

## Technical structure and influencing factors of nitrogen and phosphorus removal in constructed wetlands

Lei Yang<sup>a,b,c</sup>, Xiaohui Jin<sup>a,b,c</sup>, Yawei Hu<sup>a,b,c</sup>, Mingqi Zhang<sup>a,b</sup>, Huihui Wang<sup>a,b</sup>, Qian Jia<sup>a,b</sup> and Yafei Yang<sup>d,\*</sup>

<sup>a</sup> Yellow River Institute of Hydraulic Research, Yellow River Conservancy Commission, Zhengzhou, Henan 450003, China

<sup>b</sup> Rural Water Environmental Engineering Technology Research Center of Henan Province, Zhengzhou, Henan 450003, China

<sup>c</sup> Key Laboratory of Ecological Environment Protection and Restoration in the Yellow River Basin of Henan Province, Zhengzhou, Henan 450003, China

<sup>d</sup> Yellow River Engineering Consulting Co., Ltd., Zhengzhou, Henan 450003, China

\*Corresponding author. E-mail: 1554967065@qq.com

### ABSTRACT

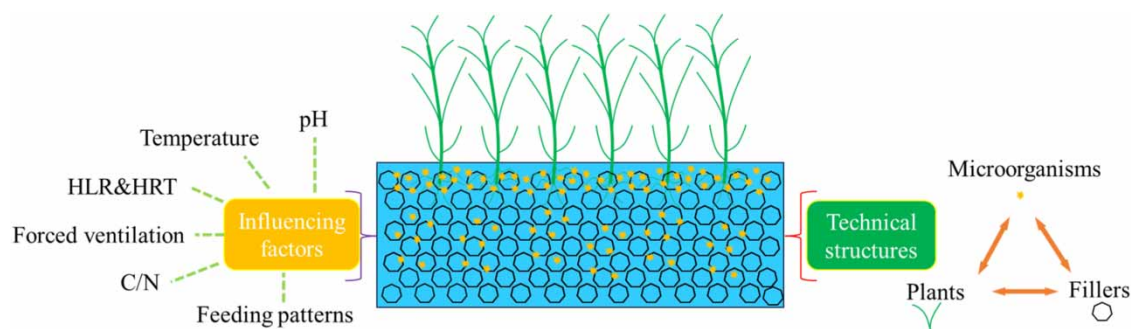
Constructed wetlands purify water quality by synergistically removing nitrogen and phosphorus pollutants from water, among other pollutants such as organic matter through a physical, chemical, and biological composite remediation mechanism formed between plants, fillers, and microorganisms. Compared with large-scale centralized wastewater treatment systems with high cost and energy consumption, the construction and operation costs of artificial wetlands are relatively low, do not require large-scale equipment and high energy consumption treatment processes, and have the characteristics of green, environmental protection, and sustainability. Gradually, constructed wetlands are widely used to treat nitrogen and phosphorus substances in wastewater. Therefore, this article discusses in detail the role and interaction of the main technical structures (plants, microorganisms, and fillers) involved in nitrogen and phosphorus removal in constructed wetlands. At the same time, it analyses the impact of main environmental parameters (such as pH and temperature) and operating conditions (such as hydraulic load and hydraulic retention time, forced ventilation, influent carbon/nitrogen ratio, and feeding patterns) on nitrogen and phosphorus removal in wetland systems, and addresses the problems currently existing in relevant research, the future research directions are prospected in order to provide theoretical references for scholars' research.

**Key words:** constructed wetland, filler, influence factor, microorganism, plant

### HIGHLIGHTS

- Plants, microorganisms, and fillers play their respective roles, and also have complex relationships in constructed wetlands.
- Environmental parameters and operating conditions are the main factors affecting nitrogen and phosphorus removal efficiency of constructed wetlands.
- From the actual operating results, there are still some shortcomings in controlling nitrogen and phosphorus pollution.

### GRAPHICAL ABSTRACT



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## 1. INTRODUCTION

In recent years, with the rapid growth of the social economy, more and more sewage has been directly discharged into water bodies without any treatment. The large amount of nitrogen and phosphorus carried in sewage accelerates the eutrophication of rivers, lakes, and estuaries (Carpenter *et al.* 1998), causing a series of problems such as massive algae proliferation, reduced biodiversity, and pollution of drinking and aquaculture water (El-Sheekh *et al.* 2021), which directly or indirectly cause serious harm to humans and ecosystems. Obviously, taking effective measures to control nitrogen and phosphorus pollution plays a very important role in the global water ecological environment security.

Up to now, the treatment methods for nitrogen and phosphorus pollution are mainly divided into three categories: physical, chemical, and biological methods. The physical method is simple and easy to operate, but the cost is high and the processing is not thorough. Chemical methods have quick results, but their costs are high and are not suitable for long-term use, which may cause secondary pollution. Biological methods have slow effects and long cycles, but they are more economical and thorough, with little environmental impact (Zhang *et al.* 2020a). Constructed wetlands in biological methods are increasingly favored by people due to their unique advantages. It is an efficient ecological technology complex that integrates water security, energy utilization, and environmental protection (Kataki *et al.* 2021). In addition, it can maintain stable operation for a long time (Parde *et al.* 2021), and can buffer sudden water pollution events through its own structure to intercept pollutants in sewage and continuously purify the water body (Li *et al.* 2018).

Many scholars have used different research methods to account for the cost of constructed wetlands, and the results show that constructed wetlands are the most cost-effective among various wastewater treatment methods (Roley *et al.* 2016; Hansen *et al.* 2021; Li *et al.* 2021a). Therefore, constructed wetlands have an important position for both humans and ecosystems. However, before using constructed wetlands for sewage treatment, the relatively large footprint of constructed wetlands should be considered. Currently, the application of constructed wetlands is being increasingly extended to address multiple types of wastewaters. Zhu *et al.* used integrated vertical flow constructed wetlands (IVCWs) for treating wastewater treatment plant tail water (Zhu *et al.* 2021b). Vymazal reported average treatment efficiencies in horizontal subsurface flow constructed wetlands (HSSF CWs) for treating agricultural wastewaters (Vymazal 2009). Qiao & Zheng conducted a study on the purification of urban runoff using a multi-stage series connected surface flow constructed wetland (Qiao & Zheng 2019). Hyeseon Choi *et al.* used a HSSF CW to treat stormwater runoff from highly impervious road and parking lot (Choi *et al.* 2021). In Italy, Gorra *et al.* established HF CW to treat wastewater from cheese production plant. One study was conducted to evaluate the performance of water quality level in stormwater runoff through a constructed wetland under tropical climate (Gorra *et al.* 2007).

A better understanding of the complex interactions involved in wetland systems can provide the best combination of basic science and existing technologies, allowing for fuller application of wetland technologies. The main objectives of this review are: (1) to explore the interactions among plants, microorganisms, and fillers in constructed wetlands, (2) to analyze the impact of major environmental parameters (such as pH, temperature) and operating conditions (such as hydraulic load and hydraulic retention time (HRT), forced ventilation, influent carbon/nitrogen ratio, and influent mode) on nitrogen and phosphorus removal in wetland systems, and (3) to address current issues and look forward to future research directions. It is hoped to provide theoretical references for scholars in the design, operation, and management of constructed wetlands, thereby maximizing the role of constructed wetlands and achieving the goal of maximizing the comprehensive benefits of the wetland system.

## 2. INTERACTION BETWEEN PLANTS, MICROORGANISMS, AND FILLERS

The technical structure of constructed wetland system includes three main elements: plants, microorganisms, fillers, which all play an important role in wastewater treatment (Koottatep & Polprasert 1997; Thammarat & Chongrak 1997; Ge *et al.* 2019; Zhuang *et al.* 2019). These three factors play their respective roles in the constructed wetland, but also have complex relationships, which can play a role of  $1 + 1 + 1 > 3$ .

### 2.1. Plants

An inherent characteristic of constructed wetland is that they need to be covered by wetland plants. Therefore, plants are essential components of constructed wetlands. The plants in the constructed wetland system mainly include three types of free-floating plants, submerged plants, and emergent plants, which are all applied in the research of constructed wetlands (Mustafa & Hayder 2021). They provide habitat for wild animals and form the topography and landscape of wetland

ecosystems, making wastewater treatment systems more aesthetically pleasing. The aboveground portion of a plant can be harvested after the growth period for resource recycling, generating economic value (Ji *et al.* 2021).

However, at present, the direct role of plants in wastewater treatment in constructed wetlands is still controversial (Liu *et al.* 2016). Although N and P in wetland systems can be removed by harvesting plants, many studies have shown that direct absorption by plants contributes less to the removal of N and P in wetland systems. In pilot scale constructed wetlands, the contribution of plants to total nitrogen (TN) removal is only 8.3% (Cui *et al.* 2019). In wetlands where emergent plants are grown, the storage of nitrogen and phosphorus in plant biomass is less than 5% (Vymazal 2011). Even in the most suitable growth environment, the contribution rate of plants is still below 15% (Wei *et al.* 2019). It can be seen that the proportion of pollutants N and P removed by plants in wetlands is indeed low. Therefore, many scholars believe that the most important removal processes in most treatment wetland systems are based on physical and microbial processes (Brix 1997).

However, the indirect role of plants in wetlands cannot be ignored. For example, plants can reduce the water flow rate in wetland systems, stabilize the surface of the filler bed, provide good conditions for physical filtration (Brix 1997; Brisson & Chazarenc 2009), and also isolate the filler, reduce the risk of ice formation in winter, and play a role in thermal insulation (Brix 1997; Brisson & Chazarenc 2009; Vymazal 2011; Tsihrintzis 2017). In addition, plants also have a certain impact on microorganisms and fillers; it plays a positive role in improving the removal rate of pollutants. Many studies have indeed shown that systems with plants have higher pollutant removal efficiency than systems without plants (Vymazal 2011; Sandoval *et al.* 2019).

### 2.1.1. Effects of plants on microorganisms

Wetland plants can enhance the abundance and diversity of rhizosphere microorganisms through their own structures and functions, affect the growth and activity of microorganisms (Zheng *et al.* 2020; Carrillo *et al.* 2022), and regulate the internal structure and composition types of microorganisms, thereby strengthening the water purification capacity of constructed wetlands (Xie *et al.* 2021).

First, plant roots have a large available specific surface area, which can provide a good habitat for microbial growth, increasing the abundance and quantity of microorganisms (Hu *et al.* 2021). Calheiros pointed out that the presence of vegetation has a significant impact on community diversity, as lower bacterial diversity is observed in wetland systems without vegetation (Calheiros *et al.* 2009). Moreover, mixed planting of multiple plants has a better effect. Compared to single planting, mixed planting wetland systems exhibit a higher percentage of bacteria related to nitrogen metabolism, as well as higher microbial diversity and abundance (Huang *et al.* 2019).

Second, the roots of wetland plants can secrete a series of degradable organic compounds (including sugars, organic acids, and amino acids), which can provide a carbon source for the growth and activity of microorganisms, especially for the denitrifying bacteria in the wetland system (Dong *et al.* 2016), thereby promoting denitrification.

Then, the oxygen secretion function of plants changes the oxygen environment near the root system, forming multiple small-scale wastewater treatment systems similar to aerobic, anoxic, and anaerobic systems in the plant root system (Rehman *et al.* 2017), improving the treatment efficiency of pollutants. The research result shows that through photosynthesis, constructed wetlands with vegetation obtain an average of 22.22% more dissolved oxygen than constructed wetlands without vegetation (Pan *et al.* 2019). The oxygen release of reed varies between 108.89 and 404.44 mg O<sub>2</sub>/(m<sup>2</sup>·d) during different growth periods. The oxygen provided by wetland plants may support the removal of 300.37 mg COD/(m<sup>2</sup>·d) or 55.87 mg NH<sub>4</sub><sup>+</sup> – N/(m<sup>2</sup>·d) (Zhang *et al.* 2014). In two pilot scale horizontal subsurface flow wetlands, the nitrification intensity, the number of nitrite oxidizing bacteria, and ammonia oxidizing bacteria in the rhizosphere are significantly higher than those in the non-rhizosphere, and the number and denitrification intensity of denitrification bacteria in the non-rhizosphere soil are higher, which is related to the release of oxygen from plant roots (Hua *et al.* 2017). Similarly, there are significant differences in the microbial community structure of constructed wetlands without planting and planting plants. The presence of plants significantly changes the composition of bacteria at the class and genus levels (Chen *et al.* 2015).

### 2.1.2. Effect of plants on filler

The main impact of plants on the filler in wetland systems is the impact of roots on the hydraulic properties of the filler. Some studies have shown that when the original effective porosity is relatively high, plant roots can hinder water flow. When the blockage is relatively severe, growing plant roots may open new pore spaces in the filler, increasing hydraulic conductivity. In addition, compared to non-planting systems, planting systems have shorter residence times and lower hydraulic efficiency

factors in the late stages of clogging, indicating that root growth can offset filler clogging (Hua *et al.* 2014). Similarly, there are similar conclusions, when the filler is severely clogged, the flow distribution of linear and uniform planting systems is more uniform than that of non-planting systems, because in the case of severe filler clogging, root growth will open new pore spaces and promote the hydraulic conductivity of the filler (Liu *et al.* 2018). According to Antonio Torrens, in a vertical flow constructed wetland, a small portion of water flows through the planted bed faster than through the non-planted bed, possibly because the presence of roots creates a preferential path (Torrens *et al.* 2009). In summary, plants can reduce the risk of clogging the wetland system by affecting the hydraulic conductivity of the filler, thereby improving the service life of the wetland system filler (Tsihrintzis 2017). In addition, plant roots can also absorb soluble nutrients adsorbed on the surface of the filler, thereby increasing the adsorption point of the filler, alleviating filler saturation, and improving the purification efficiency of the wetland system.

Based on the above statements, there is no doubt about the contribution of plants in constructed wetlands. However, the mechanism of action of plants in constructed wetlands is relatively abstract and complex, and this contribution is difficult to quantify.

## 2.2. Microorganisms

There are a wide variety and a large number of microorganisms in the constructed wetland system. They are the main decomposers in the wetland system. They utilize their own metabolic functions to decompose and transform pollutants in water, promoting the material circulation within the wetland, thereby achieving the goal of purifying water quality (Tang *et al.* 2020). These microorganisms mainly include bacteria, actinomycetes, and fungi, among which bacteria account for more than 70% of all microorganisms in the wetland, making them the absolute dominant species in the wetland system. Then there are actinomycetes, which have a strong ability to decompose amino acids and can secrete antibiotic substances to keep the number and community of microorganisms in a balanced state. Finally, fungi, which mainly exist in the matrix, can produce a variety of enzymes, such as fungal lipases that can hydrolyze oil, proteases that can decompose protein compounds (Peng 2011). Research results have shown that the number of microorganisms and enzymes (FDA, catalase, urease, etc.) on the filler surface has a significant impact on the removal regularity and effectiveness of major pollutants in constructed wetlands (Wang *et al.* 2018).

At the same time, microorganisms react quickly to environmental disturbances, therefore, the composition and function of microbial communities can be used as biological indicators for wetland health (Tang *et al.* 2020). Previous studies have shown that when wetlands enter the initial stage of clogging, the abundance of denitrifying bacteria in microorganisms significantly increases, as does the abundance of organic matter degrading bacteria in the phylum Thickwalled and Curvularia. Therefore, the above bacteria are expected to become early warning indicators of clogging in constructed wetland systems (Zhang *et al.* 2021).

When microorganisms in a wetland system act on themselves, they also have a certain impact on plants and fillers.

### 2.2.1. Effects of microorganisms on plants

Due to the high concentration of amino acids and sugars near the plant rhizosphere, which provides rich nutrients for the growth of microorganisms, the microorganisms in the plant rhizosphere are rich and diverse, and an extremely complex microecosystem is formed in the rhizosphere, with a strong rhizosphere effect (Penn *et al.* 2017). Studies have shown that a class of microorganisms known as 'plant rhizosphere growth promoting bacteria' have various effects on plants, such as reducing the toxic effects of plant pathogens on plants, synthesizing substances beneficial to plant absorption using inorganic nutrients in the environment, and providing trace element ions to plants, enhance the solubility of certain minerals through a series of chemical actions (Elsgaard *et al.* 2001; Tan *et al.* 2021; Zhu *et al.* 2021a, 2021b). At the same time, some studies have shown that the combined action of rhizosphere microorganisms can convert organic phosphorus into inorganic phosphorus, facilitating plant absorption, and thus accelerating phosphorus removal. Their research indicates that the increment of phosphorus content in the roots, stems, and leaves of plants with rhizosphere microorganisms is higher than that of plants with rhizosphere microorganisms removed (Cao 2018).

### 2.2.2. Effect of microorganisms on filler

A large number of microorganisms/biofilms exist on the surface of the wetland filler and in the roots of plants. When wastewater flows through the wetland, suspended solids are blocked and retained by the filler and roots, and soluble organic matter is adsorbed by microorganisms in the biofilm and removed through assimilation and dissimilation (U.S.EPA 1986). However,



these large amounts of microbial membranes can lead to a decrease in the porosity of the filler, or even clogging, which can hinder the operation of the wetland system. Therefore, when selecting fillers for constructed wetlands, it is necessary to select fillers with higher porosity and specific surface area, which can provide both greater surface area for biofilm adhesion and higher hydraulic conductivity, thereby reducing the risk of wetland system blockage (Meng *et al.* 2014).

### 2.3. Fillers

In constructed wetland systems, filter is the aggregate in the wetland bed. It plays an important role in wetland systems, providing support for plant growth, as well as providing a surface for microorganisms in the system to adhere to. It can also directly remove pollutants through adsorption, sedimentation, and filtration (Sandoval *et al.* 2019), and is the main venue for wastewater treatment. At the same time, the filler extends the residence time of pollutants in the wetland system through adsorption, sedimentation, and filtration, which is more conducive to plant absorption and transformation, as well as microbial decomposition and utilization. In addition, the matrix can also be used as a support for dispensing and monitoring devices, such as water distributors, pipelines, level gauges, and microbial fuel cells (Ji *et al.* 2022).

Currently, fillers used in wetlands mainly include natural materials such as soil, zeolite, gravel, sand, shale, limestone, volcanic rock, calcite, apatite (Stefanakis & Tsihrintzis 2012; Lu *et al.* 2016), agricultural or industrial wastes such as alum sludge, oyster shells, fly ash, waste bricks, blast furnace slag (Anderson & Paul 2011; Zhao *et al.* 2011, 2018; Zhang *et al.* 2020b), artificial materials, such as activated alumina (Tan *et al.* 2021), activated carbon, biochar (Zhou *et al.* 2019), ceramsite (Fu *et al.* 2017; Cheng *et al.* 2018), modified clay (Ferreira *et al.* 2017), and Filtralite P<sup>TM</sup> (Ádám *et al.* 2005). These fillers all have one or more of the following characteristics: (1) they contain abundant cations such as Ca<sup>2+</sup>, Mg<sup>2+</sup>, Al<sup>3+</sup>, Fe<sup>3+</sup>, the chemical deposition process between phosphate and these cations is considered the main mechanism for phosphorus removal in artificial wetlands (Ji *et al.* 2020); (2) having a large porosity, specific surface area, and a certain degree of surface roughness can provide larger attachment surface area for microorganisms, improve the adsorption sites of the substrate, and improve the hydraulic performance of wetland systems, thereby enhancing the removal capacity of pollutants; (3) affordable, lightweight, and stable to reduce the cost of wetland systems and enhance their stability (Vohla *et al.* 2011; Lu *et al.* 2016; Yin *et al.* 2017). The main specifications and parameters of the above fillers are shown in Tables 1–4. It is worth emphasizing that many studies have confirmed that inorganic compound iron and sulfur and their compounds can be used as wetland substrates to improve nitrogen removal performance, such as natural pyrite (Li *et al.* 2021b; Xu *et al.* 2021), elemental sulfur and iron, pyrite (Li *et al.* 2020; Ma *et al.* 2020; Chu *et al.* 2022) and other substances to increase autotrophic denitrification in wetlands, in which sulfur or iron is used as electron donor of autotrophic denitrification bacteria, without additional carbon sources. In addition, due to the strong reducibility of iron, it can maintain reduction and hypoxia conditions for iron removal. Dissolved iron ions can also promote bacterial growth and precipitation of phosphate groups (Zhuang *et al.* 2019). Considering the different demands of different pollutants on the types of substrates, it is usually done by making several substrates into one substrate in different proportions or directly combining them to improve efficiency (Cheng *et al.* 2018; Abedi & Mojiri 2019). Therefore, the selection of fillers is of great significance for the operational efficiency of artificial wetland sewage treatment systems.

The role of filler in wetlands is self-evident. In addition to the above effects, it also has a certain impact on plants and microorganisms.

#### 2.3.1. Effect of filler on plants

For the growth and reproduction of most submerged and emergent plants, the support function of the filler is essential (Ji *et al.* 2022). However, due to the different physical and chemical properties of the filler itself, types and usage of fillers have varying degrees of impact on plant growth and physiological activities. For example, zeolite is more suitable for the growth of canna compared to biological ceramsite and anthracite (Wu *et al.* 2019). In surface flow constructed wetlands, when the amount of biochar added is less than 10%, the impact on *Bitter Grass* is not significant. When too much biochar is added, it is not conducive to the growth of *Bitter Grass*, mainly manifested by a significant decrease in the total biomass, relative growth rate, and chlorophyll content of *Bitter Grass*, and a slight increase in root activity and root and leaf biomass compared to the first and then decreased (Zheng *et al.* 2021). Meanwhile, in a pilot system for treating acidic starch wastewater, the relative greenness of leaves, stem root ratio, and average plant height of calamus and reed grown in shale are better than those of plants grown in zeolite. The root activity of calamus and reed grown in shale is 3.7 and 1.6 times higher than that of plants grown in zeolite (Zhang *et al.* 2006).

**Table 1** | Main specification parameters of wetland matrix

Materials	Bauxite	Shale	Limestone	Zeolite	LECA	Fly ash	Filtralite P™	
Composition	Rich in hydrated aluminum oxides and ferric oxides	–	Composed largely of calcium carbonate	A hydrated aluminum-silicate mineral	Expanding special clay minerals	Consisting mainly of silica (SiO <sub>2</sub> ), alumina (Al <sub>2</sub> O <sub>3</sub> ) and iron oxides.	A high Ca and Mg content	
Hydraulic conductivity (ms <sup>-1</sup> × 10 <sup>-4</sup> )	5.9 (0.3)	10.0 (0.7)	2.4 (0.1)	29.4 (0.5)	7.0 (0.2)	2.8 (0.3)	11.57	
Porosity (%)	34.5 (2.8)	37.7 (1.9)	33.0 (1.6)	54.4 (1.0)	40.4 (2.9)	45.3 (3.0)	68	
<sup>a</sup> CEC (cmol·kg <sup>-1</sup> )	8.5 (1.3)	19.4 (2.8)	22.8 (1.3)	23.0 (1.1)	9.5 (2.0)	9.3 (0.4)	–	
pH	5.9 (0.1)	4.5 (0.1)	7.8 (0.1)	6.5 (0.2)	8.2 (0.1)	8.3 (0.2)	>10	
<sup>b</sup> Uniformity coefficient (d60/d10)	–	–	–	–	–	–	2.6	
Particle size distribution of substrates <sup>c</sup> (% w/w)	0.0–2.8 mm 2.9–4.0 mm 4.1–6.7 mm 6.8–12.6 mm >12.70 mm	0.2 0 2.6 75.7 21.5	1.9 5.3 25.6 41.9 25.3	1.2 2.2 8.6 26.3 61.7	0 0 4.2 83.6 12.2	0.4 0.1 4.3 25 70.2	77.8 3.5 6.5 12.2 0	0–4 mm
Surface area (m <sup>2</sup> ·g <sup>-1</sup> )	6.8 (0.4)	19.9 (1.6)	7.4 (0.5)	31.4 (0.2)	–	–	–	
P adsorption maximum (g·kg <sup>-1</sup> )	0.61 (0.08)	0.65 (0.07)	0.68 (0.09)	0.46 (0.08)	0.42 (0.04)	0.86 (0.06)	0.25	
Reference	<i>Drizo et al. (1999)</i>						<i>Adám et al. 2006</i>	

<sup>a</sup>Cation exchange capacity.<sup>b</sup>d10 and d60 are the diameters of particle sizes of a substrate material at which 10 and 60% of the particles pass through the sieves based on the accumulative frequency, and d60/d10 is the uniformity coefficient.<sup>c</sup>Weight of size fraction as a percentage of total weight of sample.

Values shown are averages with standard deviations in parentheses.

**Table 2** | Physico-chemical characteristics of filter media

Materials		Coarse sand	Broken stone	Calcite	Soil	Sand	Furnace slag	Blast furnace slag
Component content (mg.kg <sup>-1</sup> )	C	–	–	–	7,890	–	–	–
	TN	74	3.9	11	790	–	–	–
	TP	118	184	1,071	260	–	–	–
	K	1,638	1,394	32	–	–	–	–
	Ca	2,212	1,031	407,540	–	56,300	–	19,249
	Mg	489	1,942	9,555	–	1,260	–	126.7
	Al	–	–	–	1,340	1,340	–	80,350
	Fe	–	–	–	2,950	6,340	–	4,190
Density (g.cm <sup>-3</sup> )	–	1.58	1.47	1.6	–	–	–	0.946
Porosity (%)	–	29	38	36	–	–	–	62.63
EC (dS.m <sup>-1</sup> )	–	0.05	0.04	5.65	–	–	–	–
pH	–	7.9	7.8	11.9	4.94	8.08	12.3	9.34
Organic matter (%)	–	0.54	4.13	0.2	–	–	–	22.92
Hydraulic conductivity (cm.s <sup>-1</sup> )	–	–	–	–	–	–	–	170
Particle size distribution of substrates (% w/w)	0–0.15 mm	–	–	–	–	7.6	22.9	–
	0.16–0.25 mm	–	–	–	–	11.6	6.2	–
	0.26–0.50 mm	–	–	–	–	37	10.8	–
	0.51–11.0 mm	–	–	–	–	22.8	19.1	–
	1.1–2.0 mm	–	–	–	–	10.4	9.8	–
	2.1–5.0 mm	–	–	–	–	10.6	31.2	–
	d10 (mm)	–	1.2	1.3	1.4	–	–	–
d60 (mm)	–	3.5	2.7	3	–	–	–	1.75
Uniformity coefficient (d60/d10)	–	2.92	2.07	2.14	–	–	–	5.3
N and P adsorption maximum (mg.kg <sup>-1</sup> )	NH <sub>4</sub> – N	28.2–40.4	27.11–30.20	226.66–426.24	–	–	–	–
	PO <sub>4</sub> – P	9.67–11.52	25.59–30.88	929.11–1,541.55	970	290	8,890	1,598
Reference		Seo <i>et al.</i> (2008)			Xu <i>et al.</i> (2006)			Cui <i>et al.</i> (2008)

Therefore, when selecting fillers, it is necessary not only to investigate the physical and chemical properties of the fillers, but also to evaluate the impact of the filler on plants, which is very necessary for building an overall efficient and stable wetland system.

### 2.3.2. Effect of filler on microorganisms

The types of fillers vary, and their physical and chemical properties vary. They are also one of the carriers of microbial growth, and the growth environment of microorganisms varies. Therefore, the type and depth of fillers have a certain impact on the species diversity and community structure of microorganisms. According to the researching report, in wetland systems where the filler is sand, zeolite, and gravel, bacterial community diversity and structure show significant spatial differences in sand and zeolite wetland systems, while in gravel wetland systems, there is little change (Guan *et al.* 2015). In wetland systems with three different fillers (gravel, steel slag, and zeolite), the filler type has a significant impact on bacterial abundance, but has a small impact on bacterial diversity. Archaea abundance and diversity are affected by the filler type and wetland depth (Long *et al.* 2016). Ge claims that the types of fillers (pyrite, limestone) change the community structure of microorganisms at the genus level (Ge *et al.* 2019). Similarly, studies have shown that constructed wetlands constructed from expansive clay

**Table 3** | Main specification parameters of wetland matrix

Materials	Component content(g.kg <sup>-1</sup> )					Grain size distribution (mass fraction/%)				P sorption maximum (g.kg <sup>-1</sup> )	Reference
	Fe	Ca	Al	Mg	pH	0.0–0.5 mm	0.5–1.0 mm	1.0–2.0 mm	2.0–5.0 mm		
Quartz sand I	3.34	56.4	1.34	1.26	7.38	19.1	47.7	22.8	10.4	0.30 ± 0.02	Dai & Hu (2017)
Quartz sand II	2.27	35.4	1.51	1.12	7.08	19.6	50.1	18.7	11.6	0.21 ± 0.01	
Quartz sand III	2.65	37.6	1.45	1.39	7.19	13.9	15.0	27.7	43.4	0.18 ± 0.01	
Quartz sand IV	12.31	47.3	1.37	1.65	7.61	18.5	46.3	22.7	12.5	0.33 ± 0.03	
Activated carbon	–	–	–	–	6.95	0.0	0.0	67.1	32.9	4.64 ± 0.05	
Zeolite	15.1	12.3	71.6	3.14	10.4	0.0	0.9	58.1	41.0	6.78 ± 0.09	
Ceramic	1.32	22.7	82.8	1.39	9.84	0.0	1.5	43.0	55.5	7.24 ± 0.12	
Furnace slag	19.4	15.66	38.8	0.41	12.1	5.9	7.9	34.3	61.9	8.11 ± 0.10	

aggregates and fine gravel have an important impact on the structure and species richness of bacterial communities (Calheiros *et al.* 2009).

### 3. FACTORS AFFECTING NITROGEN AND PHOSPHORUS REMOVAL IN CONSTRUCTED WETLANDS

There are many factors affecting the efficiency of nitrogen and phosphorus removal in constructed wetlands, mainly including two categories: environmental parameters and operating conditions. The effects of different conditions on nitrogen and phosphorus removal in constructed wetlands are shown in Table 5.

#### 3.1. Environmental parameters

##### 3.1.1. pH

pH plays a key role in the process of nitrogen and phosphorus removal in wetland systems. The research results of Aldrew Alencar Baldovi show that in a free surface constructed wetland system with an average inlet pH of  $5.7 \pm 0.2$  and an average outlet pH of  $6.1 \pm 1.0$ , the TP removal rate can reach about 90%, and it is speculated that this pH range is suitable for plant growth (Baldovi *et al.* 2021). Junhong Bai also pointed out that the lower the pH, the more conducive to phosphorus absorption by plants (Bai *et al.* 2017). However, extreme pH values can also affect plant growth and microbial diversity, with pH values ranging from 4 to 12 for wetland system stability (Li *et al.* 2018). The optimal pH for nitrogen removal is 7.0–7.5. When  $\text{pH} < 6.0$  and  $\text{pH} > 8.0$ , nitrogen removal efficiency will be reduced (Saeed & Sun 2012). This is because the nitrification process consumes the alkalinity in the system, which significantly decreases the acidity in the system, thereby hindering denitrification, currently, alkaline substances such as lime are mainly added to the system to neutralize the acidity and maintain pH stability (Ahn 2006).

##### 3.1.2. Temperature

Temperature is one of the main factors affecting nitrogen and phosphorus removal rates in wetland systems. As temperature increases, the average removal efficiency of TN,  $\text{NH}_3\text{-N}$ , and TP increases significantly, because lower temperatures inhibit the growth and activity of plants and bacteria related to nitrogen and phosphorus removal (Shui *et al.* 2013). When water temperature varied between 15 and 20 °C, the TN removal efficiency was as high as 92.58%. With temperature dropping to 10–15 °C, the reactor went into a transition state, causing a significant variation in TN removal and an accumulation of nitrite. When temperature was further down to the range of 5–10 °C, the TN removal efficiency was reduced to 19.19% with a significant accumulation of nitrite detected in the effluents, indicating lower water temperatures were inhibitory to the microbial activity but not so much as to halt denitrification process (Xu *et al.* 2016). The research results of Yumei Hua show that the removal rates of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  in both horizontal subsurface flow wetlands are significantly positively correlated with water temperature, on the one hand because low temperature inhibits the release of oxygen from plant



**Table 4** | Main specification parameters of wetland matrix

Materials	Slag	Gravel	Shale ceramicsite	Activated alumina	Volcanic rock	Corn stalk derived biochar	Active carbon
Component	C	–	–	–	–	90.2	granular active carbon made of coconut shell
content(g.kg <sup>-1</sup> )	CaO	0.3782	0.0106	–	0.1		
	SiO <sub>2</sub>	0.2182	0.6156	–	0.15		
	MgO	5.53	1.75	–	–		
	Na <sub>2</sub> O	–	1.56	–	–		
	Al <sub>2</sub> O <sub>3</sub>	16.9	17.85	–	0.43		
	Fe <sub>2</sub> O <sub>3</sub>	13.1	7.61	–	0.12		
	pH	10.55	8.76	–	–	9.7	
Surface area (m <sup>2</sup> .g <sup>-1</sup> )	4.3	2.81	2.168	334.07	2	176.6	–
Particle size (mm)			15–20;	5–8	3–5		2–4
Micro-pore volume(mm <sup>3</sup> .g <sup>-1</sup> )			2.844 mm <sup>3</sup> .g <sup>-1</sup>	393.1 mm <sup>3</sup> .g <sup>-1</sup>		184	–
Porosity (%)	–	–	–	–	73		39.98,
Density (g.cm <sup>-3</sup> )					1.1	0.7	–
Removal rate	3.15 g P kg <sup>-1</sup>	0.81 g P kg <sup>-1</sup>	NH <sub>4</sub> <sup>+</sup> – N: 64.9%; TN :47.2%; TP:27.4%	NH <sub>4</sub> <sup>+</sup> – N: 85.4%; TN: 72.8%; TP: 96.4%	NH <sub>4</sub> <sup>+</sup> – N: 98.42%; TP:82.19%	NH <sub>4</sub> <sup>+</sup> – N: 84.2% ± 3.2%; TN: 45.3% ± 2.2%; PO <sub>4</sub> <sup>3-</sup> – P: 84.6% ± 2.8%	NH <sub>4</sub> <sup>+</sup> – N: 53.70 ± 8.91%; TN: 47.06 ± 9.97%,
Reference	<i>Ge et al. (2015)</i>		<i>Tan et al. (2019)</i>		<i>Wei &amp; Ren (2023)</i>	<i>Chen &amp; Zhang (2019)</i>	<i>Xu et al. (2022)</i>

**Table 5** | Effects of different conditions on nitrogen and phosphorus removal in constructed wetlands

Wetland type	Wastewater type	Wetland design and operation						Removal performance			Reference
		Dimension L × W × D	Feed type	HRT	HLR	temperature	influence factor	NH <sub>4</sub> <sup>+</sup> – N,	TN	TP	
SF	Synthetic wastewater	67 × 47 × 35 cm	Batch	–	–	10 °C	C/N: 1.57, 4.74, 9.87, 11	–	C/N = 9.87,48.5%	–	Ding <i>et al.</i> (2017)
SF	Synthetic wastewater	Diameter of 40 cm, depth of 50 cm	Sequencing fill-and-draw batch mode	–	–	–	C/N: 0:1, 1:1, 3:1, 6:1, 12:1	C/N = 6:1, 98.82%	C/N = 12:1, 89.81%	–	Li <i>et al.</i> (2017)
HSSF	Synthetic wastewater	2 × 0.5 × 0.65 m	Intermittent	10 d	0.018 m/d	1.6–1.9 °C	C/N:1, 2, 3, 4, 5, 6, 7	The removal efficiency of NH <sub>4</sub> <sup>+</sup> – N decreased with increasing C/N ratio	C/N = 5,89.9%	–	Zhu <i>et al.</i> (2014)
CW	Domestic sewage	2 m length × 1 m width	Intermittent	1 d	1 m <sup>3</sup> /(m <sup>2</sup> .d)	–	T < 10 °C, 10 < T < 20 °C, T > 20 °C	T > 20 °C, 53.6 ± 10.8%	T > 20 °C, 56.8 ± 12.7%	T > 20 °C, 53.1 ± 16.1%	Shui <i>et al.</i> (2013)
HSSF	Domestic sewage	8 m length × 3 m Width, bottom slope:1%.	Intermittent	2.5 d	0.15 m <sup>3</sup> m <sup>-2</sup> d <sup>-1</sup>	–	T:12 –29 °C	The removal efficiencies of NH <sub>4</sub> <sup>+</sup> – N was significantly positively correlated with water temperature; 21.5%–66.9%	Mean:0.837 g N m <sup>-2</sup> d <sup>-1</sup>	–	Hua <i>et al.</i> (2017)
HSSF	Synthetic sewage	3 × 0.75 × 1 m	–	–	–	–	T:2 < T < 26 °C	T < 15 °C, 58.5%; T > 15 °C, 69.1%	T < 15 °C, 58.5% T > 15 °C, 73.9%	T < 15 °C, 41.8% T > 15 °C, 70.1%	Akratos & Tsihrintzis (2007)
SSF	Urban wastewater	1.1 × 0.7 × 0.38 m	–	3.3 d	26 mm/d	14 °C	Flow modes: intermittent and continuous	Intermittent: 80–99% continuous: 71–85%	–	–	Caselles-Osorio & García (2007)
VF	Synthetic domestic	Height of 60 cm and diameter of 24 cm	Intermittent	3 d	0.21 m <sup>3</sup> m <sup>-2</sup>	–	Aeration and non-aerated	–	Aerated:91.31–93.91% Non-aerated: 12.22–53.92%	–	Liu <i>et al.</i> (2019)
HSSF	Domestic wastewater	3 × 0.7 × 1 m	–	–	–	–	Aeration and non-aerated	Aerated:89.1% ± 7.2% Non-aerated:68.3% ± 13.7%	Aerated:86.0 ± 5.6% Non-aerated:73.8 ± 8.2%	–	Zhang <i>et al.</i> (2010)
SSF	Synthetic domestic	Height of 65 cm and an inner diameter of 20 cm	A sequencing fill-and-draw batch mode	3 d	0.21 m <sup>3</sup> m <sup>-2</sup> batch <sup>-1</sup>	–	Aeration and non-aerated	Aerated:98.8% ± 1.0% Non-aerated:27.1% ± 6.1%	Aerated:85.8 ± 9.2% Non-aerated:32.3 ± 5.7%	–	Wu <i>et al.</i> (2016)
VF	Domestic wastewater	30 cm long and 24.5 cm in diameter	–	–	–	–	HLR:21, 14, 7 cm d <sup>-1</sup>	–	–	HLR = 7,71% HLR = 21,60%	Cui <i>et al.</i> (2010)

(Continued.)

Table 5 | Continued

Wetland type	Wastewater type	Wetland design and operation					influence factor	Removal performance			Reference
		Dimension L × W × D	Feed type	HRT	HLR	temperature		NH <sub>4</sub> <sup>+</sup> – N,	TN	TP	
HSF	A mixture of domestic sanitary wastewater, grey water	12 × 1.6 × 1.1 m	-	-	-	-	HLR:31, 62, 104, 146 mm d <sup>-1</sup>	HLR = 31, 91 ± 3% HLR = 62, 69 ± 13% HLR = 104, 65 ± 4% HLR = 146, -1.4%	HLR = 31, 84 ± 3% HLR = 62, 61 ± 3% HLR = 104, 62 ± 7% HLR = 146, 16 ± 9%	HLR = 31, 99 ± 1% HLR = 62, 98 ± 1% HLR = 104, 85 ± 5% HLR = 146, 72 ± 6%	Trang <i>et al.</i> (2010)
SF	Synthetic domestic	50 cm in depth and 40 cm in diameter	Sequencing fill-and-draw batch mode	5 d	0.0398 m d <sup>-1</sup> .	-	pH:7.5, 8.5, 9.5, 10.5	-	pH = 7.5, 76.3 ± 0.04% pH = 8.5, 66.2 ± 0.04% pH = 9.5, 56.0 ± 0.07% pH = 10.5, 51.8 ± 0.04%		Yin <i>et al.</i> (2016)
Batch.P adsorption tests	Artificial.P solution	-	Batch	-	-	-	pH:4.3, 6.0, 7.0, 8.5, 9.0	-		<sup>a</sup> PH = 4.3, 22.4 mg-P/g.sludge <sup>a</sup> PH = 9.0, 0.9 mg-P/g.sludge	Zhao <i>et al.</i> (2009)

<sup>a</sup>Maximum P adsorption capacity.

roots into the system, and on the other hand because low temperature inhibits the activity of microorganisms (Hua *et al.* 2017).

The above studies have shown that low temperatures do have a negative impact on nitrogen and phosphorus removal efficiency. To improve nitrogen removal rates at low temperatures, currently, different isolation or thermal insulation measures are mainly taken to maintain suitable temperatures in wetlands (Li *et al.* 2011), or to increase HRT to allow microorganisms more time to complete the nitrogen removal process (Xu *et al.* 2016), or to use microbial inoculums and biological disturbances to promote microbial activity, alternatively, different plant configurations (such as planting cold tolerant plants) may be used (Ji *et al.* 2020).

## 3.2. Operating conditions

### 3.2.1. Hydraulic load rate and HRT

Hydraulic load rate (HLR) refers to the volume ( $\text{m}^3$ ) of sewage that can be digested per unit area ( $\text{m}^2$ ) and per unit time (d). HRT refers to the average time that water stays in a wetland. These two parameters are very critical in wetland design and performance evaluation (Almukhtar *et al.* 2018). Because they directly affect the contact time between living or non-living things and wastewater, thereby affecting the removal efficiency of pollutants, in addition, they also affect the floor area of wetland systems (Li *et al.* 2018). For instance, Li Dan conducted statistical regression analysis on nitrogen and phosphorus removal, HLR, and HRT, and the results showed that with the decrease of HRT or the increase of HLR, the efficiency of nitrogen and phosphorus removal showed a slow downward trend. Therefore, reducing HLR and extending HRT can improve the removal efficiency of pollutants in subsurface flow constructed wetland (Li *et al.* 2018). Baldovi Aldrew Alencar's study had similar results. The average removal of total phosphorus in surface flow constructed wetlands is up to 85% within a HRT of 0.5–56 days. Compared to the same type of wetland, the total phosphorus removal rate is 60% higher in HRT of 1–3 days (Baldovi *et al.* 2021). This occurred because a longer HRT resulted in more contacts and interactions of TP in the influent with the substrates and roots, which in turn promoted more adsorption, transformation, and uptake of nutrient in the CWs (Cui *et al.* 2010). Dong also claimed that the high removal rate of various pollutants in wetland systems is due to the long HRT of 92 days (Dong *et al.* 2011). However, when the HRT is too long, plants may die, causing pollutants to return to the wetland system again (Sudiarto *et al.* 2019).

### 3.2.2. Forced ventilation

Forced ventilation is one of the effective measures to solve the lack of dissolved oxygen in wetland systems. Due to the unique structure of constructed wetlands, the content of dissolved oxygen in the system is relatively low. Oxygen is the main influencing factor for nitrogen removal in constructed wetlands (Lu *et al.* 2020), because oxygen has a significant impact on the metabolic activity of aerobic nitrifying bacteria, thereby affecting nitrogen removal efficiency. Therefore, it is particularly important to improve the removal of nitrogen by oxygen in wetland systems through technical means such as forced ventilation. There are studies show that the removal rate of  $\text{NH}_4^+\text{-N}$  in different constructed wetlands does not change with the increase of the influent C/N ratio (Zhou *et al.* 2019), mainly because intermittent aeration enhances the oxygen supply capacity, promotes the growth and development of nitrifying bacteria, and thus promotes nitrification reactions (Zhou *et al.* 2017). Kyonghak Hyunab pointed out that aeration at the inlet caused rapid nitrification in the wetland system, and the generated nitrate was reduced in the middle and end of the wetland through denitrification, thereby improving the nitrogen removal efficiency (Hyun *et al.* 2015).

### 3.2.3. Carbon nitrogen ratio of influent water

The influent carbon nitrogen ratio (C/N) represents the relative amount of organic carbon sources in constructed wetland systems, and is considered a key factor in nitrogen conversion by significantly affecting microbial nitrification and heterotrophic denitrification (Li *et al.* 2017).  $\text{NH}_4^+\text{-N}$  removal is highly sensitive to changes in influent C/N (Mohammed & Babatunde 2017). In surface flow constructed wetlands, with the continuous increase of C/N, the removal efficiency of TN and  $\text{NH}_4^+\text{-N}$  gradually increased. Under the condition of C/N of 12, there is a good removal rate for  $\text{NH}_4^+\text{-N}$  (98%) and TN (90%) (Li *et al.* 2017). In a subsurface flow constructed wetland system, with the increase of C/N, the removal rates of TN and nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) increase, and when the C/N is 5, the removal rate is the highest. This is because the input of carbon sources promotes the activity of denitrification microorganisms (Zhu *et al.* 2014). In a vertical subsurface

flow constructed wetland system, when C/N is in the range of 2.5–5, there is a high nitrogen removal efficiency (Zhao *et al.* 2010).

As mentioned above, the C/N of different types of constructed wetlands are not always consistent when achieving the optimal nitrogen removal rate. This is because different types of artificial wetlands have different reaeration abilities. The stronger the reaeration ability the higher the C/N at the highest removal rate, and the higher the nitrogen removal rate. When the oxygen content is constant, with the increase of C/N, the degradation of excess organic matter will consume more dissolved oxygen, which will inhibit the activity of nitrifying microorganisms (Li *et al.* 2017), as a result, the denitrification efficiency is reduced. When the increase in influent C/N leads to a decrease in nitrification rate, it can be solved by aeration into the wetland system (Zhou *et al.* 2019). When the C/N of influent water is low, if heterotrophic denitrification technology is used, the nitrogen removal efficiency will be low due to insufficient carbon sources, and a better treatment effect cannot be achieved. However, autotrophic denitrification technology can be used to promote nitrogen removal, thereby achieving deep nitrogen removal of low-carbon wastewater (Chu *et al.* 2022).

### 3.2.4. Water feeding patterns

The feeding patterns mainly affect the reoxygenation ability of the wetland system, while oxygen affects the removal of pollutants in the wetland system. Therefore, selecting a suitable feeding pattern plays an important role in improving the removal rate of the wetland system. Currently, the main feeding patterns of constructed wetlands are as follows: intermittent, continuous and tidal flow (TF). ‘Intermittent flow’ implies a repeated fed-batch operation, in which CWs are periodically flooded sewage and then periodically drained. ‘TF’ is a new type of water feeding patterns evolved from intermittent flow. It adds an additional idle time compared to intermittent flow, where the TF process is ‘flooded – drained – idle’ and continuously cycles through this process.

In the TF device, different idle/response times (12 h: 12 h, 8 h: 16 h, 16 h: 8 h) were set, and the average  $\text{NH}_4^+\text{-N}$  removal rate ( $98.04 \pm 0.94\%$ ,  $98.56 \pm 0.73\%$ ,  $99.09 \pm 0.67\%$ ) and average nitrification intensity were better than those in the continuous flow device ( $94.58 \pm 2.73\%$ ), this is mainly due to the TF type of water intake, which improves the oxygen content inside the constructed wetland and is conducive to nitrification reaction (Zhang 2015). The TF CW reactor designed by Chen *et al.* adopts a method of flooded 24 h and idled 24 h, compared with the continuous flow (TN: 17.6%, TP: 45.5%) inlet method, the operation mode of TF (TN: 37.6%, TP: 54.1%) can improve the system’s nitrogen and phosphorus removal efficiency (Chen *et al.* 2017). Jian Zhou showed that under the same conditions, compared to the continuous flow mode, the TN removal rate increased by 32% when using intermittent water feeding mode (Zhou *et al.* 2011). The research results of Aracelly show that in the first and second stages, the ammonium removal rates of the continuous influent system are 71 and 85%, and the ammonium removal rates of the intermittent influent system are 80 and 99%, because the intermittent system has better oxidation environmental conditions (Caselles-Osorio & Garcia 2007). Ling’s research suggests that under intermittent flow operation with an HRT of 1 day, the average removal rates of  $\text{NH}_4^+\text{-N}$  and TN by volcanic rocks are 16.45% and 7.25%, respectively and the average removal rates of  $\text{NH}_4^+\text{-N}$  and TN by sponge balls are 11.13% and –2.67%, respectively. Under the operation mode of flooded/drained time ratio of 12 h/12 h a cycle, the removal efficiency of  $\text{NH}_4^+\text{-N}$  and  $\text{NH}_4^+\text{-N}$  is as follows: volcanic rock (35.49%, 20.01%) > sponge ball (27.33%, 14.86%) (Ling 2022).

In general, the use of TF inflow has a higher efficiency in treating nitrogen and phosphorus, especially in nitrogen removal. This is because in TF CWs, influent is controlled to ensure the wetland bed alternates between flood and drainage. As the substances can sufficiently contact with air in drainage period, oxygen supply could be intensified in the whole bed. Thereby increasing the oxygen content in the bed, which is beneficial for the aerobic metabolism and nitrification reaction of microorganisms. Studies have shown that the average oxygen supply capacity of traditional wetlands is less than  $100 \text{ g}/(\text{m}^2\cdot\text{d})$ , far lower than  $350 \text{ g}/(\text{m}^2\cdot\text{d})$  of TF constructed wetlands (Wu *et al.* 2011).

## 4. CONCLUSION AND OUTLOOK

So far, the use of constructed wetlands in the treatment of nitrogen and phosphorus pollution has been widely recognized at home and abroad, and has been proven to have good social, ecological, and economic benefits. However, from the perspective of actual operation results, there are still some shortcomings in the treatment of nitrogen and phosphorus pollution by constructed wetlands. Future research on the treatment of nitrogen and phosphorus pollution by constructed wetlands can focus on the following aspects:



- (1) The direct absorption of nitrogen and phosphorus by plants has a small contribution to the TN and phosphorus removal rate of the wetland system. Therefore, it is possible to cultivate or train plant varieties with high pollution resistance, high absorption, and high biomass through transgenic technology or training technology to enhance the nitrogen and phosphorus removal effect of the wetland system.
- (2) Rhizosphere growth-promoting bacteria have a positive role in wetland systems, which can directly or indirectly promote plant growth, control diseases and insect pests, etc. However, at present, there is less research and application of rhizosphere growth promoting bacteria in constructed wetlands. In the future, it is possible to strengthen the screening of rhizosphere growth promoting bacteria and application research in wetland systems, thereby improving the removal efficiency of pollutants in wetland systems.
- (3) Filler saturation and clogging are key issues that affect the purification capacity and lifespan of wetland systems. In the future, further attention can be paid to the development of new fillers or the design of new wetland structures to delay filler saturation and clogging.
- (4) Plants, microorganisms, and fillers play an important role in nitrogen and phosphorus removal in wetland systems, but systematic research on these three elements in the same wetland system is still rare. It is necessary to further study the independent roles of plants, fillers, and microorganisms in the same wetland system and their coupling effects, which plays an important role in optimizing the purification capacity of the wetland system.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## CONFLICT OF INTEREST

The authors declare there is no conflict.

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