerably increase the purifying efficiency of CWs (Karungamye, 2022; Srivastava et al., 2008).

Phragmites australis is a perennial herbaceous plant widely distributed in estuarine and coastal wetlands, renowned for its adaptability and wide ecological range. It exhibits a strong ability to utilize nutrients, particularly N and P (Rickey and Anderson, 2004), and has been extensively employed in CW for the treatment and purification of N and P pollutants (Li and Guo, 2017; Wang et al., 2021). *Scirpus mariqueter* is a perennial halophytic herbaceous plant endemic to China, found in the Yangtze River estuary and Hangzhou Bay regions (Ou et al., 1992). Although its application in CW is relatively limited, recent research has confirmed its effectiveness in removing N and P pollutants (Cheng et al., 2023), presenting new possibilities for plant selection in CWs. *Spartina alterniflora*, another perennial halophytic herbaceous plant, is native to the Atlantic coast of North America and the Gulf of Mexico (Chen et al., 2012). Unlike *P. australis* and *S. mariqueter*, *S. alterniflora* is a C4 plant with a strong capability for carbon sequestration (Jin et al., 2017). During the last centuries, *S. alterniflora* has been promoted for land reclamation and coastal protection in various countries, including Europe, Asia, and Australia (Lin and Mendelssohn, 1998). Its vigorous reproductive and competitive abilities have led to rapid expansion, posing significant threats to native species and local ecosystems (Callaway and Josselyn, 1992; Chen et al., 2004), resulting in substantial economic losses and the degradation of ecosystem structure and function (Wan et al., 2009). Nevertheless, some studies have shown that CWs planted with *S. alterniflora* can achieve favorable results in removing N and P pollutants (Sousa et al., 2011; Tanner et al., 1998; Wu et al., 2019). Moreover, as the strongest competitor among native plants such as the *P. australis* and *S. mariqueter* in the Shanghai tidal marshes, *S. alterniflora* has been reported to have higher N and P contents in its above-ground parts during the growing season compared to *P. australis* and *S. mariqueter* (Quan et al., 2007). Li et al. (2020) also found that *S. alterniflora* in the Yangtze River estuary significantly enhances the N removal efficiency in the 0–50 cm sediments layer when compared to native plants such as *P. australis* and *S. mariqueter*.

While each of the three plants mentioned above in the CW showed some degree of denitrification and P removal potential, there remains uncertainty regarding the variations in N and P removal effects among these plants in the CW and their respective denitrification mechanisms. This lack of clarity is particularly significant in the context of sewage treatment systems, as the specific contributions of plants to other components (such as sediments adsorption or other fillers) and processes (like denitrification involving microorganisms) have not been fully elucidated yet. To bridge this knowledge gap, this study implemented a lab-scale horizontal subsurface flow CW using three types of plants: *S. alterniflora*, a native plant, along with *P. australis* and *S. mariqueter*, two commonly found local plants from the Yangtze River estuary. The primary objective of this study was to compare and analyze the efficiency of these three plants in removing N and P pollutants from simulated domestic wastewater. Additionally, this study sought to quantify and analyze the contributions of various components (plants, substrates, and microorganisms) in the CW to denitrification and phosphorus removal, as well as understand their respective microbial mechanisms. To achieve these goals, this study utilized ^{15}N stable isotopes to label the N present in the simulated domestic wastewater, enabling the tracking and analysis of its abundance in the substrates (cinders, sediments) and plant components (roots, stems, and leaves) within the treatment system. Furthermore, the quantitative PCR (qPCR) analysis were conducted to explore the involvement of key denitrification-related microbial functional genes in the CW sediments. The findings from this study are expected to offer theoretical insights into improving the purification of domestic wastewater and selecting appropriate plants for use in CWs.

2. Materials and methods

2.1. Design and construction of the CW system

In an artificial climate chamber, 12 lab-scale CW systems of the same size were set up using polypropylene (PP) material. As shown in Fig. 1, each CW system was composed of a CW and a circulating pump. The simulated domestic wastewater was continuously pumped from the outlet to the inlet, achieving a circulation in the system. Each CW system measured 0.6 m in length, 0.3 m in width, and 0.7 m in height. It was divided into two parts by a baffle capable of filtering impurities. The baffle was designed with 44 circular filtering holes, arranged in a 4 by 11 grid, each with a diameter of 5 cm and a layer of 300-mesh nylon filter net. The left side represented the wastewater treatment zone (0.55 m long), and the right side served as the water collection zone (0.05 m long). To ensure the permeability and water permeability of the CW system, the wastewater treatment zone was filled with a substrate consisting of native sediments from the habitats of the three plants (collected from the coastal Yangtze River estuary) and purchased cinders (2–3 cm in diameter) to a height of 0.55 m. The filling process alternated between 5 layers of 3 cm thick cinders and 5 layers of 7 cm thick tidal flat sediments, topped with a final layer of 5 cm thick cinders. Inlet and outlet pipes (0.02 m in diameter) were respectively set above the wastewater treatment zone and below the water collection zone to simulate the inflow and outflow of domestic wastewater. The 12 CW systems were divided into four groups: a control group without plants, a group with *P. australis*, a group with *S. mariqueter*, and a group with *S. alterniflora*. Each group had three replicates. The CWs in the *S. alterniflora* and *P. australis* groups were planted with 20 plants each, resulting in a planting density of approximately 121 plants per square meter. Considering the smaller plant height and biomass of the *S. mariqueter* the *S. mariqueter* group was planted with 40 plants, resulting in a planting density of approximately 242 plants per square meter. The control group did not have any plants planted.

2.2. Collection, cultivation, and domestication of plants

In August 2022, young seedlings of *S. alterniflora* and *P. australis*, as well as seeds of *S. mariqueter*, were collected at the coastal wetland in Fengxian District, Shanghai. The collected *S. alterniflora* and *P. australis* seedlings were stored in tap water for future use. The seeds of *S. mariqueter* were placed in a constant-temperature incubator at 30 °C for germination treatment. After the seeds of *S. mariqueter* germinated, they were transplanted separately into corresponding CWs, along with the *S. alterniflora* and *P. australis* seedlings. The above-ground parts of the *S. alterniflora* and *P. australis* seedlings were trimmed to approximately 10 cm. After transplantation, the plants were irrigated with an equal volume of pure water for two weeks. Upon transferring the plants to CW, the initial above-ground height was recorded, and the above-ground height was measured at the same time every week until the end of the experiment. To measure the height above ground of three plant species, start by positioning a ruler vertically at the plant's base and inserting it into the sediment. Determine the height by measuring the vertical distance from the sediment level to the stem's highest point. This procedure should be repeated for each plant in every CW system to determine the average above-ground height, which represents the absolute height of the plants in that system. Then, consistently every seven days, measure and average out the absolute heights of all plants across the 12 CW systems.

In this study, to facilitate the adaptation of plants to the simulated domestic wastewater environment, the irrigation water in the cultivation phase was initially modified to include 25% simulated domestic wastewater. The concentration was incrementally increased each week (to 50%, then 75%, and ultimately 100%). Over a period of one month,

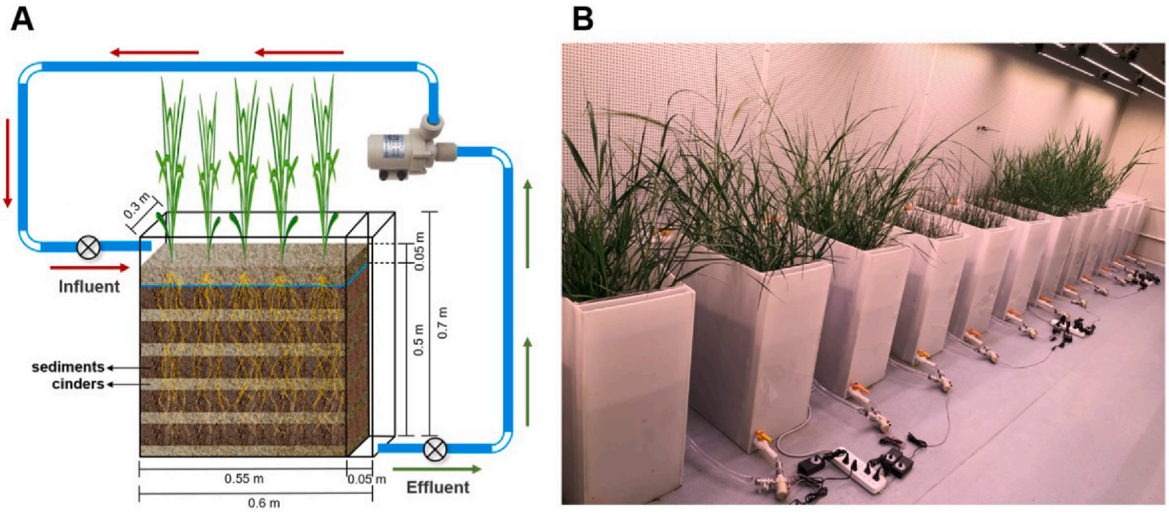

Fig. 1. Schematic diagram of (A) a laboratory-scale CW system and (B) an experimental site image.

the plants were acclimatized to this final concentration. The water used in the experiment was sterile deionized water. The formula used to simulate domestic wastewater during the experiment is shown in Table S1 (Jia, 2013). In the influent, the COD, NH4+-N, and TP exhibited theoretical concentrations of 400 mg/L, 50 mg/L, and 5 mg/L, respectively, while the pH ranged between 7.2 and 7.5. This concentration is similar to many studies on CW treatment of urban domestic wastewater (Prochaska et al., 2007; Zhao et al., 2010). The concentration of the simulated domestic wastewater was increased weekly (25%, 50%, 75%, 100%) until reaching the final concentration, and the plants were acclimated to this concentration for two weeks. During the acclimation phase, a trial run was conducted on the CWs by activating the water pump switch, allowing the simulated domestic wastewater to circulate within the CWs for two weeks. This ensured that the system had a favorable operating environment and stable purification performance during the formal experiment. At the same time, the same procedures were carried out for the control group.

2.3. *Comparison of N and P removal rate among different CWs*

This experiment was divided into two treatment periods, with each period lasting 7 days. At the beginning of each treatment period, the simulated domestic wastewater in the CWs was first emptied, and then 16 L of 100% concentrated simulated domestic wastewater was reintroduced. The water pump switch was turned on to circulate the wastewater within the CWs. The hydraulic retention time was set to 7 days, and the water level was maintained at around 0.5 m throughout the experiment.

Before each treatment period, untreated simulated domestic wastewater was collected as the original sample for Day 0. Subsequently, water samples were gathered on Days 1, 3, 5, and 7 to obtain both influent and effluent samples. The physicochemical parameters, including temperature (T), pH, dissolved oxygen (DO), salinity (Sal), electrical conductivity (EC), oxidation-reduction potential (Eh), total dissolved solids (TDS), chemical oxygen demand (COD), total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), and orthophosphate (PO_4^{3-}-P), were closely monitored. A multifunctional water quality analyzer (AZ86031, Hengxin) was used for in-situ measurements of T, pH, DO, Sal, EC, and TDS in the water samples. A portable oxidation-reduction potential meter (CT-8022 model, Kedida) was used for in-situ measurements of Eh in the water samples. The COD of the water samples was determined using the rapid closed-catalytic digestion method, and the measurements were taken at a wavelength of 600 nm (Ministry of Environmental Protection of the People's Republic of China, 2002).

The TN in the water samples was measured using a total organic carbon analyzer (TOC-V, Japan). The concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N in the water samples were measured using a SAN++ 4-channel physicochemical analyzer (San Plus System, Netherlands). The concentration of PO_4^{3-}-P in the water samples was determined using the phosphomolybdate blue spectrophotometric method at a wavelength of 882 nm (General Administration of Quality Supervision, 2007). Different forms of N (TN, NH_4^+-N, NO_2^--N, and NO_3^--N), and phosphorus (PO_4^{3-}-P) in influent and effluent samples collected at various time points were used to calculate the removal rate of N and P in the CW system. The equation for calculating the removal rate (RR) of different forms of N and P in CWs is as follows:

$$RR_{N,P} = \frac{I_{N,P} - E_{N,P}}{I_{N,P}} \times 100\%$$

In the equation, $I_{N,P}$ and $E_{N,P}$ represent the concentrations of N and P pollutants in the influent and effluent, respectively. If the calculation results are negative, the RR for that particular form of N and P is considered as 0.

2.4. *Comparison of N allocation in different components of the CWs*

After completing the previous stage of the experiment, the simulated domestic wastewater in the CWs was drained, and the storage tanks were washed and dried. Using an analytical balance, 12 equal portions, each weighing 108.32 mg, of ^{15}N-labeled potassium nitrate ($K^{15}NO_3$) were weighed out (equivalent to an addition of 15 mg ^{15}N per CW treatment). These labeled samples of $K^{15}NO_3$ were then separately added to each CW containing 16 L of 100% concentrated simulated domestic wastewater. The solutions were thoroughly mixed by stirring. Next, the water pump switches were turned on to initiate the circulation of the ^{15}N-labeled wastewater in the CWs. The timing started from the moment the influent was introduced. The system was set to operate continuously for 14 days during the experimental period without any stop intervals or changes in the influent.

After this stage of the experiment, the system was stopped, and all the water in the CWs was drained. The loose substrate was carefully removed, and the plants along with their root systems were taken out of the CWs. The substrate around the plant roots was washed clean. The plants were then separated into three parts: roots, stems, and leaves. In this study, 'stems' and 'leaves' of the *S. mariqueter* specifically denote the plant's above-ground components, encompassing its upright stem and foliage. The stem, measuring approximately 20–90 cm in height, exhibits a sharply triangular and smooth surface. Typically, this plant sports 1 to 2 leaves that are each 2–3 mm in width, shorter than the stem, and possess a flat structure (Zhang et al., 2023). Each part was dried at 60 °C until a constant weight was reached and then weighed. Using a five-point sampling method, cinder and sediments samples were collected from different depths (0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, 40–50 cm) within the CWs.

After mixing samples of different depths separately to obtain homogenized samples, they are used as final total sediments and final total cinder samples. The collected final cinder, sediments, and plant (roots, stems, leaves) samples and the initial cinder, sediments, and plant (roots, stems, leaves) samples retained before the experiment are dried at 60 °C until constant weight. After passing through a sieve with a pore size of 0.125 mm, TN content is determined using an elemental analyzer (Vario EL III, Germany), and then the TN increment of different samples is calculated. Both the initial and final samples are subjected to microwave digestion, and the total phosphorus (TP) content is measured using an inductively coupled plasma optical emission spectrometer (iCAP 7400, America), and then the TP increment of different samples is calculated. For the final cinder, sediments, and plant (roots, stems, leaves) samples, the relative abundance of ^{15}N is determined using elemental analyzer-isotope ratio mass spectrometry (Flash-2000 Delta V Advantage, America), and the corresponding proportion of ^{15}N content was calculated using the measured TN increment of different samples.

2.5. Microbial community analysis

After draining all the water from the CWs, a five-point sampling method was used to collect plant rhizosphere and bulk sediments from different depths within the CWs: 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, 40–50 cm. The samples from each depth were mixed thoroughly to create composite samples for both rhizosphere and bulk sediments. Along with the total sediment samples from the control group, these samples were sent to Majorbio, a biotechnology company in Shanghai, for high-throughput sequencing of bacterial 16S rDNA and qPCR analysis of ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and the nirS gene (denitrifying bacteria). The primers used for amplification are listed in Table S2 (Francis et al., 2005; Rotthauwe et al., 1997; Throbäck et al., 2004; Xu et al., 2016).

2.6. Statistical analyses

In this experiment, all the data presented are the mean values and standard deviations of three replicate treatments. GraphPad Prism 8.0.1 was used for one-way analysis of variance (One-Way ANOVA) within the same treatment group and two-way repeated measure ANOVA among different treatment groups. Post-hoc comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test for multiple comparisons. Correlation plots were created, and significance was considered when $p < 0.05$. The structure and diversity of the bacterial community were analyzed using R software (version 3.6.1; R Core Team, 2019). Principal coordinate analysis (PCoA) was conducted to explore the similarity among bacterial communities. The relative abundance of representative nitrifying and denitrifying microbial genera was calculated, and the results were visualized in the form of a heatmap. The "functional annotation of prokaryotic taxa (FAPROTAX) (Louca et al., 2016) on Major Bio's online analytical platform was used to perform functional annotation of taxonomic groups associated with N cycle.

3. Results

3.1. The growth of the plants

Throughout the entire duration of the experiment, the three plant species, namely *S. alterniflora*, *P. australis*, and *S. mariqueter*, exhibited continuous growth. During the course of the experiment, both *S. alterniflora* and *P. australis* showed significantly greater aboveground heights compared to *S. mariqueter*. Furthermore, starting from the 5th week, the aboveground height of *S. alterniflora* was significantly higher than that of *P. australis* (Figure S3A). At the end of the experiment, there were no significant differences between *S. alterniflora* and *P. australis* regarding root, stem, leaf, and total biomass. However, the biomass of both *S. alterniflora* and *P. australis* was significantly higher than that of *S. mariqueter* (Figure S3B).

3.2. The changes in the physicochemical parameters of the wastewater

Throughout the experiment, the temperature of each wastewater group was carefully maintained within the range of 27.3–28.0 °C, which closely resembled the ambient temperature in the artificial climate chamber (Figure S1A). The pH levels of all groups remained relatively stable, fluctuating within a range of 7.42–8.68 (Figure S1B). Following the initiation of the experiment, the DO concentration in the control group showed a significant decrease compared to the plant groups. Conversely, the DO concentration in the plant groups exhibited a notable increase from the initial levels, with a trend observed as *P. australis* > S. mariqueter > S. alterniflora (Figure S1C). Both the Sal and EC of each wastewater group experienced a significant increase, with a more pronounced upward trend observed in the plant groups (Figure S1D & S1E). The general pattern observed in the Eh of each CW group demonstrated an upward trend (Figure S1F).

The TDS concentration of each wastewater group showed a significant decrease on the first day of each cycle, followed by a slow increase thereafter (Figure S2A). Upon passing through the CW, the wastewater exhibited a swift reduction in COD concentration within each group. Notably, the plant groups exhibited significantly lower COD concentrations compared to the control group. Both S. alterniflora and *P. australis* had similar COD concentrations, which were significantly lower than those of the S. mariqueter group (Figure S2B).

The two-way repeated measures ANOVA revealed that treatment time, CW type, and the interaction between treatment time and CW type all have an extremely significant impact on the TDS and COD of the wastewater ($p < 0.0001$) (Table S4).

3.3. The removal of N

In both experimental cycles, the plant groups demonstrated TN removal rates exceeding 77%, which were significantly higher than those of the control group (45.92–50.30%). Within the plant groups, both *S. alterniflora* and *P. australis* exhibited similar TN removal rates, approximately 95%, which were significantly higher than the TN removal rate observed in the *S. mariqueter* group (77.24–83.21%) (Fig. 2A). The plant groups achieved NH_4^+-N removal rates surpassing 94%, significantly outperforming the control group (66.33–72.64%). *S. alterniflora* and *S. mariqueter* groups exhibited similar NH_4^+-N removal rates, both around 95%, whereas *P. australis* displayed the highest NH_4^+-N removal rate (>98.7%) (Fig. 2B).

In both experimental cycles, except for the S. alterniflora group, the concentrations of NO_2^--N in all other groups showed a significant increase followed by a fluctuating decrease after the experiment began (Fig. 2C). Similarly, the concentrations of NO_3^--N in all four groups also exhibited a significant increase followed by a fluctuating decrease, and the differences in concentration between NO_2^--N and NO_3^--N were nearly consistent (Fig. 2D). Among the different plant groups, the S. alterniflora group demonstrated the best removal rate for both NO_2^--N and NO_3^--N. The initial and final concentrations of TN, NH_4^+-N, NO_2^--N, and NO_3^--N, as well as their removal rates in the four types of CWs are detailed in Table S3.

The two-way repeated measures ANOVA showed that treatment time, CW type, and the interaction between treatment time and CW type all had a highly significant impact on the TN concentration and removal rate, NH_4^+-N concentration and removal rate, as well as the NO_2^--N and

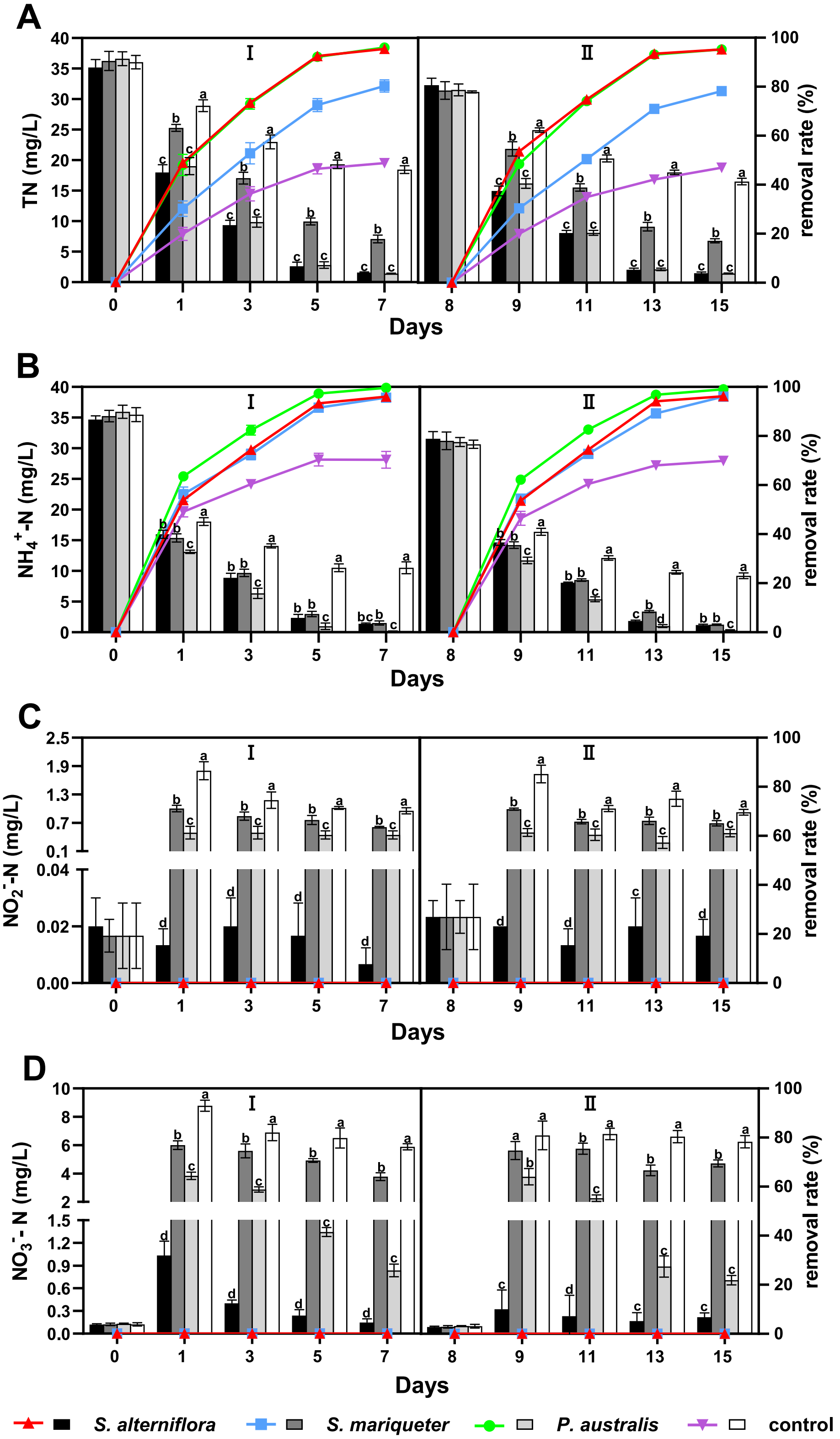

Fig. 2. Variation in TN, concentrations of various inorganic N species, and removal rate of the simulated domestic wastewater. For each treatment, data with different letters are significantly different at $p < 0.05$.

NO_3^--N concentrations in the wastewater ($p < 0.0001$) (Table S4).

3.4. The removal of P

In both experimental cycles, the concentrations of PO_4^{3-}-P in the wastewater from the four types of CW showed a rapid decrease on the first day after the wastewater was introduced. Subsequently, the concentrations slightly increased or remained stable with fluctuations. The plant groups and the control group showed similar removal rates for PO_4^{3-}-P, with removal rates exceeding 95% (Fig. 3). The initial and final concentrations of PO_4^{3-}-P and their removal rates in the four types of CWs are detailed in Table S3.

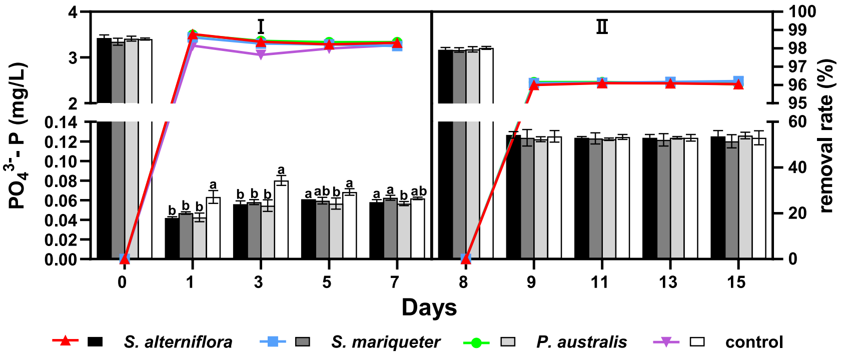

Fig. 3. Variation in concentration and removal rate of reactive phosphates in the simulated domestic wastewater. For each treatment, data with different letters are significantly different at $p < 0.05$.

The two-way repeated measures ANOVA showed that the CW type had a highly significant impact on the PO_4^{3-}-P concentration in the wastewater ($p < 0.0001$). Additionally, treatment time and the interaction between treatment time and CW type both had a highly significant effect on the PO_4^{3-}-P removal rate in the wastewater ($p < 0.0001$) (Table S4).

3.5. Allocation of N and P among different CW components

3.5.1. TN and TP increments and proportions in each component

By the end of the experiment, TN increment in the CW system of the plant groups was significantly higher than that in the control group ($p < 0.0002$). Both the *S. alterniflora* and *P. australis* groups showed significantly higher TN increments compared to the *S. mariqueter* group ($p < 0.001$), with the *P. australis* group exhibiting the highest TN increment (Fig. 4A). However, there were no significant differences in TP increments among the plant groups (Fig. 4B). When examining the

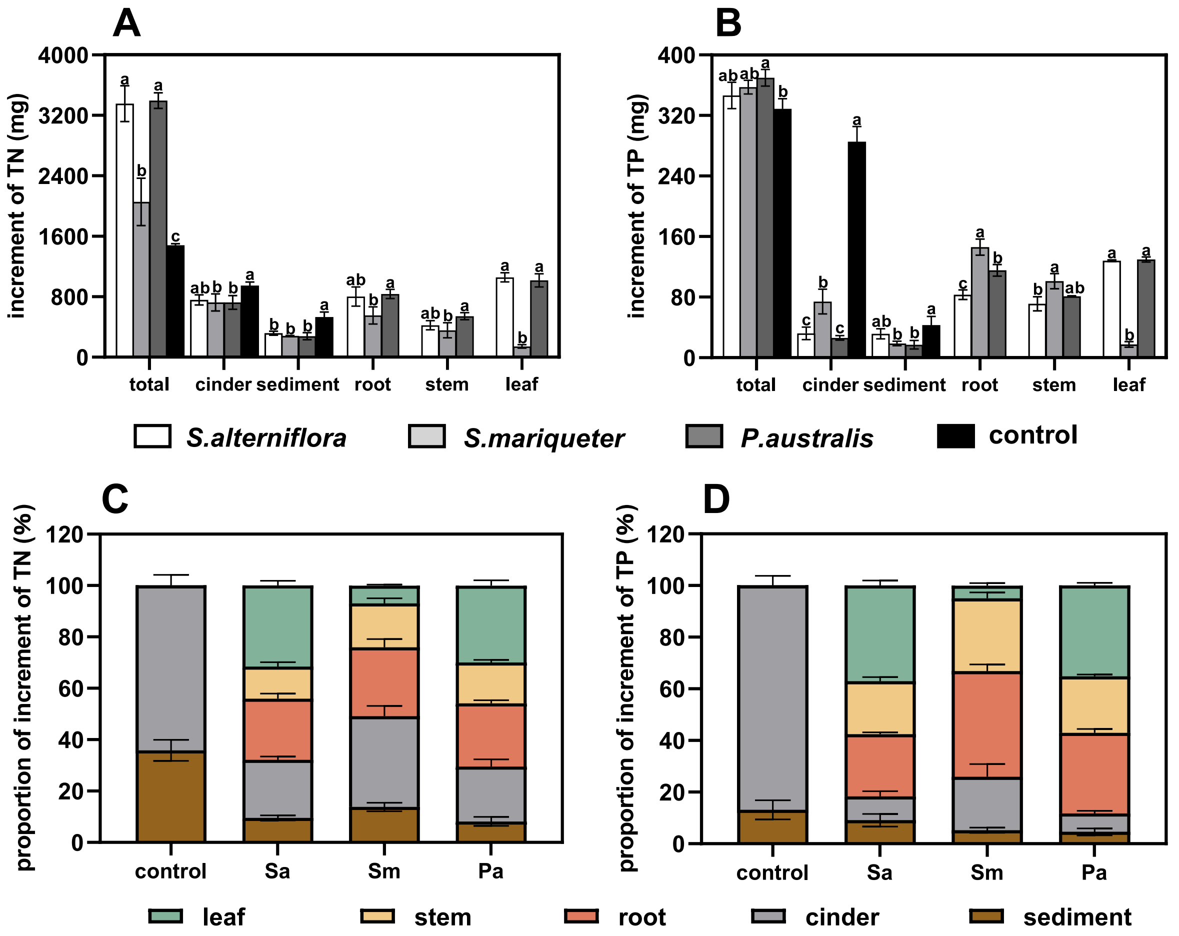

Fig. 4. Increment of TN (A) and TP (B) and the proportion of increment of TN (C) and TP (D) in different system components of different CWs. For each treatment, data with different letters are significantly different at $p < 0.05$.

increment of each component, the control group had higher TN and TP increments in cinders and sediments compared to the plant groups. The TN increments in the roots, stems, and leaves of the *S. alterniflora* and *P. australis* groups were similar but significantly higher than those of the *S. mariqueter* group ($p < 0.05$). Regarding the distribution proportion of TN and TP increments among different components within each system, in the control group, cinders had the highest proportion in both TN and TP increments, exceeding 59% for both, with the TP increment in cinders accounting for as much as 86.8% (Fig. 4C & D). In the plant groups, the combined proportion of roots, stems, and leaves in TN and TP increments exceeded 45% and 67%, respectively (Fig. 4C & D). Among the different plant groups, *P. australis* had the highest combined proportion of TN and TP increments in roots, stems, and leaves, with averages of 70.5% and 88.3%, respectively. *S. alterniflora* ranked second with averages of 67.9% and 81.6%, while *S. mariqueter* had the lowest proportions, with values of 50.9% and 74.0%, respectively.

3.5.2. *Proportion of 15N content in each component*

At the end of the experiment, the control group demonstrated the highest percentage of ^{15}N distribution across other parts or pathways (Fig. 5). Consequently, the control group exhibited the lowest proportion of enriched ^{15}N (inclusive of sediments and cinders), approximately at 27.0%. Among these constituents, cinders contributed to a relatively larger share, accounting for about 17.4% of the ^{15}N content. The distribution of ^{15}N content across substrates within the three plant groups (including sediments and cinders) was notably similar, showing no significant disparities, at approximately 18.63%. However, this was markedly lower than the control group at 26.98% ($p = 0.0014$). Among the plant groups, the cumulative ^{15}N content in *P. australis*' roots, stems, and leaves constituted approximately 44.58%, slightly surpassing *S. alterniflora* (42.44%), with both significantly exceeding *S. mariqueter* (19.28%) ($p < 0.0001$). The distribution of ^{15}N content across roots, stems, and leaves varied among the three plant species. In the case of *P. australis* and *S. alterniflora*, the leaves exhibited the highest proportion of ^{15}N content, followed by roots and stems, respectively. Conversely, *S. mariqueter* displayed the highest proportion of ^{15}N content in roots and the lowest in leaves, aligning with the proportions of TN increments in roots, stems, and leaves for the three plant species.

3.6. *Structure and diversity of bacterial communities in plant rhizospheres and bulk sediments*

The Shannon index showed no significant differences among the three plant rhizosphere sediments, rhizosphere adjacent sediments, and the control group, indicating little variation in bacterial community diversity (Table S5). The Chao index for all plant groups was higher than that of the control group, suggesting that the presence of plants enhances the abundance of sediments bacterial communities. Among the three plant species, *S. alterniflora* had the most significant effect (Table S5). PCoA revealed that the rhizosphere and bulk sediment bacterial communities of *S. alterniflora* were distinctly separated and distant from other groups along PcoA axis 1, indicating significant differences in bacterial community structure compared to the other groups (Figure S5).

From the perspective of bacterial community composition, there were significant differences in some bacterial community structures between the rhizosphere and bulk sediments of *S. alterniflora* and other groups. At the phylum level, the rhizosphere and bulk sediments of *S. alterniflora* were enriched with more Desulfobacterota and Campilobacterota compared to other groups, with Desulfobacterota and Campilobacterota significantly higher in the rhizosphere compared to all other groups ($p < 0.05$) (Figure S4). The control group and *P. australis* rhizosphere had significantly higher *Nitrospirota* than the *S. alterniflora* rhizosphere ($p < 0.05$). At the genus level, most typical nitrifying bacteria genera in the *S. alterniflora* rhizosphere and bulk sediments, such as *Nitrospira* and *Nitrosomonas*, were lower than the average value, with *Nitrospira* in the *S. alterniflora* rhizosphere significantly lower than in all other groups ($p < 0.05$). The relative abundance of *Nitrosomonas* in the *S. alterniflora* rhizosphere was significantly lower than in the rhizospheres of *S. mariqueter* and *P. australis* ($p < 0.05$). Additionally, the relative abundance of *Nitrospina* and *Nitrospira* in the *S. alterniflora* rhizosphere was significantly lower than in the control group ($p < 0.05$) (Fig. 6). Compared to other groups, the relative abundance of most typical denitrifying bacteria genera, such as *Sulfurovum*, *Sulfurimonas*, and *Desulfatitalea*, was higher in the *S. alterniflora* rhizosphere and bulk sediments, with *Sulfurovum* and *Desulfatitalea* in the *S. alterniflora* rhizosphere significantly higher than in other groups ($p < 0.05$). The relative abundance of *Paracoccus* in the *S. alterniflora* rhizosphere sediments was also significantly higher than in other groups ($p < 0.01$) (Fig. 6).

3.7. *Expression of functional genes related to nitrification and denitrification*

Quantitative analysis of amoA and nirS genes for AOA, AOB and denitrifying bacteria showed significant differences between the control group and the plant groups. The copy numbers of amoA genes (AOA and AOB) in the control group were significantly lower than in the plant groups ($p < 0.0208$ and $p < 0.0005$, respectively). Additionally, for all three plant species, the copy numbers of amoA genes (AOA and AOB) were consistently higher in the rhizosphere than in the bulk sediments (Fig. 7A & B). In particular, the copy numbers of amoA genes (AOA) in the rhizosphere of *P. australis* were higher than in the rhizosphere of S. alterniflora and significantly higher than in the rhizosphere of S. mariqueter ($p = 0.0062$), and these differences were also observed in the bulk sediments, although they were not significant (Fig. 7A). Regarding the amoA gene (AOB), the copy numbers were significantly higher in the rhizosphere of S. alterniflora and *P. australis* than in their respective bulk sediments ($p < 0.05$). Moreover, the copy numbers of the amoA gene (AOB) in the rhizosphere of *P. australis* were significantly higher than in the rhizosphere of S. alterniflora and S. mariqueter ($p < 0.0001$). Additionally, the rhizosphere of S. mariqueter had significantly higher amoA gene (AOB) copy numbers than the rhizosphere of S. alterniflora ($p < 0.01$). Similar significant differences were observed in the copy numbers of the amoA gene (AOB) in the bulk sediments of all three plant species (Fig. 7B).

In contrast to the amoA gene, the nirS gene of all three plant species showed a consistent pattern of being more abundant in their respective bulk sediments compared to their rhizospheres (Fig. 7C). The nirS gene abundance in the rhizosphere of *P. australis* was higher than in the rhizosphere of S. mariqueter and S. alterniflora, and a similar trend was observed in the bulk sediments of different plant species. Furthermore, except for the significantly higher nirS gene abundance in the rhizosphere of *P. australis* compared to its bulk sediments ($p < 0.05$), no significant differences were observed in the other groups.

4. Discussion

Horizontal subsurface flow CWs are a special type of artificial wetland where wastewater flows horizontally below the substrate layer. The water in the system often does not directly contact the air throughout the treatment process, relying solely on the oxygen supply from plant roots. As a result, both the substrate and the water lack oxygen, leading to less than satisfactory N removal results (da Costa et al., 2013; Vymazal, 2009). However, in this experiment, due to the continuous circulation of wastewater, the effluent enters the collection area and comes into direct contact with the air before flowing back into the system. This significantly increases the oxygen content in the entire system, ultimately achieving a good N removal rate (Fig. 2). The substrate used in this experiment was sandy sediments (Watts et al., 1991), and the addition of wastewater significantly increased the sediments moisture content (Bauder and Schneider, 1979), leading to strong leaching effects in the sediments. As a result, after the start of the first stage of the experiment, the Sal and EC of all wastewater groups increased significantly (Figure S1D & S1E). The TDS concentration in all groups also decreased significantly and then gradually increased (Figure S2A), which was also due to the strong leaching effect, causing the originally adsorbed dissolved solids in the substrate to gradually dissolve into the water.

The choice of appropriate plants is a crucial step in the creation of CWs (Karungamye, 2022; Srivastava et al., 2008). Kohzu et al. (2003) studied the variation of δ^{15}N values in the roots of plant communities in two wetlands in central Japan with increasing root depth and found that the deeper the plant roots, the higher the δ^{15}N values. In this study, we observed that the root depth of *S. alterniflora*, *S. mariqueter*, and *P. australis* in the CWs was similar to their natural state, with *S. alterniflora* and *P. australis* having deeper roots than *S. mariqueter*. This difference could be due, in part, to the significantly higher ^{15}N content proportion in the plant tissues of *S. alterniflora* and *P. australis* compared to *S. mariqueter* (Fig. 5). Additionally, although there was no significant difference in the TN content per unit biomass between *S. mariqueter* and *S. alterniflora* or *P. australis*, the lower biomass of *S. mariqueter* (Figure S3B) resulted in a significantly lower TN increment in its roots, stems, and leaves compared to *S. alterniflora* and *P. australis* (Fig. 4A). Qing et al. (2015) conducted single and mixed-culture experiments with *S. alterniflora* and *P. australis* under two N levels in a greenhouse. After 17 weeks of growth, they exposed both plants to ^{15}N-labeled ammonium chloride for 48 h and found no significant difference in the uptake of ^{15}N between the two plants under single-culture conditions. Similarly, in our study, we found that the ^{15}N content proportion in the roots, stems, and leaves of *P. australis* and *S. alterniflora* was similar, and there was no significant difference between them (Fig. 5). In addition, 'others' in Fig. 5 represent two aspects: the percentage of ^{15}N removed from the system by microbial denitrification and the percentage of ^{15}N remaining in the simulated domestic wastewater. According to Fig. 5, the presence of plants in each of the three studied groups notably decreased their respective 'others' percentages, with the *P. australis* and *S. alterniflora* groups showing the most significant reductions. When we correlate these findings with those from Fig. 2, it's evident that in all three plant groups, the percentage of ^{15}N removed by microbial denitrification outweighs the percentage remaining in the simulated wastewater. This underscores the crucial role of plants in influencing both the quantity and variety of microbes within the entire CW system. Consistent with the TN increment and ^{15}N content proportions observed in our study for the three plant species, *P. australis*, one of the most commonly planted wetland plants in the world (Vymazal, 2011), demonstrated good N removal rate in our study. The CW system with *P. australis* achieved a TN removal rate of over 95% and an NH$_4^+$-N removal rate of around 99% (Table S3). The CW system with *S. alterniflora* showed a similar TN removal rate to *P. australis* but a slightly lower NH$_4^+$-N removal rate (Fig. 2B). However, it is worth noting that the effluent concentrations of NO$_2^-$-N and NO$_3^-$-N in the CW system with *S. alterniflora* were significantly lower than in the other groups (Fig. 2C & D). Although the CW system with *S. mariqueter* had a significantly lower N removal rate compared to the other two plant groups (Fig. 2). A large number of cinders with good permeability and aeration was used as the substrate for the CWs in this study, and Zhang et al. (2005) demonstrated that cinders can adsorb and precipitate soluble P from domestic wastewater. Therefore, due to both the relatively low initial concentration of PO$_4^{3-}$-P in the simulated domestic wastewater used in our experiment and the strong adsorption capacity of cinders for P, all four types of CW achieved a PO$_4^{3-}$-P removal rate of over 95% (Fig. 3), which might have masked the specific contribution of plants in removing PO$_4^{3-}$-P in the CWs. For future applications, materials with strong air and water permeability but relatively weak P adsorption capabilities, like quartz sand, could be considered as alternatives to cinders (Jiang et al., 2014).

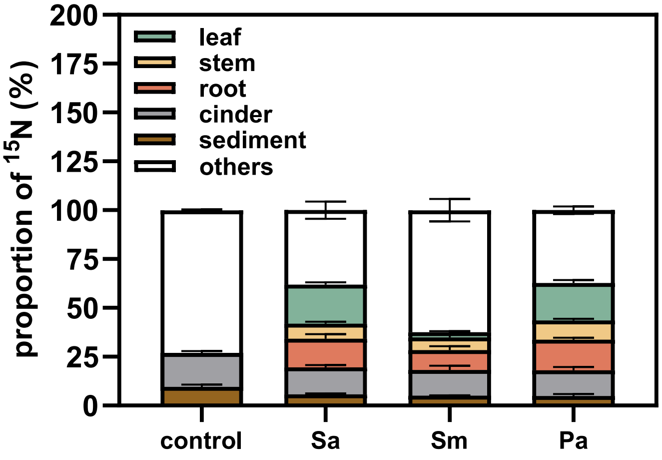

Fig. 5. Proportions of ^{15}N content in different components of four types of constructed wetlands (note: Pa, Sa, and Sm represent the *P. australis* group, the *S. alterniflora* group, and the *S. mariqueter* group, respectively).

The bacterial community structure in the sediments of the CWs in this study (Figure S4) shows similarities to several studies conducted on CWs (Cheng et al., 2023; Hua et al., 2018). Proteobacteria is the dominant phylum, and many bacteria within this phylum are involved in nitrification, denitrification, and sulfur oxidation processes (Li et al., 2015; Miao et al., 2015). However, there are some differences from the study conducted by Meng et al. (2007) in the Yangtze River estuary, where they found sequences related to nitrifying bacteria *Nitrosomonas* and *Nitrosospira* in the rhizosphere sediments of *S. alterniflora* but not in the rhizosphere sediments of *P. australis* and *S. mariqueter*. In contrast, our study detected several sequences related to *Nitrospira* in the rhizosphere sediments of *S. mariqueter* (Fig. 6). *Nitrospira* is known to be the most diverse genus of nitrifying microorganisms (Latocheski et al., 2022) and the dominant genus of nitrite-oxidizing bacteria in wastewater treatment (Mehrani et al., 2020), while *Nitrosomonas* is the primary ammonia-oxidizing genus (Foesel et al., 2008). In our study, the relative abundance of *Nitrospira* and *Nitrosomonas* in the rhizosphere and bulk sediments of *S. alterniflora* was lower than the overall average. Notably, the rhizosphere of *S. alterniflora* had significantly lower *Nitrospira* abundance compared to other groups, and the rhizosphere of *S. alterniflora* also had significantly lower *Nitrosomonas* abundance compared to the rhizosphere and bulk sediments of *S. mariqueter* and *P. australis* (Fig. 6). These trends were consistent with the results obtained from qPCR, which showed significantly lower copies of the *amoA* gene (AOB) in the rhizosphere and bulk sediments of *S. alterniflora* compared to *P. australis* and *S. mariqueter* (Fig. 7B). These findings suggest that the artificially high N conditions may influence the abundance and even the types of nitrifying bacteria in the rhizosphere sediments of these three plant species. Rolando et al. (2022) found that the core microbial community in the rhizosphere and bulk sediments of *S. alterniflora* was dominated by taxa capable of sulfur oxidation and sulfate reduction, such as sulfur-oxidizing bacteria from the genus *Sulfurovum* and sulfate-reducing bacteria from the genus *Desulfatitalea*. Our study also yielded similar results, with relatively high relative abundances of *Paracoccus*, *Sulfurovum*, *Sulfurimonas*, and *Desulfatitalea* in the rhizosphere and bulk sediments of *S. alterniflora*. Specifically, *Paracoccus*, *Sulfurovum*, and *Desulfatitalea* in the rhizosphere of *S. alterniflora* were significantly more abundant than in other groups, and *Sulfurimonas* in the rhizosphere of *S. alterniflora* was significantly more abundant than in other groups except its own bulk sediments (Fig. 6). It has been reported that heterotrophic denitrifying bacteria like *Paracoccus* and autotrophic denitrifying bacteria like *Sulfurovum* and *Sulfurimonas* can utilize NO$_3^-$-N or NO$_2^-$-N as electron acceptors and various sulfur compounds as electron donors to facilitate simultaneous desulfurization-denitrification (SDD) reactions, leading to the removal of NO$_3^-$-N or NO$_2^-$-N from wastewater (Zheng et al., 2022). In light of the findings from this study, it can be observed that the abundant cinders

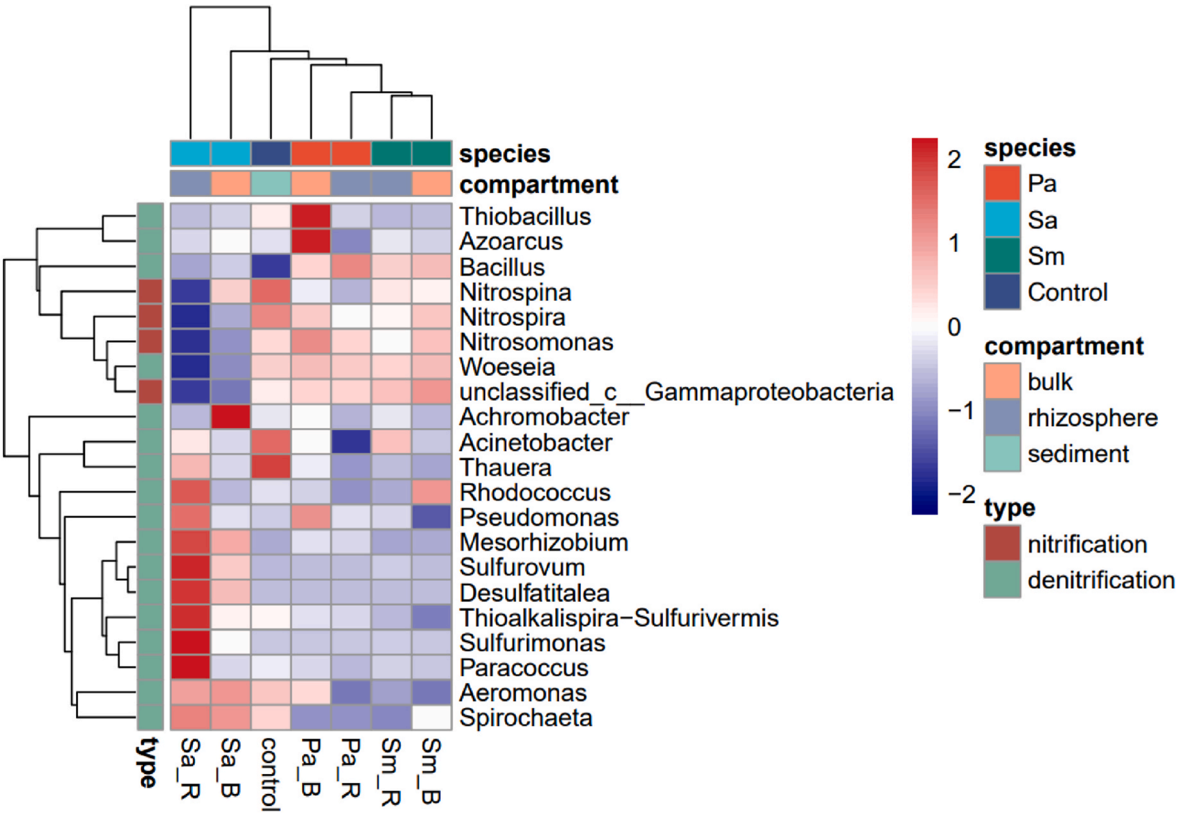

Fig. 6. Heatmap of the relative abundance of dominant nitrifying and denitrifying bacterial genera in the rhizosphere and bulk sediments for each treatment.

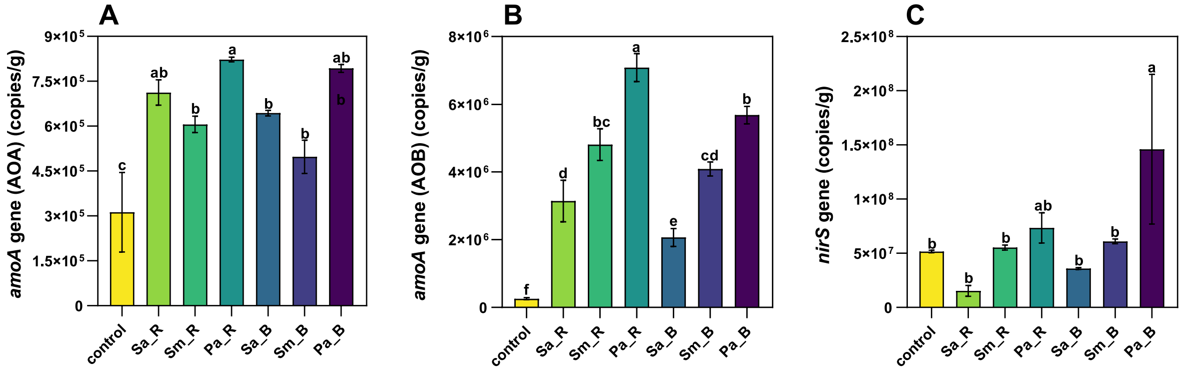

Fig. 7. Copy numbers of *amoA* (AOA and AOB types) and *nirS* genes. Data with different letters are significantly different at $p < 0.05$.

used in the experiment's matrix contains sulfur, providing essential material conditions for SDD reactions in bacterial genera like *Paracoccus*, *Sulfurovum*, and *Sulfurimonas* (Zhang et al., 2005).

In this experiment, the abundance of *nirS*-type denitrifying bacteria in the rhizosphere and bulk sediments of *S. alterniflora* (Fig. 7C) was the lowest. However, the functional prediction analysis using FAPROTAX revealed that *S. alterniflora* rhizosphere had the highest proportion of denitrifying bacteria (Figure S6B). This indicates that the rhizosphere and bulk sediments of *S. alterniflora* might host a higher diversity of denitrifying bacteria, including *nirK* and *nosZ* types. This also explains the lower effluent concentrations of NO_2^--N or NO_3^--N from the CW with *S. alterniflora* compared to other groups (Fig. 2C & D). Hua et al. (2017) compared the effects of two types of CW (planted with *Acorus calamus* L. and *P. australis*) on denitrification efficiency and microbial communities in treating domestic wastewater. They observed that the abundance of AOB was significantly higher in the rhizosphere substrate of both plants compared to the non-rhizosphere media, while the abundance of denitrifying bacteria was higher in the non-rhizosphere substrate. These findings are consistent with the qPCR results for the rhizosphere and bulk sediments of *P. australis* in this study (Fig. 7B & C). Furthermore, in this study, due to the enrichment of AOA and AOB in the rhizosphere and bulk sediments of *P. australis* (Fig. 7A & B), along with *P. australis* itself having the highest TN absorption capacity (Fig. 4A), the *P. australis* group exhibited the highest removal rate for NH_4^+-N. On the other hand, the abundance of ammonia-producing and AOB in the rhizosphere and bulk sediments of *S. mariqueter* was significantly higher than that of *S. alterniflora* (Figure S6A & 7B), and *S. mariqueter* had significantly lower TN absorption capacity than *S. alterniflora* (Fig. 4A). As a result, the two species had similar NH_4^+-N removal rate (Fig. 2B).

Stable isotope techniques have been frequently used to quantitatively investigate N cycling processes in wetlands (Cheung and Wang, 2008; Rejmánková et al., 2004). Harrison et al. (2012) employed the ^{15}N tracing method to assess N removal processes in two wetlands during different seasons, they observed that during the summer, plants and algae in both wetlands accounted for 42% and 63%, respectively, of the absorbed $^{15}NO_3^-$. In the present study, our results indicated that the combined ^{15}N content in the roots, stems, and leaves of *P. australis* represented the highest proportion, approximately 44.58%, slightly surpassing *S. alterniflora* (42.44%), and both were considerably higher than *S. mariqueter* (19.28%) (Fig. 5). These outcomes suggest that *S. alterniflora*, compared to the conventional wetland plant *P. australis*, also plays a significant role in denitrification in CWs. In the future, *S. alterniflora* could be considered as one of the plant options for treating high-N domestic wastewater in CWs, alongside *P. australis*, and can be practically implemented.

5. Conclusion

This study revealed the differences in the N and P removal capabilities of lab-scale CWs constructed with different wetland plant species for treating simulated domestic wastewater. The results indicated that CWs planted with three different plant species, *S. alterniflora*, *P. australis*, and *S. mariqueter,* showed significantly better N removal rate compared to the unplanted control group. This improvement was mainly attributed to the significant increase in the relative abundance of certain denitrifying microorganisms, such as AOA and AOB, facilitated by the presence of these three plant species. Furthermore, the plants could absorb N, P, and other nutrients. Among the three plant species, CWs planted with *S. alterniflora* and *P. australis* had equal total N removal rate, which outperformed CWs planted with *S. mariqueter*. *P. australis* enriched the rhizosphere and bulk sediments with more AOA and AOB, and its own N absorption capacity was high, resulting in the highest NH_4^+-N removal rate in *P. australis*-planted wetlands, outperforming *S. alterniflora* and *S. mariqueter* wetlands. Although *S. mariqueter* had more ammonifying bacteria and AOB in its rhizosphere than *S. alterniflora*, its own N uptake capacity was weaker, resulting in a similar final NH_4^+-N removal rate. When compared to the other groups, *S. alterniflora*-planted CWs considerably enriched more bacterial communities with denitrifying capabilities, including SDD bacteria, resulting in significantly lower amounts of NO_3^--N or NO_2^--N in the effluent. The four types of CWs exhibited similar and non-significant PO_4^{3-}-P removal rate due to the strong adsorption capacity of cinders in the substrate. The presence of plants did not lead to a significant enhancement in the system's P removal rate based on the experimental approach employed in this study. To gain further insights into the contributions of different plants to P removal and their associated processes, future research could focus on refining the substrate ratio in CW systems, like exploring alternative materials to replace cinders.

CRediT authorship contribution statement

Xiaoqing Gao: Formal analysis, Investigation, Writing - original draft. **Yuxin Bi:** Investigation, Resources. **Lin Su:** Investigation. **Ying Lei:** Investigation. **Lv Gong:** Investigation. **Xinhan Dong:** Investigation. **Xiuzhen Li:** Supervision. **Zhongzheng Yan:** Conceptualization, Supervision, 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.119864.

References

Bauder, J., Schneider, R., 1979. Nitrate-nitrogen leaching following urea fertilization and irrigation. Soil Sci. Soc. Am. J. 43 (2), 348–352.

Callaway, J.C., Josselyn, M.N., 1992. The introduction and spread of smooth cordgrass (*Spartina alterniflora*) in South San Francisco Bay. Estuaries 15, 218–226.

Chen, J., Wang, L., Li, Y., Zhang, W., Fu, X., Le, Y., 2012. Effect of *Spartina alterniflora* invasion and its controlling technologies on soil microbial respiration of a tidal wetland in Chongming Dongtan, China. Ecol. Eng. 41, 52–59.

Chen, Z., Li, B., Zhong, Y., Chen, J., 2004. Local competitive effects of introduced *Spartina alterniflora* on *Scirpus mariqueter* at Dongtan of Chongming Island, the Yangtze River estuary and their potential ecological consequences. Hydrobiologia 528, 99–106.

Cheng, M., Li, X., Gao, X., Zhao, Z., 2023. Effects of two plant species combined with slag-sponges on the treatment performance of contaminated saline water in constructed wetland. Environ. Sci. Pollut. Res. 30 (23), 63592–63602.

Cheung, M.S., Wang, W.-X., 2008. Analyzing biomagnification of metals in different marine food webs using nitrogen isotopes. Mar. Pollut. Bull. 56 (12), 2082–2088.

da Costa, J.F., de Paoli, A.C., Seidl, M., von Sperling, M., 2013. Performance and behaviour of planted and unplanted units of a horizontal subsurface flow constructed wetland system treating municipal effluent from a UASB reactor. Water Sci. Technol. 68 (7), 1495–1502.

Dotro, G., Langergraber, G., Molle, P., Nivala, J., Puigagut, J., Stein, O., Von Sperling, M., 2017. Treatment Wetlands. IWA Publishing, London.

Duan, H., Tao, M., Loiselle, S.A., Zhao, W., Cao, Z., Ma, R., Tang, X., 2017. MODIS observations of cyanobacterial risks in a eutrophic lake: implications for long-term safety evaluation in drinking-water source. Water Res. 122, 455–470.

Foesel, B.U., Gieseke, A., Schwermer, C., Stief, P., Koch, L., Cytryn, E., De La Torré, J.R., Van Rijn, J., Minz, D., Drake, H.L., 2008. *Nitrosomonas* Nm143-like ammonia oxidizers and *Nitrospira marina*-like nitrite oxidizers dominate the nitrifier community in a marine aquaculture biofilm. FEMS Microbiol. Ecol. 63 (2), 192–204.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc. Natl. Acad. Sci. U. S. A. 102 (41), 14683–14688.

General Administration of Quality Supervision, 2007. Inspection and quarantine of the People's Republic of China. In: The Specification for Marine Monitoring Part 4: Seawater Analysis (GB17378.4-2007) (In Chinese).

Harrison, M.D., Groffman, P.M., Mayer, P.M., Kaushal, S.S., 2012. Nitrate removal in two relict oxbow urban wetlands: a ^{15}N mass-balance approach. Biogeochemistry 111, 647–660.

Hua, G., Cheng, Y., Kong, J., Li, M., Zhao, Z., 2018. High-throughput sequencing analysis of bacterial community spatiotemporal distribution in response to clogging in vertical flow constructed wetlands. Bioresour. Technol. 248, 104–112.

Hua, Y., Peng, L., Zhang, S., Heal, K.V., Zhao, J., Zhu, D., 2017. Effects of plants and temperature on nitrogen removal and microbiology in pilot-scale horizontal subsurface flow constructed wetlands treating domestic wastewater. Ecol. Eng. 108, 70–77.

Jayalakshmi, R., Soundaranayaki, K., Subhash Kannan, M., 2023. Removal of Methylene Blue dye from textile wastewater using vertical flow constructed wetland. Mater. Today: Proc. 77, 365–370.

Jesus, J.M., Danko, A.S., Fiúza, A., Borges, M.-T., 2018. Effect of plants in constructed wetlands for organic carbon and nutrient removal: a review of experimental factors contributing to higher impact and suggestions for future guidelines. Environ. Sci. Pollut. Res. 25, 4149–4164.

Jia, W.L., 2013. N_2O Emission Characteristics and Mechanisms during Simultaneous Nitrification and Denitrification Process. Shandong University (In Chinese).

Jin, B., Lai, D.Y.F., Gao, D., Tong, C., Zeng, C., 2017. Changes in soil organic carbon dynamics in a native C_4 plant-dominated tidal marsh following *Spartina alterniflora* invasion. Pedosphere 27 (5), 856–867.

Jiang, C., Jia, L., Zhang, B., He, Y., Kirumba, G., 2014. Comparison of quartz sand, anthracite, shale and biological ceramsite for adsorptive removal of phosphorus from aqueous solution. J. Environ. Sci. 26 (2), 466–477.

Karungamye, P.N., 2022. Potential of *Canna indica* in constructed wetlands for wastewater treatment: a review. Conservation 2 (3), 499–513.

Knight, R.L., Payne, V.W.E., Borer, R.E., Clarke, R.A., Pries, J.H., 2000. Constructed wetlands for livestock wastewater management. Ecol. Eng. 15 (1), 41–55.

Kohzu, A., Matsui, K., Yamada, T., Sugimoto, A., Fujita, N., 2003. Significance of rooting depth in mire plants: evidence from natural ^{15}N abundance. Ecol. Res. 18, 257–266.

Kulshreshtha, N.M., Verma, V., Soti, A., Brighu, U., Gupta, A.B., 2022. Exploring the contribution of plant species in the performance of constructed wetlands for domestic wastewater treatment. Bioresour. Technol. Rep. 18, 101038.

Latocheski, E.C., da Rocha, M.C.V., Braga, M.C.B., 2022. *Nitrospira* in wastewater treatment: applications, opportunities and research gaps. Rev. Environ. Sci. Biotechnol. 21 (4), 905–930.

Li, C., Ren, H., Xu, M., Cao, J., 2015. Study on anaerobic ammonium oxidation process coupled with denitrification microbial fuel cells (MFCs) and its microbial community analysis. Bioresour. Technol. 175, 545–552.

Li, N., Li, B., Nie, M., Wu, J., 2020. Effects of exotic *Spartina alterniflora* on saltmarsh nitrogen removal in the Yangtze River Estuary, China. J. Clean. Prod. 271, 122557.

Li, X., Guo, R., 2017. Comparison of nitrogen removal in floating treatment wetlands constructed with *Phragmites australis* and *Acorus calamus* in a cold temperate zone. Water, Air. Soil Pollut 228, 1–8.

Lin, Q., Mendelssohn, I.A., 1998. The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands. Ecol. Eng. 10 (3), 263–274.

Louca, S., Parfrey, L.W., Doebeli, M., 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353 (6305), 1272–1277.

Mehrani, M.-J., Sobotka, D., Kowal, P., Ciesielski, S., Makinia, J., 2020. The occurrence and role of *Nitrospira* in nitrogen removal systems. Bioresour. Technol. 303, 122936.

Meng, W., Jia-Kuan, C., Bo, L., 2007. Characterization of bacterial community structure and diversity in rhizosphere soils of three plants in rapidly changing salt marshes using 16S rDNA. Pedosphere 17 (5), 545–556.

Miao, Y., Liao, R., Zhang, X.X., Wang, Y., Wang, Z., Shi, P., Liu, B., Li, A., 2015. Metagenomic insights into Cr (VI) effect on microbial communities and functional genes of an expanded granular sludge bed reactor treating high-nitrate wastewater. Water Res. 76, 43–52.

Ministry of Environmental Protection of the People's Republic of China, 2002. Editorial board on methods of monitoring and analysis of water and wastewater. In: Methods of Monitoring and Analysis of Water and Wastewater, fourth ed. China Environmental Science Press, Beijing (In Chinese).

Ohore, O.E., Qin, Z., Sanganyado, E., Wang, Y., Jiao, X., Liu, W., Wang, Z., 2022. Ecological impact of antibiotics on bioremediation performance of constructed wetlands: microbial and plant dynamics, and potential antibiotic resistance genes hotspots. J. Hazard Mater. 424, 127495.

Ou, S., Fang, Y., Shen, G., 1992. Studies on the effects of the environmental factors of Shanghai beach on the growth of sea-bulrush (*Scirpus*× *mariqueter*) and its productivity. J. Shanghai Norm. Univ., Nat. Sci. 21 (Suppl. l.), 4–21.

Prochaska, C.A., Zouboulis, A.I., Eskridge, K.M., 2007. Performance of pilot-scale vertical-flow constructed wetlands, as affected by season, substrate, hydraulic load and frequency of application of simulated urban sewage. Ecol. Eng. 31 (1), 57–66.

Qing, H., Cai, Y., Xiao, Y., Yao, Y., An, S., 2015. Nitrogen uptake and use efficiency of invasive *Spartina alterniflora* and native *Phragmites australis*: effect of nitrogen supply. Clean: Soil, Air, Water 43 (2), 305–311.

Quan, W., Han, J., Shen, A., Ping, X., Qian, P., Li, C., Shi, L., Chen, Y., 2007. Uptake and distribution of N, P and heavy metals in three dominant salt marsh macrophytes from Yangtze River estuary, China. Mar. Environ. Res. 64 (1), 21–37.

Rejmánková, E., Komárková, J., Rejmánek, M., 2004. δ^{15}N as an indicator of N_2-fixation by cyanobacterial mats in tropical marshes. Biogeochemistry 67, 353–368.

Rickey, M.A., Anderson, R.C., 2004. Effects of nitrogen addition on the invasive grass *Phragmites australis* and a native competitor *Spartina pectinata*. J. Appl. Ecol. 41 (5), 888–896.

Rolando, J.L., Kolton, M., Song, T., Kostka, J.E., 2022. The core root microbiome of *Spartina alterniflora* is predominated by sulfur-oxidizing and sulfate-reducing bacteria in Georgia salt marshes, USA. Microbiome 10 (1), 37.

Rotthauwe, J.-H., Witzel, K.-P., Liesack, W., 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63 (12), 4704–4712.

Sharma, M.K., Tyagi, V.K., Singh, N.K., Singh, S.P., Kazmi, A.A., 2022. Sustainable technologies for on-site domestic wastewater treatment: a review with technical approach. Environ. Dev. Sustain. 24, 3039–3090.

Sousa, W.T.Z., Panitz, C.M.N., Thomaz, S.M., 2011. Performance of pilot-scale vertical flow constructed wetlands with and without the emergent macrophyte *Spartina alterniflora* treating mariculture effluent. Braz. Arch. Biol. Technol. 54, 405–413.

Srivastava, J., Gupta, A., Chandra, H., 2008. Managing water quality with aquatic macrophytes. Rev. Environ. Sci. Biotechnol. 7, 255–266.

Strokal, M., Bai, Z., Franssen, W., Hofstra, N., Koelmans, A.A., Ludwig, F., Ma, L., van Puijenbroek, P., Spanier, J.E., Vermeulen, L.C., van Vliet, M.T.H., van Wijnen, J., Kroeze, C., 2021. Urbanization: an increasing source of multiple pollutants to rivers in the 21st century. NPJ Urban Sustain 1 (1), 24.

Tanner, C.C., Sukias, J.P., Upsdell, M.P., 1998. Organic matter accumulation during maturation of gravel-bed constructed wetlands treating farm dairy wastewaters. Water Res. 32 (10), 3046–3054.

Throbäck, I.N., Enwall, K., Jarvis, Å., Hallin, S., 2004. Reassessing PCR primers targeting *nirS, nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiol. Ecol. 49 (3), 401–417.

Vymazal, J., 2009. The use constructed wetlands with horizontal sub-surface flow for various types of wastewater. Ecol. Eng. 35 (1), 1–17.

Vymazal, J., 2010. Constructed wetlands for wastewater treatment. Water 2 (3), 530–549.

Vymazal, J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia 674 (1), 133–156.

Vystavna, Y., Frkova, Z., Marchand, L., Vergeles, Y., Stolberg, F., 2017. Removal efficiency of pharmaceuticals in a full scale constructed wetland in East Ukraine. Ecol. Eng. 108, 50–58.

Wan, S., Qin, P., Liu, J., Zhou, H., 2009. The positive and negative effects of exotic *Spartina alterniflora* in China. Ecol. Eng. 35 (4), 444–452.

Wang, J., Chen, G., Fu, Z., Qiao, H., Liu, F., 2021. Assessing wetland nitrogen removal and reed (*Phragmites australis*) nutrient responses for the selection of optimal harvest time. J. Environ. Manag. 280, 111783.

Watts, D.G., Hergert, G.W., Nichols, J.T., 1991. Nitrogen leaching losses from irrigated orchardgrass on sandy soils. J. Environ. Qual. 20, 355–362.

Wu, J.Z., Wang, Y.Y., Li, Y.Y., Zhu, J.X., Qu, K.M., Cui, Z.G., 2019. Correlation between nitrogen removal effect and matrix enzyme activity in seawater constructed wetland system. Mar. Sci. (Beijing, China) 43 (5), 36–44 (In Chinese).

Xu, N., Tan, G., Wang, H., Gai, X., 2016. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. Eur. J. Soil Biol. 74, 1–8.

Yang, C., Zhang, X., Tang, Y., Jiang, Y., Xie, S., Zhang, Y., Qin, Y., 2022. Selection and optimization of the substrate in constructed wetland: a review. J. Water Process Eng. 49, 103140.

Zhang, J., Huang, X., Liu, C., Shi, H., Hu, H., 2005. Nitrogen removal enhanced by intermittent operation in a subsurface wastewater infiltration system. Ecol. Eng. 25 (4), 419–428.

Zhang, M., Xu, D., Bai, G., Cao, T., Liu, W., Hu, Z., Chen, D., Qiu, D., Wu, Z., 2021. Changes of microbial community structure during the initial stage of biological clogging in horizontal subsurface flow constructed wetlands. Bioresour. Technol. 337, 125405.

Zhang, S., Wang, Y., Liu, W., 2023. Study on × *Bolboschoenoplectus mariqueter* and related species based on DNA barcoding. Guihaia 1–15 (In Chinese).

Zhao, Y.J., Liu, B., Zhang, W.G., Ouyang, Y., An, S.Q., 2010. Performance of pilot-scale vertical-flow constructed wetlands in responding to variation in influent C/N ratios of simulated urban sewage. Bioresour. Technol. 101 (6), 1693–1700.

Zheng, S., Liu, X., Yang, X., Zhou, H., Fang, J., Gong, S., Yang, J., Chen, J., Lu, T., Zeng, M., 2022. The nitrogen removal performance and microbial community on mixotrophic denitrification process. Bioresour. Technol. 363, 127901.