scientific reports

OPEN

# The impact of biochar and activated carbon on the purification efficiency of two wetland systems under varying pollution loads

Wei Zhou[1], Hao Chen[1], Xinjuan Cui[2], Dongxu Cui[1] & Qingqing Cao[1]✉

**The water quality purification of constructed wetlands (CWs) with adsorbent materials has been extensively studied, yet the impact on the improvement in water quality of natural sediment wetlands (SWs) remains unclear. In this study, CWs and SWs were established with biochar and activated carbon substrates, and high and low nitrogen (N) and phosphorus (P) pollution loads were applied. Additionally, relevant studies on the purification efficiencies of artificial wetlands under different pollution loads were reviewed. Results showed that the purification capacities of biochar and activated carbon were affected by the pollution load, and that there was significant interaction between the pollution load and wetland type (P = 0.000). The removal efficiencies of $NH_4^+$, $NO_3^-$, and total P in CWs were significantly higher than those in SWs. In particular, the removal stability of $NO_3^-$ was higher in the BCW (77.12%) and ACW (75.94%) under high pollution loads (P = 0.000). As the pollution load increased, the TP removal efficiency of SWs decreased from 86.56% to 75.97%, while the addition of biochar significantly enhanced removal efficiency (94.57%). The BSW experiment was more efficient in purifying P and $NH_4^+$ than ASW or SW. ACWs presented lowest microbial diversity and richness while higher metabolism of chemoheterotrophy and nitrate reduction. This study confirms that the addition of B can promote the sustainability of SW purification, and that both biochar and activated carbon exhibited varying adsorption characteristics in different wetland types. It demonstrates the improved purification potential in biochar added SWs.**

**Keywords** Activated carbon, Biochar, Constructed wetlands, Natural sediment wetlands, Pollution load

With increasing urbanization and industrialization, various human activities, including fertilizer and pesticide use, the food industry, and heavy engineering, have resulted in large quantities of nitrogen (N) and phosphorus (P) entering the aquatic environment. N and P serve as fundamental indicators for water pollution, and their excessive accumulation in aquatic systems may lead to eutrophication[1]. China's average annual sewage discharge is in the tens of billions of tons. Although treated at sewage treatment plants, the contents of N and P remain relatively high, and the large discharge of sewage causes pollution problems in rivers, lakes, and seas[2].

At present, constructed wetlands (CWs) and natural sediment wetlands (SWs) are widely adopted as efficient and economical methods of sewage treatment. CWs have significantly higher plant species and abundance, microbial diversity and metabolic functions, as well as absorptive materials as substrate. As a result, compared with SWs, CWs have a distinct advantage in the removal of ammonia ($NH_4^+$), nitrate ($NO_3^-$), and total P (TP). The filling substrate, a vital component of CWs, not only provides a conducive environment for plant and microbial growth, but also contributes significantly to the removal of N, P, and organic substances through processes like filtration and adsorption[3]. Differences in the surface microenvironment of different substrates affect biofilm formation[4]. However, traditional substrates, such as limestone, zeolite, and plastic, face challenges including low permeability, susceptibility to clogging, and low adsorption capabilities for N and P, as well as easy saturation[5].

In recent years, biochar-based substrates have shown significant efficacy in water purification due to their adsorption capacity and stability[6]. Meanwhile, activated carbon is also gaining popularity because of its strong ability to remove wastewater pollution[7]. Both of these wetland substrate materials, i.e., activated carbon and biochar, are currently being widely researched for their wastewater purification functions. Most studies show that the addition of biochar can effectively promote the removal of N and P in CWs[8], but some research has revealed

[1]School of Architecture and Urban Planning, Shandong Jianzhu University, Jinan 250000, China. [2]Jinan Motor Vehicle Pollution Prevention and Control Center, Jinan 250000, China. ✉email: caoqingqing18@sdjzu.edu.cn

opposite results, i.e., that biochar not only fails to promote total N (TN) removal, but also further inhibits the microbial metabolism and degradation, which may be related to multiple factors[9]. This discrepancy in findings is probably related to the pollution load and wetland features. As reported, CWs are effective in transforming and removing N when faced with low to moderate pollutant loads[10], and wetlands have a range of tolerance for pollution loads in the influent water[11]. Meanwhile, the effluent water quality shows a decreasing pattern with the an increase in the influent pollution load[12]. Moreover, different wetland types have various operating modes and microbial activities, resulting in distinct removal efficiencies for N and P[11]. Also, the addition of biochar or activated carbon results in significantly different removal characteristics in response to pollutant loads[13,14]. However, most wastewater purification studies focus on CWs, while neglecting the purification response of SWs when adsorbent materials are added. Furthermore, the purification potential of SWs remains unknown when faced with different pollution loads.

In the above context, and building on the above research findings to date, we explored the response of CWs and SWs to low and high pollution loads, as well as the effectiveness of adding biochar and activated carbon. We hypothesized that (i) the addition of biochar and activated C would significantly enhance the purification efficiency of SWs; and (ii) the purification capacities of biochar and activated carbon would differ significantly depending on the pollution load, and that there would also be significant interaction between the pollution load and wetland type.Therefore, in this study, using biochar and activated carbon as substrates, CWs and SWs units were set up to carry out the removal rates of TP, $NH_4^+$ and $NO_3^-$, from wetlands under high and low pollutant loads. This study will promote a deep understanding of the effluent purification process, and provide a theoretical basis for the application of wetland wastewater purification scenarios and model development.

## Results

### The sewage purification under low pollutant load

The TP removal in all wetland units were above 80% when facing low pollution load (Fig. 1A), with the highest value observed in BCW. However, the addition of activated carbon and biochar in SWs actually limited the TP removal. In contrast, TP concentrations in BSW, ASW, and ACW exhibit a pronounced logarithmic decline over time, signifying a systematic enhancement in purification efficiency.

The purification efficiency of $NH_4^+$ in CWs is 84.90%, significantly surpassing that in SWs (77.88%, $P = 0.030$). Whether in CWs or SW, the effect of different substrate additions on $NH_4^+$-N removal is inconspicuous. With the increase in sewage purification period, $NH_4^+$-N contents exhibit a logarithmic decreasing trend (Fig. 1B).

The average purification efficiency of $NO_3^-$ in CWs is 66.79%, significantly surpassing that in SWs (33.13%, $P = 0.000$), indicating a notable advantage of CWs in nitrate removal. The addition of biochar or activated carbon in CWs was not effective in nitrate removal (Fig. 1C). The nitrate removal rates in SWs are sorted as: ASW (39.00%) > BSW (36.82%) > SW (23.58%). Thus addition of biochar and activated carbon significantly enhances $NO_3^-$ removal in SWs.

### The sewage purification under high pollutant load

The TP removal efficiency in CWs is significantly superior to that in SWs under high pollutant load ($P = 0.000$, Fig. 2A). The specific performances of the different treatments are as follows: CW (97.18%) > BCW (94.57%) > BSW (93.13%) > ACW (91.93%) > SW (75.97%) > ASW (70.79%). The CWs show the most robust stability in TP removal rate during the sewage purification cycles, and achieve an average effluent concentration of 0.08 mg $L^{-1}$, further indicating that the addition of activated carbon and biochar in CWs limited the TP removal. While the addition of biochar in SW significantly promotes the TP removal.

CWs show average $NH_4^+$ purification efficiency of 91.28% under high pollutant loads, which significantly higher than SWs (67.68%, $P = 0.000$, Fig. 2B). Specifically, the removal rates are as follow: CW (92.31%) > BCW (91.67%) > ACW (89.84%) > BSW (79.19%) > SW (65.33%) > ASW (58.53%). The $NH_4^+$-N removal efficiency in BCWs, ACWs and CWs remains very stable with the sewage purification cycles, while the removal rates increase in SWs. Biochar addition benefits the $NH_4^+$-N purification but activated carbon limited the metabolism in SW. In addition, the adaptability of SWs to high purification load is significantly lower than CWs.

Under high sewage load conditions, the specific performance in $NO_3^-$ removal efficiency is as follows: BCW (77.12%) > ACW (75.94%) > CW (51.00%) > BSW (14.11%) > SW (8.28%) > ASW (8.08%). The $NO_3^-$ removal rates of BCW and ACW remain relatively stable during effluent purification cycle, which are significantly higher than constructed wetlands (Fig. 2C). While nitrate removals are in very low levels in SWs, and the prompting of biochar or activated carbon is less effective.

The removal rates of N and P are significantly differed under high/low pollutant loads or two wetland types ($P < 0.05$, Fig. 3 and Table 1), and pollutant loads and wetland types also present a significant interaction effect. When comparing high and low sewage loads, it is observed that ASW and SW exhibit poorer purification levels for TP and $NH_4^+$ under high loads, while BSW shows stronger purification potential.

### Microbial analysis in CWs

Microbial high-throughput sequencing in the CWs units are conducted. Results show that Proteobacteria, Actinobacteria, Bacteroidetes, Planctomycetes, and Chloroflexi are the main phyla. Proportions of Actinobacteria and Planctomycetes are significantly higher BCWs and ACWs. While CWs show the higher relative abundance of Bacteroidetes and Chloroflexi. ACWs also have the highest relative abundance of Proteobacteria (Fig. 4A). At the genus level, *Thauera* is dominant, accounting for as much as 43% in ACWs, 23% in CWs, and 17% in BCWs. Additionally, there is a significant abundance of unidentified *nitrosomonadaceae* in CWs.

The bacterial alpha diversity index indicates the highest bacterial richness and diversity in CWs, which are followed by BCWs and ACWs, indicating that adding biochar or activated carbon as substrates seem unfavourable for the growth of microbial communities. Significant differences in the dominant metabolism

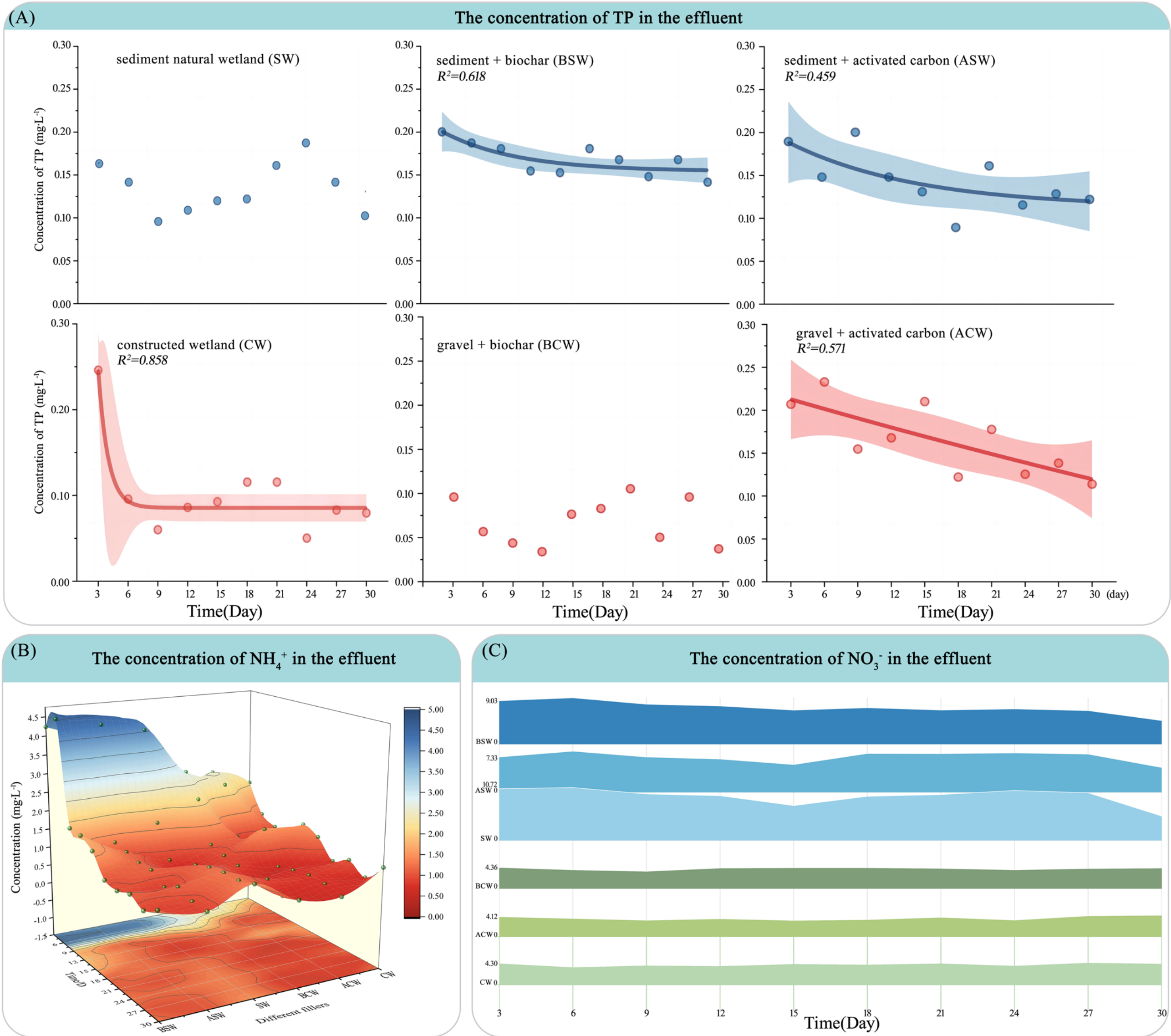


**Fig. 1**. Trends of TP, $NH_4^+$ , $NO_3^-$ concentration and removal rate over time in CWs and SWs under low-load sewage inflow. (**A**) The concenteation of TP in the effluent; (**B**) The concenteation of $NH_4^+$ in the effluent; (**C**) The concenteation of $NO_3^-$ in the effluent.

functions are found (Fig. 4C). It suggests that addition of biochar increases the bacterial N fixation, while the addition of activated carbon increases the nitrate reduction and heterotrophic metabolic activities.

# Discussion

## The TP purification mechanism in CWs

In the text, we reviewed the papers on the purification efficiency of CWs under different pollution loads, and obtained 37, 32 and 22 sets of data for $NH_4^+$, $NO_3^-$ and TP respectively[3,15–33]. The study found that the TP removal efficiency in all biochar-amended CWs and non-biochar CWs ranged from 17.10 to 89.99% (Fig. 5A). The TP removal efficiency was significantly higher in biochar-amended CWs compared to those without biochar. Biochar, with its high specific surface area, porous structure, and abundant surface functional groups, facilitates the adsorption of P and provides a larger surface area for microbial attachment, thereby enhancing P removal[34,35]. The TP removal effectiveness of biochar depends on the concentration of P in the wetland and the hydraulic retention time[36]. At low influent concentrations (0 mg $L^{-1}$ to 2 mg $L^{-1}$), the TP removal efficiency was relatively high, generally ranging from 34.48 to 94.97%. However, as the pollution load increased, TP removal efficiency decreased (Fig. 5B). Under low concentration conditions, adsorption sites in the wetland system are abundant, allowing for effective P capture and resulting in higher purification efficiency. As the influent concentration increases, adsorption sites gradually become saturated, leading to a decline in purification efficiency[37]. Notably, the overall TP removal efficiency in this study was relatively high and significantly increased with the pollution load. This may be because, under low concentration conditions, microbial activity

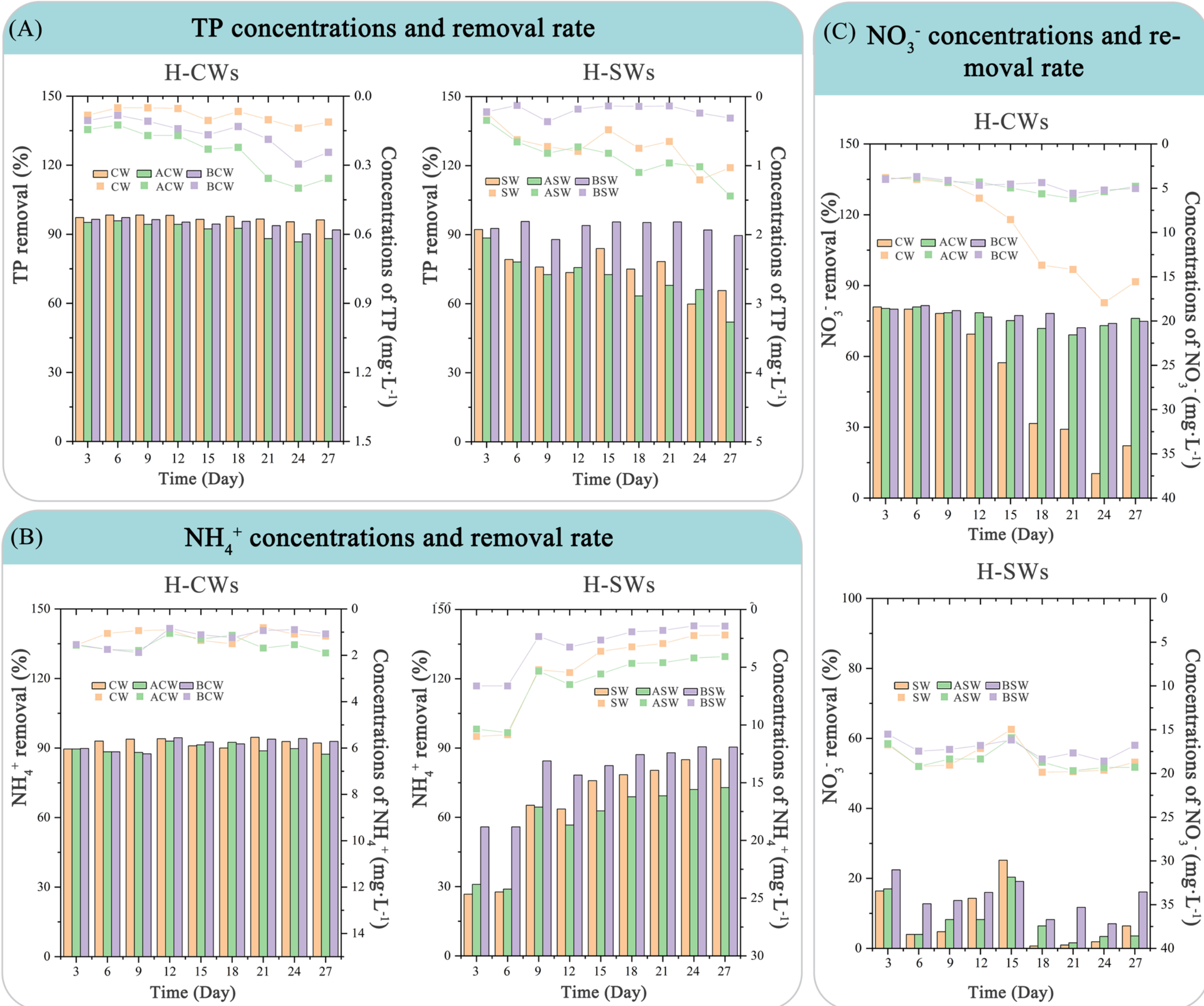


**Fig. 2**. Trends of TP, $NH_4^+$, and $NO_3^-$ concentrations and removal rates over time in CWs and SWs under high-load sewage inflow. (**A**) TP concentrations and removal rate; (**B**) $NH_4^+$ concentrations and removal rate; (**C**) $NO_3^-$ concentrations and removal rate.

may be lower due to insufficient P sources, leading to less efficient P removal. As the P concentration increases, microbial activity may be stimulated, allowing polyphosphate bacteria to more efficiently absorb and fix P, thus improving the purification efficiency. Additionally, this experiment was conducted during the summer, when plant and microbial growth were most active.

Studies have shown that the addition of biochar and activated carbon primarily exerts a synergistic effect on TP purification[25,38]. This study found that as the pollution load increased, TP purification efficiency significantly improved, while the addition of biochar and activated carbon mainly had a constraining effect. Research indicates that the substrate plays a key role in P deposition and adsorption, while the larger pore volume of gravel enhances the development of microbial biofilms and microbial P uptake[39]. The addition of biochar and activated carbon undermines the stability of microbial biofilms, and the decrease in microbial richness and diversity supports this hypothesis. Interestingly, in this experiment, activated carbon exhibited the strongest inhibitory effect on TP purification in CWs. The α-diversity of microbes was lowest, and based on the primary functional microbial communities, the addition of activated carbon altered the microbial community structure and enhanced the metabolic functions related to the degradation of aromatic compounds[40].

### The TP purification mechanism in SWs

As the pollution load increased, the TP removal efficiency of SWs decreased from 86.56% to 75.97%, while the addition of biochar significantly enhanced removal efficiency (94.57%). The self-purification ability of SWs depends on natural filtration mechanisms, including plants, microorganisms, and sediments[41]. Under low pollution loads, wetland plants and microorganisms effectively absorb and process pollutants in the water. However, as the pollution load increases, the wetland system's purification capacity gradually becomes saturated,

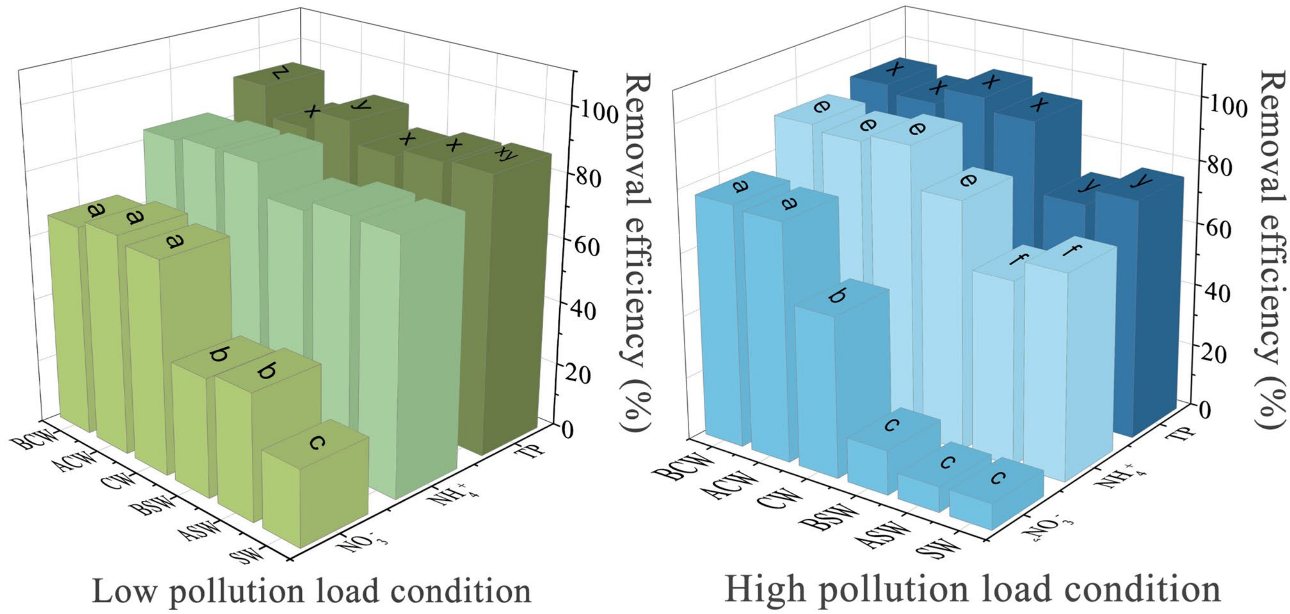


**Fig. 3**. N and P removal efficiency under low and high pollution loads. Different lowercase letters for TP, $NH_4^+$ or $NO_3^-$ represent significant differences in $\alpha < 0.05$ level.

| | Low load scenario | | High load scenario | | | | |
|---|---|---|---|---|---|---|---|
| Variables | SW | CW | SW | CW | p(High and low loads) | p(wetland type) | p(Wetland type * high and low loads) |
| $NH_4^+$ | 77.88 | 84.9 | 67.68 | 91.28 | 0.028 | 0.000 | 0.000 |
| $NO_3^-$ | 33.13 | 66.79 | 10.16 | 68.02 | 0.000 | 0.000 | 0.000 |
| TP | 85.13 | 88.82 | 79.96 | 94.56 | 0.000 | 0.000 | 0.000 |

**Table 1**. Multifactor analysis of N and P removal efficiency under high and low loads and different wetland types.

and the treatment capacity of plants and microorganisms is limited, leading to decreased purification efficiency. Unlike native plants and microorganisms in SWs, biochar, with its unique physicochemical properties, effectively complements the treatment capacity of the wetland system and maintains high TP removal efficiency under high pollution loads. Therefore, in practical applications, adding biochar can significantly improve the TP purification capacity of SWs. However, adding activated carbon limits TP removal in SWs. Some studies suggest that activated carbon is not the optimal medium for P removal[42]. The surface charge properties and pore structure of activated carbon are unsuitable for P adsorption. Adding activated carbon can affect microbial abundance in SWs and the stability of water pH. Microorganisms in the sediment can metabolize organic P in both the sediment and overlying water, potentially promoting the conversion of organic P to inorganic P and increasing the release rate of P from the sediment[43,44]. In contrast, artificial wetlands dominated by gravel do not encounter this issue, which significantly contributes to the superior P removal efficiency in CWs.

## The TP purification mechanism in wetland system

According to the literature, the addition of biochar improved $NH_4^+$ removal efficiency but had no significant effect on $NO_3^-$ removal efficiency (Fig. 5A). As the pollution load increased, the purification efficiency of $NH_4^+$ and $NO_3^-$ fluctuated, while the activity of nitrifying and denitrifying bacteria was suppressed.Competitive adsorption and kinetic limitations became the primary factors[45] (Fig. 5B). Generally, pollutant removal efficiency decreases as the pollution load rises[11]. Interestingly, in this study, $NH_4^+$ and $NO_3^-$ removal efficiency increased with the pollution load. Related studies show that when N concentrations in CWs are too low, essential nutrients for biological growth and reproduction become insufficient. Conversely, when N concentrations are too high, exceeding the needs of microorganisms and plants, denitrification efficiency decreases[39].Therefore, pollution load and wetland purification efficiency are interrelated.

This study indicates that, under low-load conditions, there were no significant differences in $NH_4^+$ and $NO_3^-$ removal rates in CWs, nor between different substrates. This is mainly due to the low carbon-to-N ratio in low-concentration wastewater, which negatively affects the wetland's denitrification efficiency[46]. As the pollution load increased, denitrification efficiency significantly improved, and the addition of biochar and activated carbon notably enhanced $NO_3^-$ removal. This is primarily due to the direct influence of microbial community structure

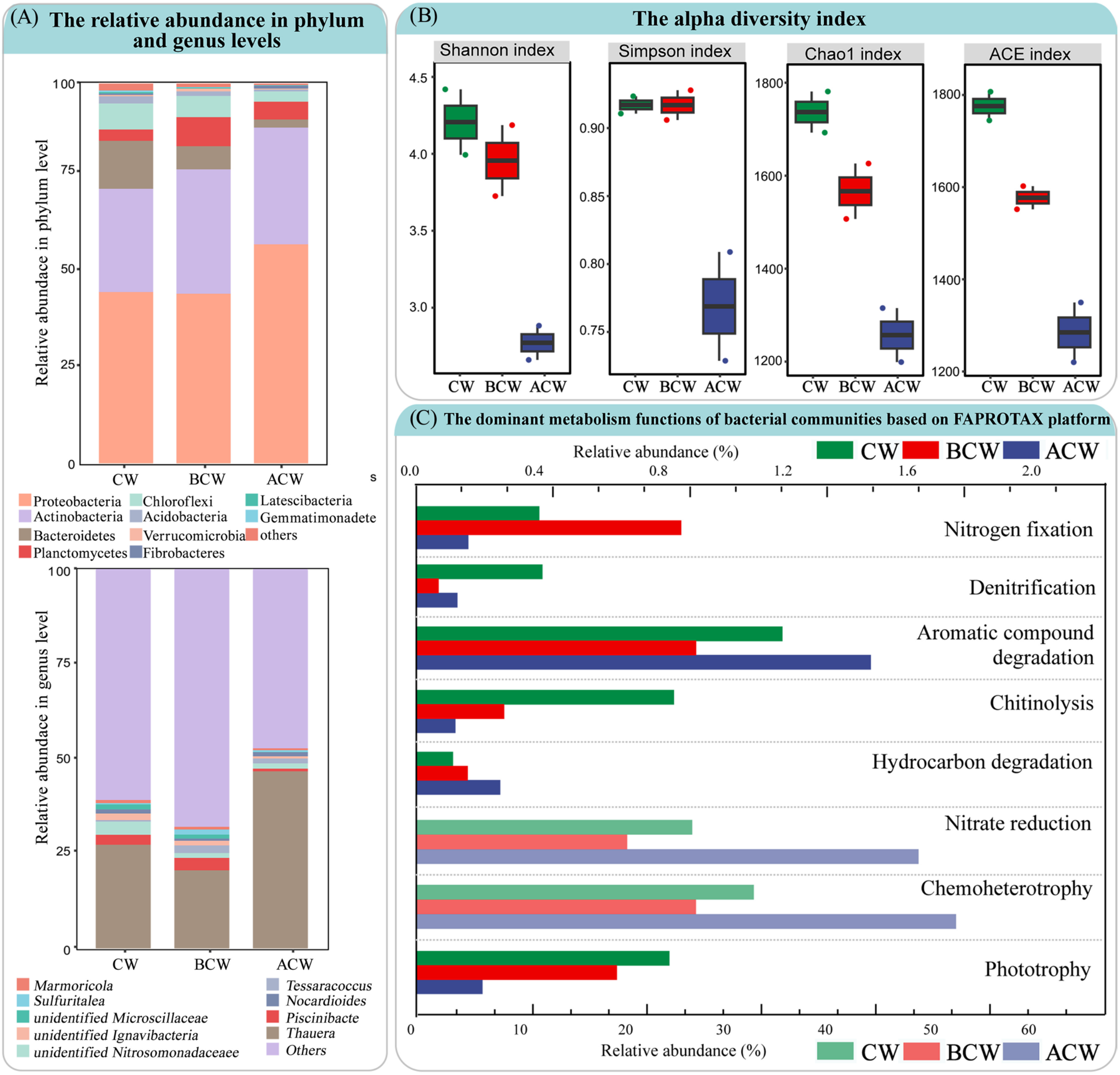


**Fig. 4**. The composition, alpha diversity index and dominant metabolism functions of bacterial communities in CWs. (**A**) The relative abundance in phylum and genus levels; (**B**) The alpha diversity index; (**C**) The dominant metabolism functions of bacterial communities based on FAPROTAX platform.

and metabolic functions on the system's denitrification capacity[47]. Under high pollution loads, microorganisms in CWs may experience higher N loads, suppressing their metabolic activity. However, the substrate's porous structure supports good microbial growth[48].

As the pollution load increased, $NH_4^+$ removal efficiency in SWs decreased from 77.88% to 67.68%, and $NO_3^-$ removal efficiency dropped from 33.13% to 10.16%, consistent with previous studies[11]. SWs have limited capacity to handle pollution loads. However, adding biochar slightly improved denitrification, especially for ammonia N, due to its unique surface properties that offer a favorable habitat for microorganisms[49]. In contrast, adding activated carbon further limited denitrification, likely due to the significantly reduced microbial diversity and abundance.

## Conclusion

We investigated the purification efficiency of TP, $NH_4^+$, and $NO_3^-$ in CWs and SWs under both low and high pollution loads, and examined the effects of adding biochar and activated carbon. The literature review indicates that the addition of biochar significantly improved TP removal efficiency compared to CWs without biochar, and enhanced $NH_4^+$ removal efficiency. However, there was no significant effect on $NO_3^-$ removal efficiency.

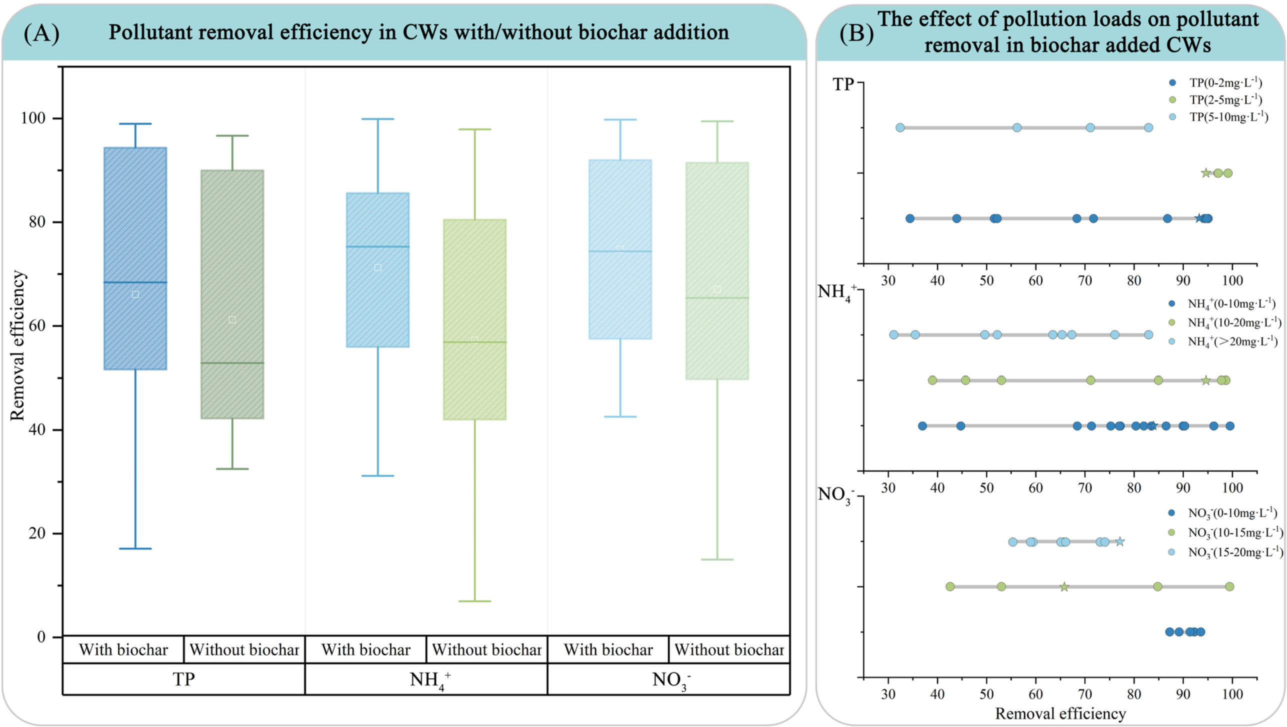


**Fig. 5**. Analysis of wastewater purification in biochar-based CWs based on relevant literature search. (**A**) Pollutant removal efficiency in CWs with/without biochar addition; (**B**) The effect of pollution loads on pollutant removal in biochar added CWs. The stars in (**B**) present the results in this study.

As the pollution load increased, the N and P removal efficiencies fluctuated. In contrast, study found that the removal efficiencies of N and P in CWs increased significantly with higher pollution loads. CWs demonstrate a significant advantage in sewage purification under high pollution loads, with the addition of biochar and activated carbon enhancing $NO_3^-$ removal stability. The BSWs exhibited higher tolerance to high pollution loads compared to ASWs and SW. However, the addition of biochar and activated carbon reduced bacterial richness and diversity, while metabolic activities related to N and P removal were not significantly enhanced. The findings of this study contribute to the theory of wastewater adsorption and offer valuable practical insights for sustainable operations.

## Materials and methods

### Experimental design

Most of the SWs use sediment as substrate, while artificial wetlands mostly use gravel as substrate. In order to study the effect of biochar and activated carbon on effluent purification efficiency in different wetland systems, we designed customised units to simulate artificial and SWs. The CWs used gravel, gravel + activated carbon, and gravel + biochar as filling substrates. For the SWs, the sediment, sediment + activated carbon, and sediment + biochar were employed as filling substrates. *Acorus calamus*, a typical wetland plant, was selected for both the CWs and the SWs. The experiments were conducted in the test field at the Environment Research Institute of Shandong University.

The experiment employed intermittent water inflow operations with a hydraulic retention time of 3 days. Initially, seedlings of *Acorus calamus* were cultivated, and tap-water was introduced. The experiment commenced after stable growth of *Acorus calamus* was achieved during the mature growth stage (September to November). To initiate the experiment, low-load wastewater (meeting the first-class B standard of water quality) was slowly pumped into all wetland units. This process aimed to incubate three hydraulic retention cycles to stabilize the effluent water quality parameters before conducting experimental water quality analyses. After one month, high-load wastewater (meeting the second-class standard of water quality) was introduced to the wetland units. Water quality monitoring experiments were conducted after the effluent water quality stabilized (Fig. 6).

Throughout the experiment, the ratio of wastewater inflow volume to substrate volume was 1:2. For SWs, the wastewater inflow volume was 30 L, with an overlying wastewater depth of approximately 15 cm. For CWs, the wastewater inflow volume was 10 L, with the water level approximately 5 cm above the substrate.

CWs exhibits a certain capacity to tolerate pollutant loads, making pollutant loading one of the primary factors influencing the efficiency of pollutant removal in wetlands[50]. We determined the pollutant loads that the wetland could sustain by varying the concentration of various pollutants in the influent water and setting different pollutant concentration levels. Because of the high variability of the daily load of pollutants in the real wastewater, the synthetic wastewater provided a more stable condition than the real wastewater for the

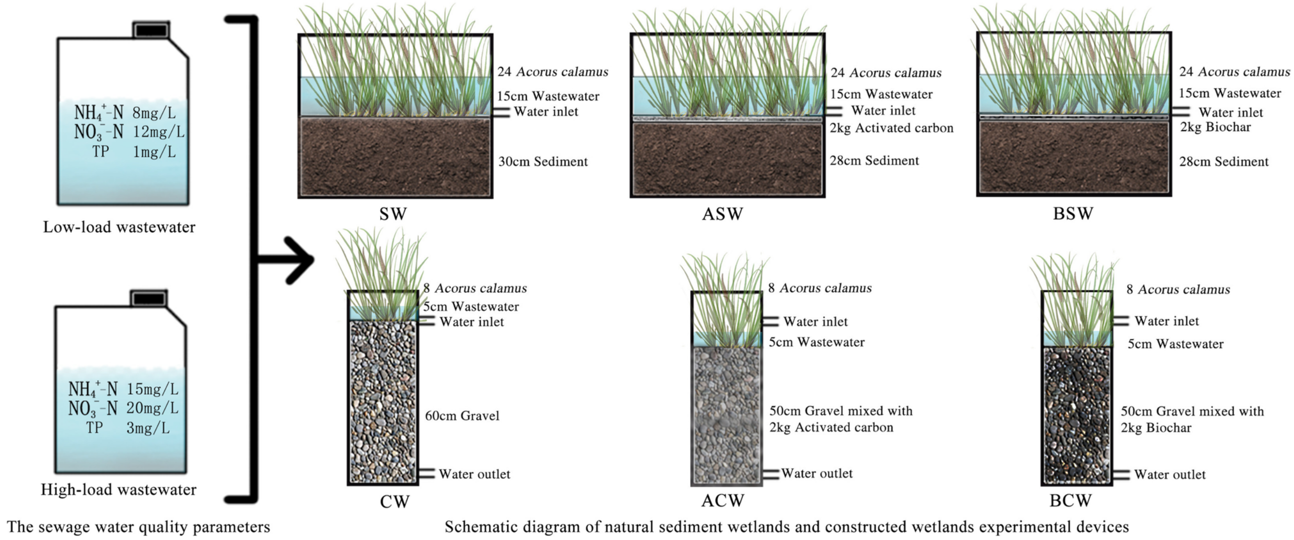


**Fig. 6**. Schematic diagram of SWs and CWs experimental devices.

| Wetland influent concentration | $NH_4^+$ | TP | $NO_3^-$ |
|---|---|---|---|
| Low pollution load(Class I-B standard) | 8 | 1 | 12 |
| High pollution load (secondary standard) | 15 | 3 | 20 |

**Table 2**. The sewage water quality parameters (unit: mg $L^{-1}$).

experimental study[51]. Therefore, the experiments were conducted to synthesize wastewater with high and low pollution loads using sucrose, $KNO_3$, $(NH_4)_2SO_4$, $KH_2PO_4$, $MgSO_4$, $CaCl_2$ and $FeSO_4 \cdot 7H_2O$ as raw materials, so that the concentrations of N and P followed the secondary and Class I-B standards in the "Standards for the Discharge of Pollutants from Municipal Wastewater Treatment Plants" (GB 18,918–2002), respectively (Table 2).

## Establishment and operation of wetland systems

SWs: Each SWs was a rectangular device measuring 70 cm in length, 50 cm in width, and 60 cm in height. Three substrates were used: sediment with a depth of 30 cm (SW), sediment mixed with 2 kg of activated carbon (ASW), and sediment mixed with 2 kg of biochar (BSW). Nine SW units were constructed, each variable having three parallel units. In each SWs unit, 24 *Acorus calamus* seedlings were evenly planted in a 4 × 6 pattern.

CWs: The CWs were cylindrical devices with a height of 70 cm and an inner diameter of 25 cm. Valves were installed beneath the units for convenient drainage and sampling. Three substrates were used: gravel with a depth of 60 cm (CW), a mixture of gravel and 2 kg of activated carbon with a depth of 50 cm (ACW), and a mix of gravel and 2 kg of biochar with a depth of 50 cm (BCW). A total of 6 units of parallel units were designed and operated. Each CWs unit accommodated 8 *Acorus calamus* seedlings based on the calculated planting density in SWs.

## Water quality determination

Effluent from the wetlands was collected using 100 mL polyethylene bottles. Water samples were collected from the overlying water for SWs setups, while for CWs setups, water was collected from the bottom valve. After filtration through a 0.45 μm cellulose acetate membrane, concentrations of TP, $NH_4^+$, $NO_3^-$ and $NO_2^-$ were determined. The TP concentration in water was measured using the ammonium molybdate spectrophotometric method outlined in the GB11893-89 standard. The concentrations of $NH_4^+$, $NO_3^-$, and $NO_2^-$ in water samples were determined using Nessler's reagent spectrophotometric method specified in the HJ535-2009 standard, the ultraviolet spectrophotometric method outlined in the HJ/T346-2007 standard, and the N-(1-naphthyl)-ethylenediamine spectrophotometric method in the GB7493-87 standard, respectively.

The removal efficiency of TN, $NH_4^+$, $NO_3^-$, $NO_2^-$, and TP in different wetland types and systems was calculated using a removal efficiency (RE) formula to assess the sewage purification efficiency of wetland systems, as follows:

$$\mathrm{RE} = (C_0 - C_i)/C_0 \times 100\%,$$

where $C_i$ is the concentration of $NH_4^+$, $NO_3^-$, $NO_2^-$, and TP in the *i*th sampling, and $C_0$ is the initial concentration of $NH_4^+$, $NO_3^-$, $NO_2^-$, and TP.

### Microbial analysis

The substrate-based microbial communities of six CWs installations were analyzed in this study to investigate the mechanistic process of wastewater purification with different substrate fillers. After completing high-load sewage purification experiments, substrate samples from the CWs were collected using a five-point sampling method, sealed in bags, and freeze-dried at – 60℃ for 72 h using a vacuum freeze dryer. Subsequently, 0.5000 g of substrate samples were weighed into centrifuge tubes, deionized water was added, and the mixture was centrifuged for 30 min to facilitate microbial detachment from substrate particles. The supernatant was filtered through a 0.022 μm organic membrane, capturing microbial organisms from the substrate particles.

For DNA extraction of substrate microbes, the MOBIO PowerSand DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA) was utilized following the provided instructions. PCR amplification was performed using primers specific to the V4 region of bacterial 16S rDNA, with primer fragments 520F (5'-barcode + AYTGGGGYDTAAAGNG-3'), 802R (5'-TACNVGGGTATCTAATCC-3'), 802R (5'-TACNVGGGTATCTAATCC-3'), and 802R (5'-TACNVGGGTATCTAATCC-3'). Following PCR amplification, quantification, and gene library construction, paired-end sequencing of microbial DNA fragments was conducted using the Illumina platform. Microbial community structure analysis was outsourced to Shanghai Passiono Biotechnology Co., Ltd. The gene sequence in this study was deposited in the Sequence Read Archive at NCBI under the accession number PRJNA1160848.

### Statistical analysis

In this study, the removal efficiencies for the various indicators were calculated using Excel. Descriptive analysis and homogeneity tests were performed on all data using SPSS (version 21.0) to identify and exclude outlier data. Subsequently, data were categorized based on different wetland types (natural and constructed), differences in influent concentrations (low and high), and three variables within different wetland types (biochar addition, activated carbon addition, and no addition). Mean analysis and one-way analysis of variance (ANOVA) were conducted on all data. Additionally, multifactor analysis was performed considering different wetland types, influent concentration differences, and different substrate data. Pearson correlation analysis assessed the correlation between data, with $p$-values less than 0.05 and 0.01 indicating significant and highly significant correlations, respectively. Graphical construction was done using OriginPro (version 9.0) and Adobe Illustrator CS6. In this study, the FAPROTAX platform was utilized to calculate the abundance of potential metabolic functions.

## Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request. The gene sequence in this study was deposited in the Sequence Read Archive at NCBI under the accession number PRJNA1160848.

Received: 4 September 2024; Accepted: 29 May 2025
Published online: 06 June 2025

## References

1. Almuktar, S. A., Abed, S. N. & Scholz, M. Wetlands for wastewater treatment and subsequent recycling of treated effluent: a review. *Environ. Sci. Pollut. R.* **25**, 23595–23623. https://doi.org/10.1007/s11356-018-2629-3 (2018).
2. Seifert, C., Krannich, T. & Guenther, E. Gearing up sustainability thinking and reducing the bystander effect – a case study of wastewater treatment plants. *J. Environ. Manage.* **231**, 155–165. https://doi.org/10.1016/j.jenvman.2018.09.087 (2019).
3. Panghal, V., Singh, A., Arora, D. & Kumar, S. Biochar-modified constructed wetlands using Eclipta alba as a plant for sustainable rural wastewater treatment. *Environ Sci Pollut R.* **31**, 17299–17310. https://doi.org/10.1007/s11356-024-32144-0 (2024).
4. McKenna, P. B. et al. Remote sensing of mine site rehabilitation for ecological outcomes: A global systematic review. *Remote Sens.* **12**(21), 3535. https://doi.org/10.3390/rs12213535 (2020).
5. Shen, S., Li, X., Cheng, F., Zha, X. & Lu, X. recent developments of substrates for nitrogen and phosphorus removal in CWs treating municipal wastewater. *Environ. Sci. Pollut. Res.* **27**, 29837–29855. https://doi.org/10.1007/s11356-020-08808-y (2020).
6. Lin, G. & Ding, Y. Enhancement of immobilized biochar/FeS on nitrogen removal in constructed wetland at low temperature. *J. Water Process Eng.* **58**, 104834. https://doi.org/10.1016/j.jwpe.2024.104834 (2024).
7. Eleryan, A. et al. Mandarin biochar-CO-TETA was utilized for Acid Red 73 dye adsorption from water, and its isotherm and kinetic studies were investigated. *Sci. Rep.* **14**, 13021. https://doi.org/10.1038/s41598-024-62870-x (2024).
8. Wu, J. et al. Tertiary treatment of municipal wastewater by a novel flow constructed wetland integrated with biochar and zero-valent iron. *J. Water Process Eng.* **47**, 102777. https://doi.org/10.1016/j.jwpe.2022.102777 (2022).
9. Zhong, H. et al. Simultaneous change of microworld and biofilm formation in constructed wetlands filled with biochar. *J. Environ. Manage.* **349**, 119583. https://doi.org/10.1016/j.jenvman.2023.119583 (2024).
10. Zhao, L. et al. A novel autotrophic denitrification and nitrification integrated constructed wetland process for marine aquaculture wastewater treatment. *Chemosphere* **321**, 138157. https://doi.org/10.1016/j.chemosphere.2023.138157 (2023).
11. Zhao, L. et al. Opposite response of constructed wetland performance in nitrogen and phosphorus removal to short and long terms of operation. *J. Environ. Manage.* **351**, 120002. https://doi.org/10.1016/j.jenvman.2023.120002 (2024).
12. Boyd, M. C., Brown, M. T. & Brandt-Williams, S. Addressing pollutant load reduction goals for impaired waterbodies through biomass harvest of Gulf Coast type Phragmites australis (common reed). *Wetl Ecol Manag* **23**(3), 519–533. https://doi.org/10.1007/s11273-015-9406-6 (2015).
13. Kang, Y. et al. Enhanced benzofluoranthrene removal in constructed wetlands with iron-modified biochar: Mediated by dissolved organic matter and microbial response. *J Hazard Mater.* **443**, 130322. https://doi.org/10.1016/j.jhazmat.2022.130322 (2023).
14. Zhang, Y. et al. Distinct roles of biochar and pyrite substrates in enhancing nutrient and heavy metals removal in intermittent-aerated constructed wetlands: Performances and mechanism. *Environ. Res.* https://doi.org/10.1016/j.envres.2024.119393 (2024).
15. Zheng, F. et al. Biochar based constructed wetland for secondary effluent treatment: Waste resource utilization. *Chem. Eng. J.* **432**, 134377. https://doi.org/10.1016/j.cej.2021.134377 (2022).
16. Wang, H., Zang, S., Xu, J. & Sheng, L. Dynamic simulation analysis of city tail water treatment by constructed wetland with biochar substrate. *Environ Sci Pollut R.* **30**, 108582–108595. https://doi.org/10.1007/s11356-023-30002-z (2023).

17. Feng, L. et al. Applying biochar coupled with pyrite substrates simultaneously enhanced nutrient and heavy metal removal in constructed wetland: Performance and mechanism. *Chem. Eng. J.* **488**, 150868. https://doi.org/10.1016/j.cej.2024.150868 (2024).
18. Huang, L. et al. Pathways and biological mechanisms of N2O emission reduction by adding biochar in the constructed wetland based on 15N stable isotope tracing. *J. Environ. Manage.* **342**, 118359. https://doi.org/10.1016/j.jenvman.2023.118359 (2023).
19. Guo, X. et al. Enhanced nitrogen removal *via* biochar-mediated nitrification, denitrification, and electron transfer in constructed wetland microcosms. *Environ. Sci. Pollut.* **30**, 72710–72720. https://doi.org/10.1007/s11356-023-27557-2 (2023).
20. Guo, F. et al. Biochar boosts nitrate removal in constructed wetlands for secondary effluent treatment: Linking nitrate removal to the metabolic pathway of denitrification and biochar properties. *Bioresour. Technol.* **379**, 129000. https://doi.org/10.1016/j.biortech.2023.129000 (2023).
21. Hong, X. et al. Packing blockage and power generation of constructed wetland coupled microbial fuel cell systems using biochar as electrode and filler with different ratios. *Chem. Eng. J.* **488**, 150978. https://doi.org/10.1016/j.cej.2024.150978 (2024).
22. Abedi, T. & Mojiri, A. Constructed wetland modified by biochar/zeolite addition for enhanced wastewater treatment. *Environ. Technol. Innov.* **16**, 100472. https://doi.org/10.1016/j.eti.2019.100472 (2019).
23. Kasak, K. et al. Biochar enhances plant growth and nutrient removal in horizontal subsurface flow constructed wetlands. *Sci. Total Environ.* **639**, 67–74. https://doi.org/10.1016/j.scitotenv.2018.05.146 (2018).
24. Nguyen, X. C. et al. Combined biochar vertical flow and free-water surface constructed wetland system for dormitory sewage treatment and reuse. *Sci. Total Environ.* **713**, 136404. https://doi.org/10.1016/j.scitotenv.2019.136404 (2020).
25. Kizito, S. et al. Treatment of anaerobic digested effluent in biochar-packed vertical flow constructed wetland columns: role of media and tidal operation. *Sci. Total Environ.* **592**, 197–205. https://doi.org/10.1016/j.scitotenv.2017.03.125 (2017).
26. Deng, C. et al. Response of microbes to biochar strengthen nitrogen removal in subsurface flow constructed wetlands: Microbial community structure and metabolite characteristics. *Sci. Total Environ.* **694**, 133687. https://doi.org/10.1016/j.scitotenv.2019.133687 (2019).
27. De Rozari, P., Greenway, M. & El Hanandeh, A. Nitrogen removal from sewage and septage in constructed wetland mesocosms using sand media amended with biochar. *Ecol. Eng.* **111**, 1–10. https://doi.org/10.1016/j.ecoleng.2017.11.002 (2018).
28. Gao, Y. et al. Highly efficient removal of nitrogen and phosphorus in an electrolysis-integrated horizontal subsurface-flow constructed wetland amended with biochar. *Water. Res.* **139**, 301–310. https://doi.org/10.1016/j.watres.2018.04.007 (2018).
29. Tang, X. et al. Enhancing neonicotinoid removal in recirculating constructed wetlands: The impact of Fe/Mn biochar and microbial interactions. *J. Hazard. Mater.* **476**, 135139. https://doi.org/10.1016/j.jhazmat.2024.135139 (2024).
30. Qi, Y. et al. Subsurface constructed wetlands with modified biochar added for advanced treatment of tailwater: Performance and microbial communities. *Sci. Total Environ.* **906**, 167533. https://doi.org/10.1016/j.scitotenv.2023.167533 (2024).
31. Wang, X. et al. Biochar and zero-valent iron alleviated sulfamethoxazole and tetracycline co-stress on the long-term system performance of bioretention cells: Insights into microbial community, antibiotic resistance genes and functional genes. *Environ. Res.* **248**, 118271. https://doi.org/10.1016/j.envres.2024.118271 (2024).
32. Zhang, G. et al. Biochar and hematite amendments suppress emission of CH4 and NO2 in constructed wetlands *Sci. Total Environ.* **874**, 162451. https://doi.org/10.1016/j.scitotenv.2023.162451 (2023).
33. Zhang, Z. et al. Intensified nitrogen and phosphorus removal and mechanism revelation in constructed wetlands amended with iron-carbon based substrate towards low C/N ratio wastewater. *Chem. Eng. J.* **481**, 148931. https://doi.org/10.1016/j.cej.2024.148931 (2024).
34. Zhao, L. et al. Roles of phosphoric acid in biochar formation: Synchronously improving carbon retention and sorption capacity. *J. Environ.* **46**, 393–401. https://doi.org/10.2134/jeq2016.09.0344 (2017).
35. Xu, H. et al. Adsorption behavior and performance of ammonium onto sorghum straw biochar from water. *Sci. Rep.* **12**, 5358. https://doi.org/10.1038/s41598-022-08591-5 (2022).
36. Kuma, P. et al. Phosphate removal from aqueous solution using coir-pith activated carbon. *Sep. Sci. Technol.* **45**(10), 1463–1470. https://doi.org/10.1016/j.watres.2018.04.007 (2010).
37. Shang, Z. et al. Enhanced phosphorus removal of constructed wetland modified with novel Lanthanum-ammonia-modified hydrothermal biochar: Performance and mechanism. *Chem. Eng. J.* **449**, 137818. https://doi.org/10.1016/j.cej.2022.137818 (2022).
38. Karungamye, P. The incorporation of activated carbon as a substrate in a constructed wetland A review. *Cleaner. Water.* https://doi.org/10.1016/j.clwat.2024.100053 (2024).
39. Li, T. et al. Towards enhanced treatment of piggery biogas slurry in tidal flow constructed wetlands by regulation of hydraulic retention time. *Process. Saf. Environ.* **181**, 195–204. https://doi.org/10.1016/j.psep.2023.11.018 (2024).
40. Almanassra, I. et al. Review of phosphate removal from water by carbonaceous sorbents. *J. Environ. Manag.* **287**, 112245. https://doi.org/10.1016/j.jenvman.2021.112245 (2021).
41. Yao, X. & Yang, G. Progress on the study of water purification ability of natural wetlands. *Prog. Geogr.* https://doi.org/10.11820/dlkxjz.2009.05.022 (2009).
42. Dai, Y. et al. The adsorption, regeneration and engineering applications of biochar for removal organic pollutants: a review. *Chemosphere* **223**, 12–27. https://doi.org/10.1016/j.chemosphere.2019.01.161 (2019).
43. Jiang, Y. et al. Effects of environmental factors on phosphorus adsorption capacity and release risk in lake sediments. *Plant Soil Environ.* https://doi.org/10.17221/41/2022-PSE (2022).
44. Li, Y. et al. Diversity and phosphate solubilizing characteristics of cultivable organophosphorus-mineralizing bacteria in the sediments of sancha lake. *Int. J. Environ. Res. Public Health.* **19**, 2320. https://doi.org/10.3390/ijerph19042320 (2022).
45. Santillan, E. et al. Sustained organic loading disturbance favors nitrite accumulation in bioreactors with variable resistance, recovery and resilience of nitrification and nitrifiers. *Sci. Rep.* **10**(1), 21388. https://doi.org/10.1038/s41598-020-78314-1 (2020).
46. Zhang, D. W. et al. Pilot study on the treatment of low carbon and nitrogen ratio municipal sewage by A1/O2/A3/A4/O5 sludge-membrane coupling process with multi-point inflow. *Environ. Sci. Pollut. Res.* **29**, 609–621. https://doi.org/10.1007/s11356-021-15721-5 (2022).
47. Lee, S., Maniquiz-Redillas, M. C. & Kim, L.-H. Settling basin design in a constructed wetland using TSS removal efficiency and hydraulic retention time. *J. Environ. Sci.* **26**, 1791–1796. https://doi.org/10.1016/j.jes.2014.07.002 (2014).
48. Zhou, X. et al. Enhanced nitrogen removal of low C/N domestic wastewater using a biochar-amended aerated vertical flow constructed wetland. *Bioresour. Technol.* **241**, 269–275. https://doi.org/10.1016/j.biortech.2017.05.072 (2017).
49. Mazioti, A. A., Stasinakis, A. S., Pantazi, Y. & Andersen, H. R. Biodegradation of benzotriazoles and hydroxy-benzothiazole in wastewater by activated sludge and moving bed biofilm reactor systems. *Bioresour. Technol.* **192**, 627–635. https://doi.org/10.1016/j.biortech.2015.06.035 (2015).
50. Wu, H. et al. Constructed wetlands for pollution control. *Nat Rev Earth Env.* **4**, 218–234. https://doi.org/10.1038/s43017-023-00395-z (2023).
51. Sacco, F. C. M., Venditti, S., Wilmes, P., Steinmetz, H. & Hansen, J. Vertical-flow constructed wetlands as a sustainable on-site greywater treatment process for the decrease of micropollutant concentration in urban wastewater and integration to households' water services. *Sci. Total Environ.* https://doi.org/10.1016/j.scitotenv.2024.174310 (2024).



## Acknowledgements
We thank Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) for microbial Illumina Sequencing. This study was supported by the National Key Research and Development Program of China (NO.2019YFD1100801),

and the Natural Science Foundation of Shandong Province (No. ZR2020QC041).

## Author contributions

W.Z.: Conceptualization, Methodology, Investigation, Formal Analysis, Writing—Original Draft. H.C.: Visualization, Investigation, Software. X.C.: Methodology, Supervision, Resources, Validation. D.C.: Resources, Supervision. Q.C.: Conceptualization, Funding Acquisition, Resources, Supervision, Writing—Review and Editing.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

**Correspondence** and requests for materials should be addressed to Q.C.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.