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Review

A Review of Enhanced Nitrogen Removal Measures and Mechanisms in Constructed Wetlands

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Abstract

Constructed wetlands can treat various types of sewage and have good nitrogen removal capacity. With the deterioration of the environment, nitrogen emission requirements have also increased; how to maximize the nitrogen removal function of constructed wetlands is particularly important. Therefore, this paper aims to summarize the measures to increase the nitrogen removal capability of constructed wetlands. New nitrogen removal methods such as simultaneous nitrification and denitrification, partial nitrification-denitrification, and anaerobic ammonium oxidation are efficient ways to remove TN. The effect of nitrogen removal in constructed wetlands can be strengthened by optimizing the configuration, such as improving the water intake mode, optimization of substrate combinations, optimization of plant species configuration, and novel constructed wetland coupling process, and improving the operating conditions, such as adding external carbon sources, improving redox conditions, microbial enhancement technology, and aeration. On this basis, the mechanisms of nitrogen removal by microorganisms, substrates, and plants as well as the coupling roles played by each other in the process of wastewater purification are illustrated. This paper provides a systematic idea for increased nitrogen removal in constructed wetlands, provides some references for research in this field, and finally provides prospects for future research.

Keywords: constructed wetland, mechanism, nitrogen removal, removal effect, strengthening measures

Introduction

Excessive wastewater from human activities leads to increasing nitrogen pollution [1], and if the polluted water is discharged directly into natural water bodies without in-depth treatment, it is likely to cause algal blooms, thus threatening water quality and ecological safety. Constructed wetland, as an environmentally friendly and sustainable way of water resource management, is of great significance to the maintenance of ecological balance and the protection of the natural environment. Constructed wetlands have been widely used because of their low energy consumption, high efficiency in treating wastewater, and easy operation and maintenance [2].

Constructed wetland refers to a wetland ecosystem that is artificially designed and built according to specific needs to simulate the structure and function

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of natural wetlands, usually composed of substrates, plants, soils, microorganisms, etc., and is a sewage treatment system with economic benefits and ecological sustainability [3], which can be used for the treatment of various sewage such as domestic sewage, agricultural wastewater, industrial wastewater, and rainwater [4]. In constructed wetlands, microorganisms are the main actors in the removal and degradation of nitrogen and play a central role in the interaction with other plants and animals. Under the action of microorganisms, nitrogen, and various pollutants are eventually degraded or converted into nutrients that can be used by plants and microorganisms or released into the environment [5]. The main microorganisms associated with constructed wetlands are bacteria, yeasts, protozoa, fungi, and algae [6]. Among them, the number of bacteria is the largest, the number of fungi is the least, and the number of bacteria with nitrogen removal functions, such as ammoniating bacteria, nitrosifying bacteria, nitrifying bacteria, and denitrifying bacteria, is at a high level [7]. The substrate is the largest part of the constructed wetland and is the main support structure [8], which can remove larger particles and pollutants through physical filtration and retention, and nitrogen can be removed mainly through adsorption [9, 10]. Plants are one of the key factors affecting the effectiveness of nitrogen removal in constructed wetlands and can directly utilize nutrients from wastewater for their own growth [11]. Nitrogen in wastewater can be removed by the uptake of plants [12]. In addition, plants also provide a place for microorganisms to live and reproduce, and the large surface area of the plant root system is a good habitat for microbial adsorption and growth [13].

The nitrogen form in wastewater mainly includes ammonia nitrogen, nitrite, and organic nitrogen [14]. Nitrification-denitrification is often considered the primary pathway for nitrogen removal in constructed wetlands; more than 50% of nitrogen in constructed wetlands is removed by nitrification-denitrification by microorganisms [15, 16], and less than 25% of nitrogen is uptaken by plant roots [17]. However, both the physicochemical properties of the substrate (substrate particle size) and the plants (secretions from the root system) can indirectly affect the nitrogen removal efficiency of constructed wetlands by influencing the microbial [18, 19]. Substrate particle size affects microorganisms by influencing oxygen transfer efficiency [20]. Plant roots can secrete oxygen to promote nitrification, and organic matter secreted by roots can serve as a carbon source for denitrifying bacteria to promote denitrification [21, 22].

In constructed wetlands, different microorganisms need different environments to fully perform their respective functions. Nitrifying bacteria need nitrification under aerobic conditions, and denitrifying bacteria need organic carbon sources; however, the original dissolved oxygen and organic carbon in wastewater and the dissolved oxygen and organic carbon secreted by plant roots are always replenished at a slower rate than the consumption rate of microorganisms [23]. As a result, configuration optimization measures and improved operating conditions can be manually taken to enhance nitrogen removal.

Novel Pathways for TN Removal

Dissolved oxygen and organic carbon are the two primary substances depleted in the process of TN removal in constructed wetlands. Compared to the traditional nitrogen removal mechanism, the new nitrogen removal path has higher nitrogen removal efficiency and can save dissolved oxygen and organic carbon. The new nitrogen removal path mainly includes simultaneous nitrification and denitrification, partial nitrification-denitrification, and anaerobic ammonia oxidation [24-26].

Simultaneous Nitrification and Denitrification

Simultaneous nitrification and denitrification is the procedure of nitrification and denitrification that occurs at the same time and in the same place [27]. As a process with high nitrogen removal effectiveness and low consumption of energy, simultaneous nitrification and denitrification play an essential role in the process of nitrogen removal in constructed wetlands. Especially under aerobic conditions, denitrification can also take place, thus making simultaneous nitrification and denitrification possible. The reaction equation is as follows: equation (1), and simultaneous nitrification and denitrification avoid competing between nitrite-oxidizing bacteria and denitrifying bacteria [28, 29].

$$NH_4^+ + 0.75O_2 \rightarrow 0.5N_2 + 1.5H_2O + H^+ \quad (1)$$

Because simultaneous nitrification and denitrification can occur in the same place, conventional nitrification and denitrification need to be carried out in different environments due to different requirements for dissolved oxygen and carbon sources, thus leading to the shortcomings of traditional nitrogen removal processes [30]. Simultaneous nitrification and denitrification can be formed under intermittent aeration, low oxygen conditions, and complex hydraulic conditions [31], and can also occur under high oxygen conditions (2.43-6.84 mg/L) [32]. Yang et al. [33] found in the study of the simultaneous nitrification and denitrification experimental device of the micro-aerated constructed wetland (aeration rate is 0.33 L/h) that the effluent ammonia nitrogen, nitrate nitrogen, and total nitrogen concentrations were all low when treating the tailwater of the sewage treatment plant, and the average removal rate of total nitrogen was as high as 96.59%. When Lai et al. [34] used an integrated reactor of a moving bed and constructed a wetland to treat domestic sewage, they found that when the dissolved oxygen was 4 mg/L through intermittent aeration, the efficiency of simultaneous nitrification and denitrification increased from 5.9% to 35.5%, and the removal rates of ammonia nitrogen and total nitrogen in the reactor were 91.8±1.2% and 77.0±2.6%, respectively. Therefore, the simultaneous nitrification and denitrification rate can be adjusted by adjusting the concentration of dissolved oxygen. In addition, the C/N ratio is an important factor influencing simultaneous nitrification and denitrification effectiveness, and if we want to achieve high simultaneous nitrification and denitrification effectiveness, it is necessary to properly control the input of carbon and nitrogen [24]. Compared with conventional nitrification and denitrification, simultaneous nitrification and denitrification can significantly decrease the operating costs of constructed wetlands and improve the efficiency of nitrogen removal.

Partial Nitrification-Denitrification

The partial nitrification-denitrification procedure consists of the conversion of NH_4^+-N to NO_2^--N and the conversion of NO_2^--N to N_2, as shown in the following equations (2) and (3) [35]:

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad (2)$$

$$NO_2^- + 1/2CH_3OH + H^+ \rightarrow 1/2N_2 + 1/2CO_2 + 1.5H_2O \quad (3)$$

From equations (2) and (3), it can be seen that the nitrogen removal effect of partial nitrification-denitrification is related to ammonia nitrogen concentration, pH, and dissolved oxygen concentration. The partial nitrification-denitrification process requires precise control of dissolved oxygen concentration. Because ammonia-oxidizing bacteria have a higher affinity for oxygen than nitrite-oxidizing bacteria, nitrite oxidation is suppressed when DO is below 1.5–2 mg/L [23, 36]. In addition, high pH (7.5–9.0), low dissolved oxygen (less than 1.0 mg/L), and free ammonia (FA) (0.1–4.0 mg/L) all had inhibitory effects on nitrite-oxidizing bacteria [37, 38]. Partial nitrification-denitrification reduces oxygen and organic matter concentration requirements by 25% and 40%, respectively, in comparison with conventional nitrification and denitrification [39]. In addition, partial nitrification-denitrification is also affected by C/N. Fu et al. [40] found in an integrated vertical flow constructed wetland that an increase in the influent C/N ratio could provide more electron donors for denitrification, and when the influent C/N was greater than 2, the NH_4^+-N removal was above 96%, TN removal was 55-90%, and TOC removal was above 95%.

Anaerobic Ammonia Oxidation

Anaerobic ammonia oxidation is the process by which anaerobic ammonia-oxidizing bacteria use ammonia as an electron donor and nitrite as an electron acceptor to produce nitrogen gas under anaerobic conditions [41]. The total anaerobic ammonia oxidation reaction is shown in equation (4) [42]:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (4)$$

Anaerobic ammonia oxidation is greatly affected by various environmental and operating parameters, and the primary factors influencing the anaerobic ammonia oxidation procedure are pH, temperature, dissolved oxygen, C/N ratio, reactor configuration, and plants [43]. Therefore, in practical applications, the influence of various factors on anaerobic ammonia oxidation bacteria should be considered. Due to the cell density-dependent phenomenon of anaerobic ammonia bacteria, a higher abundance of anaerobic ammonia bacteria can better maintain the activity and operational stability of anaerobic ammonia oxidation, while *Proteobacteria*, *Firmicutes*, *Planctomycetes*, *Bacteroidetes*, and *Chloroflexi* are the dominant phyla in the process of anaerobic ammonia oxidation [44, 45]. However, denitrification plays a complex part in the growing of anaerobic ammonia oxidation bacteria, which can either promote the growth of anaerobic ammonia oxidation bacteria by converting nitrate to nitrite or compete with anaerobic ammonia oxidation bacteria for nitrite and inhibit the growth of anaerobic ammonia oxidation bacteria; overall, it may favor the growing of anaerobic ammonia oxidation bacteria [46]. Compared with traditional nitrification and denitrification, anaerobic ammonia oxidation has low energy consumption and no secondary pollution and is one of the main ways of nitrogen removal. Gao et al. [47] reported that in wetland ecosystems, the contribution of anaerobic ammonia oxidation to total nitrogen can be up to 41%.

Strengthen Measures

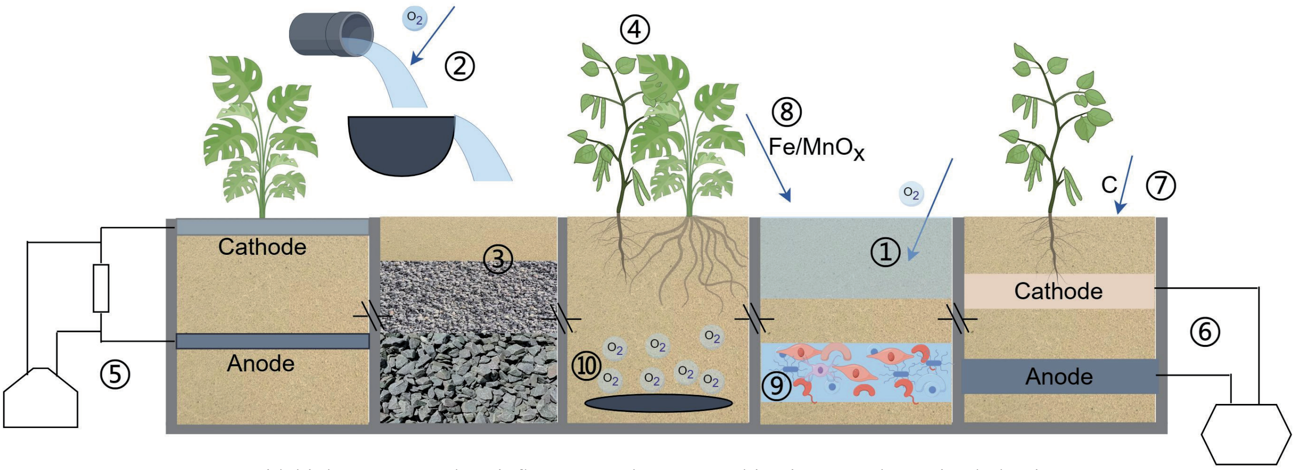
When traditionally constructed wetlands cannot meet the requirements of nitrogen removal, corresponding measures can be taken to increase the nitrogen removal capacity of constructed wetlands. Various measures for improving nitrogen removal of constructed wetlands are introduced in detail below, as shown in Fig. 1.

Configuration Optimization Measures

Improving the Water Intake Mode

The influent mode can affect the nitrification by affecting the concentration of dissolved oxygen, such as tidal influent and drop influent, which can increase the concentration of dissolved oxygen, thus strengthening the nitrification.

Tidal operation is the use of the suction generated in the alternating process of water inlet and drainage to absorb oxygen in the atmosphere in the constructed



1. Tidal inlet; 2. Water drop influent; 3. Substrate combinations; 4. Plant mixed planting;
5. Constructed wetlands coupled with microbial fuel cells; 6. Constructed wetland coupled biofilm electrode reactor;
7. Add carbon source; 8. Add Fe/MnOx; 9. Immobilized microorganism; 10. Aeration.

Fig. 1. Various enhanced nitrogen removal measures in constructed wetlands.

wetland, and the operation cycle is "influent-flood-effluent-drain" [48]. In the water inlet stage, the constructed wetland is gradually flooded, and the air in the substrate is continually depleted. During the drainage phase, the air re-enters the constructed wetland, and the substrate is re-oxygenated, which improves the efficiency of oxygen transport [49]. As wastewater and air circulate, oxygen supply and consumption in the wetland are greatly improved, and nitrification and denitrification are gradually intensified, which is conducive to maximizing TN removal efficiency [50]. Thus, wastewater can act as a timed air pump, providing alternate aerobic and anaerobic environments for nitrification and denitrification. Tidal influent allows maximum wastewater contact with the biofilm, enhancing oxygen transfer by drawing air into the wetland substrate [51]. A study claimed that the reoxygenation capacity of tidal stream-constructed wetlands was 350 $gm^{-2}d^{-1}$, which was much higher than that of traditional-constructed wetlands (<100 $gm^{-2}d^{-1}$) [52]. Li et al. [53] used tidal influent to treat low-pollution synthetic sewage. It was shown that the removal efficiency of TN and NO_3^--N in tidal influent-constructed wetland was higher than that of intermittently aerated constructed wetland, with the removal rate of NH_4^+-N above 90% and COD above 87%. Compared to aeration, tidal flow wetlands can save half of the energy and area for treating the same volume of wastewater [54]. However, tidal operation is more complex and requires skilled operation mode in order to give full play to the best nitrogen removal efficiency of constructed wetlands.

The principle of falling into the water is that the sewage is falling from a high place; when the water is falling into the air, the oxygen in the air is in full contact with the sewage, which can effectively promote the process of transferring the air-oxygen to the water. In addition, the water falling from a high place can produce waves on the surface of the water, and at the same time, it is conducive to the diffusion of air [55]. Therefore, the concentration of dissolved oxygen in wastewater increases with the increase of the drop. The research by Li et al. [56] showed that the greater the height difference, the greater the reoxygenation amount, and the best reoxygenation effect was achieved when the drop height reached 2.5 m. Zheng et al. [57] also found that the dissolved oxygen concentration increased by 5.20±0.17 mg/L when the height difference was 50 cm, and the removal of COD and NH_4^+-N by treating rural wastewater with falling water reoxygenation and tidal operation was 99.50±0.21% and 87.16%±1.76%, respectively. Falling into the water can take advantage of the potential energy present in the terrain difference to reduce the output of energy or use a pump to raise the water level to a higher level, but it requires a portion of the energy consumption [58]. In addition, the oxygenation efficiency of multi-stage drop inlet water is higher. Zou et al. [59] used a vertical flow-constructed wetland to treat rural sewage and showed that, compared with the direct fall-off water, the multi-stage, two-layer fall-off water increased the dissolved oxygen per meter by 2-6 mg/L.

Optimization of Substrate Combinations

Complementary effects and synergistic effects resulting from the combination of substrates can increase the efficiency of nitrogen removal. For instance, the combination of manganese ore and walnut shell, whose shell is rich in organic matter, can improve denitrification, and manganese ore can provide a better redox potential environment. The combination of both can simultaneously strengthen the nitrification and denitrification processes [60]. In recent years, the application of pyrite in constructed wetlands has become more and more common, and the efficient purification of wastewater has been realized. The pyrite surface has many active sites and provides nutrients for allochthonous Fe (III)-reducing microorganisms. Pyrite-based constructed wetland systems can promote denitrification using sulfur and ferrous iron as electron donors for denitrification [61]. Uniform mixing of pyrite with high porosity substrates (e.g., activated carbon, volcanic stone, etc.) can enhance microorganisms and their effective adhesion to the contact area of pyrite, promote the growth of autotrophic and heterotrophic denitrifying bacteria in the constructed wetland system, and ultimately improve the denitrification capacity of the constructed wetland [62]. Jiang et al. [63] selected pyrite, alkali-modified rice husk, quartz sand, and gravel as substrates for vertical flow-constructed wetlands and found that pyrite could enhance autotrophic denitrification and alkali-modified rice husk could enhance heterotrophic denitrification, and the combination of the two could effectively remove nitrogen from low C/N wastewater.

The combination of substrates in the appropriate proportions can provide a favorable environment for the growth and multiplication of microorganisms and plants. The addition of special porous substrates and organic substrates can provide carbon sources for denitrification and promote the removal of nitrogen. When the substrate is combined, different substrates have different abilities in different parts. When the removal ability of the upper substrate to nitrogen is gradually consumed, the removal site of nitrogen will move to the lower layer, coupled with the different properties and nitrogen removal capacity of each layer of substrate, so that the removal mechanism of nitrogen under the synergy of substrate, plants, and microorganisms is different [64]. When selecting substrate combinations, choosing substrate combinations with different particle sizes can alter the dissolution oxygen distribution in constructed wetlands. As Fig. 2, a and b show, a vertical flow-constructed wetland and a horizontal flow-constructed wetland using a combination of sand, fine gravel, and coarse gravel, with the three substrates filled to different depths. According to the porosity of the substrate and particle size, the combination of filling from small to large can form an upper aerobic, middle anaerobic, and lower hypoxic environment, produce a rich microbial community structure, and remove different forms of nitrogen by aerobic nitrification, aerobic denitrification, anaerobic denitrification, and anaerobic ammonia oxidation. On the contrary, according to the matrix porosity and particle size, from large to small order combination filling can improve the overall dissolved oxygen level, and form a high dissolved oxygen environment in the upper layer, conducive to the survival of aerobic microorganisms and various aerobic animals, produce different dissolved oxygen gradients, and promote nitrogen removal [65]. Li et al. [66] found in the subsurface flow-constructed wetland that when treating high-intensity nitrogenous wastewater, the substrate combination sequence has a great impact on the removal of pollutants, and the removal rates of COD, NH_4^+-N, and TN can be as high as 97%, 95%, and 94%, respectively, by combining gravel, slag, and wood dust in sequence.

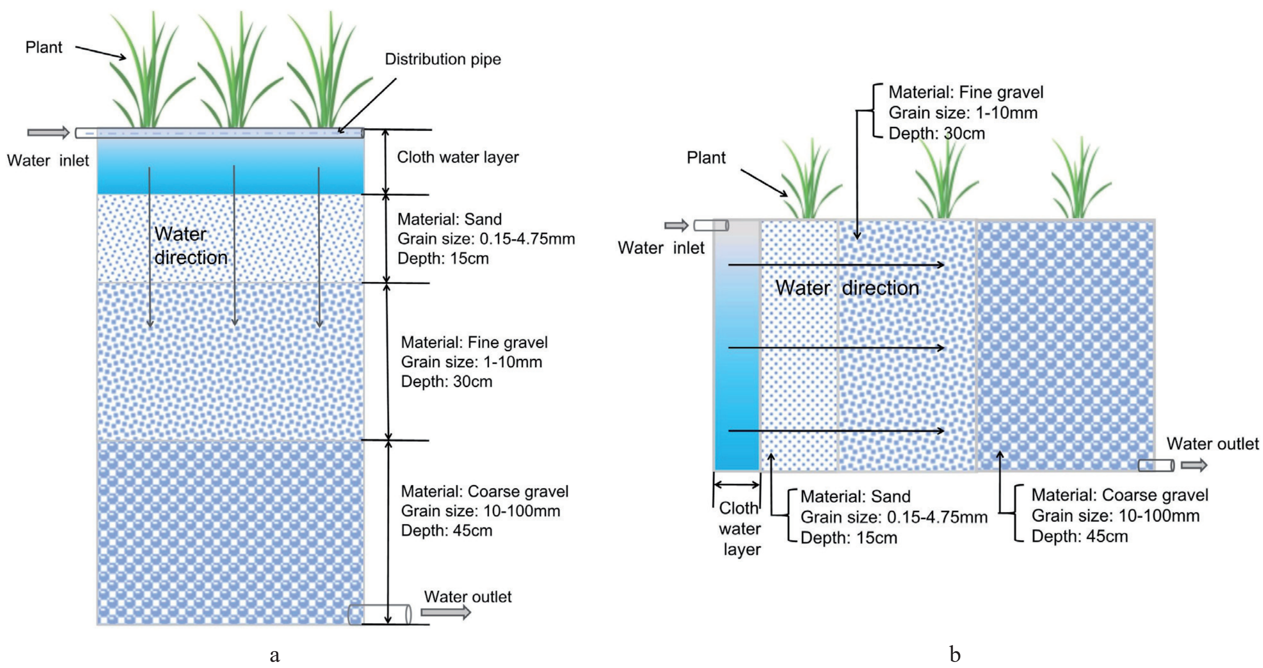


Fig. 2. Differences in the filling of the different substrate combinations.

Optimize the Plant Species Configuration

Plant species configuration is an essential factor influencing the nitrogen removal efficacy of constructed wetlands. Plants with well-developed roots can provide a larger adhesion surface for microorganisms, thus enhancing rhizosphere purification capacity and improving wetland performance [67]. However, different plants can absorb different nutrients, and the degree of absorption is also different. Therefore, constructed wetlands with monoculture and mixed planting have different removal efficiency of pollutants. Selecting the appropriate mix of plants can have complementary effects, but inappropriate selection can produce competition between species, which can affect the efficiency of nitrogen removal from constructed wetlands.

It has been found that increased plant abundance can improve biomass yield and nitrogen uptake capacity [68]. Therefore, mixed planting is one of the ways to increase the nitrogen removal effect of constructed wetlands. Tu et al. [69] showed that when free surface flow constructed wetland treated domestic sewage, the pollutant removal effect of mixed planting was significantly higher than that of single planting.

Due to plant species competition and stubble growth, monoculture and mixed planted constructed wetlands have significant differences in plant growth, biome structure, and nutrient removal. In addition, monoculture is also thought to be more susceptible to the death of plants caused by predation or disease; compared to monoculture, mixed cultivation is more resilient [70]. Relative to the monoculture wetlands, the presence of different plants in mixed-planting wetlands may provide more efficient root structures and diverse microbial habitats [71]. Therefore, the selection of suitable plant mixture planting has a good spatiotemporal compensation effect on plant growth and microbial community development, which can boost the nitrogen removal effect of wetlands [72]. As shown in Fig. 3, a and b are shown in the mixed planting and monoculture, respectively, in which the root structure of the mixed planting is richer, the nitrogen uptake is greater, and the oxygen secretion and growth status are also different. Wang et al. [73] found that mixed plant culture improved the diversity and nutrient removal efficiency of constructed wetlands, and the removal rates of COD and TN were 74.7% and 94.2%, respectively, when mixed cultivation was used with *Iris pseudacorus*, *Iris sibirica*, *Juncus effusus*, and *Hydrocotyle vulgaris*.

However, in mixed-planted wetlands, plant competition, such as competition for space, light, and nutrients, can lead to the dominance or inhibition of certain species, which can have an influence on the purification of wastewater in constructed wetlands [74]. Zhang et al. [75] found that in vertical flow-constructed wetlands, due to competition, the removal of pollutants by mixed planting is not greater than that by single planting, but mixed planting can provide other conditions conducive to plant growth, such as dissolved oxygen and pH. Therefore, when selecting plants, the competition between plant species, the growth, reproduction, regeneration, adaptation, and resource utilization capacity of plants should be fully considered, and the appropriate plant planting density should be allocated to reduce the negative impact of competition. In addition, species of the same family or genus should be avoided, as there may be additional competition between them [76].

Novel-Constructed Wetland Coupling Process

The combined use of constructed wetlands with other processes can maximize the performance of a wastewater treatment system, overcome the shortcomings of a single treatment unit, boost the effectiveness of wastewater treatment, and have a better removal efficiency of nitrogen and other pollutants [77]. In order to improve the wastewater treatment capacity of constructed wetlands, several novel wastewater treatment technologies (for example, microbial fuel cells, and biofilm electrode reactors) coupled with constructed wetlands have been proposed. Tables 1 and 2 summarize in detail the nitrogen removal effects of the two coupled processes.

Constructed wetlands coupled with microbial fuel cells are a clean energy technology for wastewater treatment and biopower generation, which can efficiently remove nitrogen from domestic wastewater [78]. Microbial fuel cells typically consist of a reaction chamber, electrodes, and external circuitry, with electrochemically active bacteria being the main biocatalysts in power generation. Electrode materials and microorganisms determine the number of electrons in the circuit and the rate of transport. Electricity production and wastewater treatment occur primarily at the anode, so microbial diversity is greater at the anode than at the cathode. The addition of conductive materials can extend the range of electroactivity of the anode to adjacent filler materials and contribute to the formation of electroactive bacterial communities and biofilms [79]. It has been found that the main dominant phyla in the treatment of synthetic wastewater by microbial fuel cells in constructed wetlands are *Proteobacteria, Actinobacteria, Chloroflexi,* and *Bacteroidetes*, with relative abundances of 76.08%, 2.02%, 8.33%, and 2.89%, respectively, and most of the electroactive bacteria belong to *Proteobacteria* [80]. In the anaerobic zone, electrochemically active bacteria generate electrons and transport them to the anode. The anode acts as an interim electron acceptor, and then the electrons are transported to the cathode through an external circuit. The aerobic region receives electrons and protons from the anaerobic region, and the final electron acceptor, such as O_2, receives electrons from the cathode [81]. Externally supplied anodes or conducting materials can act as artificial electron acceptors in constructed wetland anaerobic zones, facilitating the oxidation of pollutants

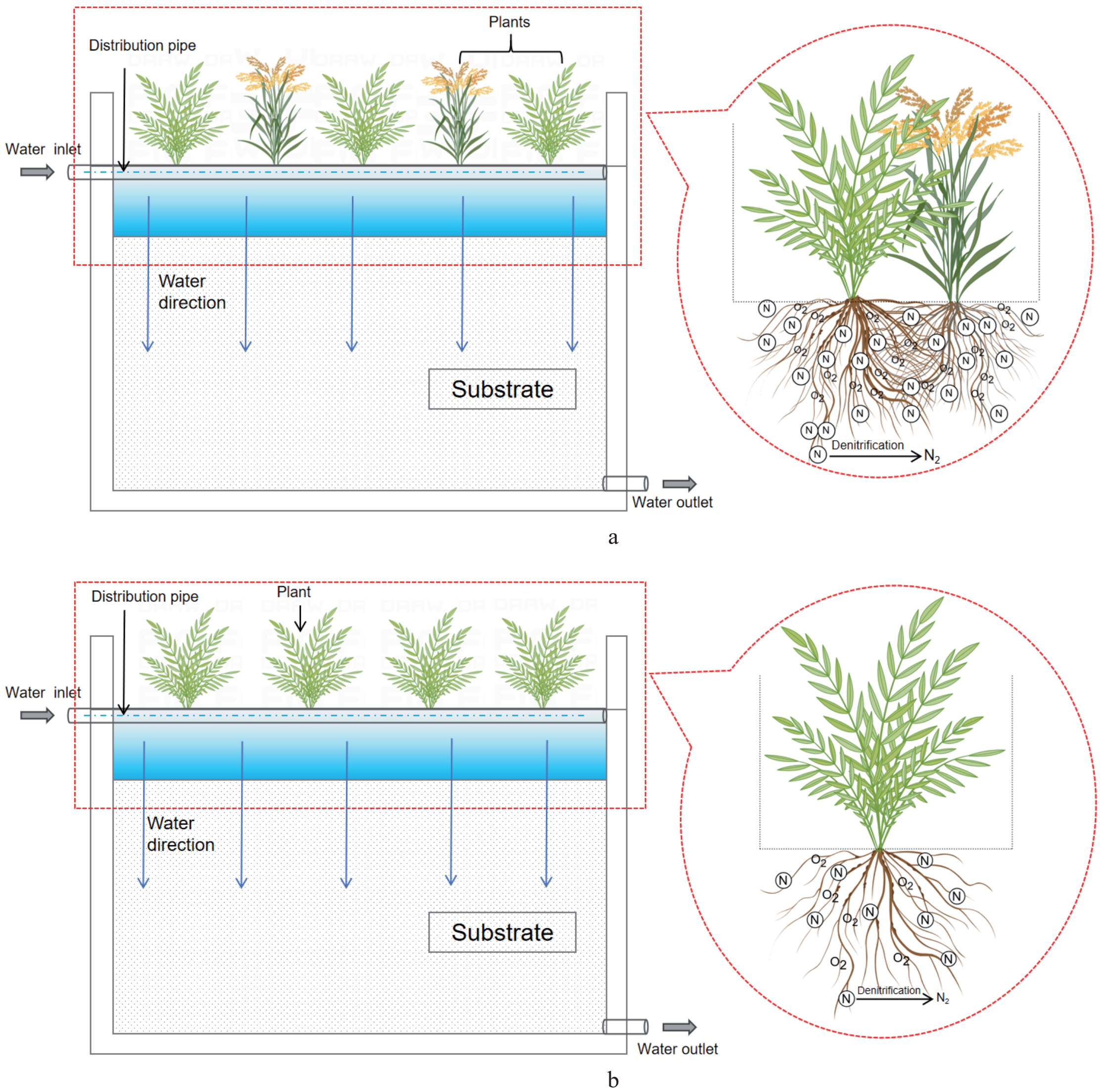


Fig. 3. Mixed and monoculture of plants.

such as NH_4^+-N and organic matter. Another aspect is that externally supplied cathodes can act as electron donors, reducing contaminants such as NO_2^--N and NO_3^--N, and electroactive microorganisms remove TN more effectively than ordinary microorganisms even at low population numbers [82]. According to Araneda et al. [83], when the microbial fuel cell combination system of a constructed wetland treats gray water, the removal rate of sCOD and nitrate can reach 91.7%±5.1%, 86.5±7.1%, respectively, and the maximum power density can reach 719.57±67.67 mWm^{-3}, which can effectively treat ash water and energy recovery.

The combination of a biofilm electrode reactor and constructed wetland can form an efficient heterotrophic/autotrophic denitrification system to promote denitrification. Nitrifying bacteria can use carbon dioxide, carbonate, and bicarbonate as carbon sources to oxidize ammonia nitrogen to nitrate and do not need to add additional organic carbon sources. Hydrogen created by water electrolysis can be utilized as an electron donor for the autotrophic denitrification process, and the coupling system can strengthen the removal of TN and NO_3^--N, and the enhanced removal rate can reach 23.26%, respectively, and 24.20% [84, 85]. In a coupled system of biofilm electrode reactors and constructed wetlands, the appropriate current intensity can provide the right amount of H_2 to facilitate the rate of autotrophic denitrification. The anode electrode material (graphite) can produce an inorganic carbon source (CO_2) to act as a pH buffer and also provide an inorganic carbon source for denitrifying bacteria [86, 87]. Wang et al. [88] found that the biofilm electrode reactor-coupled system with constructed wetland could promote about 20.8% denitrification when I=10 mA, pH=7.5. Furthermore, the growth of autotrophic denitrifying bacteria can accelerate the action of the microelectric field, thereby improving the removal rate of NH_3-N and NO_3-N by a biofilm electrode reactor-coupled system with a constructed wetland.

Improve Operating Conditions

Add Carbon Source

The denitrification process requires sufficient organic carbon sources to effectively remove nitrate [89]. At present, the main type of carbon source added to the constructed wetland is agricultural waste. Such as corn cobs, palmetto bark, and wood chips, which are easily accessible, low-cost, and have a stable release of carbon sources, can enhance denitrification and promote nitrogen removal [90, 91]. The addition of plant carbon sources can provide more attachment sites for nitrifying bacteria and increase the number of *Hao* and *NxrAB* enzymes in the wetland (42.05%, 29.99%), as well as the number of *NirK*, *NorB/NorC*, and *NosZ* genes (28.69%, 20.82%, and 29.22%) [92]. Zhang et al. [93] found that the cumulative carbon release from plants could reach 119.78-172.84 mg/g when using the vertical subsurface flow constructed wetland to treat low C/N wastewater, and the addition of a plant carbon source led to an increase in TN removal efficiency by 49.62%-61.3%.

Organic matter liberated from plant carbon sources mainly consists of tryptophans, xanthohumic acids, and humic acids [94], which have a significant organic carbon release effect. Humic acid and fulvic acids contain a variety of functional groups, such as carboxyl, phenolic, and alcohol groups [95, 96]. Different microbial genera can selectively degrade and use these functional groups. For example, *Pseudomonas* and *Thauera* have outstanding degradation capability of phenolic groups in humic acid-soluble organic compounds and serve as substrates for growth and metabolism, thus effectively promoting denitrification reactions [97, 98]. *Dechloromonas* has good degradation capability to carboxyl and alcohol groups in humic acid [99]. Tao et al. [100] found that corn cobs can provide a large area for microorganisms to attach and metabolize, and at a dosage of 70 $mg\cdot m^{-2}$, the average removal of TN, NH_4^+-N, and NO_3^--N was 99.22%, 99.25%, and 99.54%, respectively, when an up-flow constructed wetland was used to treat secondary effluent from a wastewater plant, and the corn cobs released the equivalent of 2648.17 mg of COD organic matter. In addition, the addition of glucose or sodium acetate can also regulate the microbial community, accelerate the denitrification rate, and improve the nitrogen removal rate [101]. In the case of low C/N, although the addition of a carbon source can effectively improve the removal rate of nitrogen, it may also produce secondary pollution, which can be collected uniformly for harmless or resource treatment and utilization to avoid secondary pollution [102].

Improve Redox Conditions

Redox potential is an important factor affecting microbial communities; different redox potential gradients are favorable for redox processes; aerobic microorganisms tend to have high redox potential; and anaerobic microorganisms tend to have low redox potential [103]. Therefore, the redox environment is particularly important for nitrogen removal in constructed wetlands [104]. Sulfur can act as an electron donor or electron acceptor in various dissimilatory microbial reactions, and the sulfur flowing into the constructed wetland usually exists in the form of sulfate in the oxidizing environment and sulfide in the reducing environment, as in equations (5) and (6) below [105]. Sulfur-driven autotrophic denitrification can use inorganic electron donors rather than organic electron donors [106].

$$5HS^- + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O \quad (5)$$

$$3HS^- + 8NO_2^- + 5H^+ \rightarrow 3SO_4^{2-} + 4N_2 + 4H_2O \quad (6)$$

The addition of iron and manganese oxides can facilitate the nitrogen removal process. Iron can be an electron donor for the denitrification process, maintaining reducing and anoxic conditions for nitrogen removal, and dissolved iron ions can also facilitate bacterial growth [107]. Iron is also the active center of cytochrome and most NOx reductase enzymes and conductive materials such as iron can increase the rate of electron transfer and effectively improve the removal efficiency of pollutants [108, 109]. Manganese oxides have good oxidation capacity for NH_4^+-N; Mn (II) can provide electrons for the ammonification of NH_4^+-N and the reduction of NO_3^--N resulting from the oxidation reaction. Due to the redox cycle of Mn (II) ions, the created Mn (III) or Mn (IV) oxides can furthermore oxidize NH_4^+-N. Therefore, strengthening the redox cycle of manganese can promote nitrogen removal [110]. Recently, researchers have proposed a process called Mnammox, which refers to the process by which manganese dioxide acts as an electron acceptor to mediate the oxidation of NH_4^+-N to N_2, NO_3^--N, or NO_2^--N [111], reaction equations as in (7)-(9) [112]. This new approach is of great significance for nitrogen removal, but there are few studies on it [113].

$$4MnO_2 + NH_4^+ + 6H^+ \rightarrow 4Mn^{2+} + NO_3^- + 5H_2O \quad (7)$$

$$3MnO_2 + NH_4^+ + 4H^+ \rightarrow 3Mn^{2+} + NO_2^- + 4H_2O \quad (8)$$

$$3MnO_2 + 2NH_4^+ + 4H^+ \rightarrow 3Mn^{2+} + N_2 + 6H_2O \quad (9)$$

In addition, the bioelectrochemical system combined with the constructed wetland helps adjust the aerobic and anaerobic areas of redox reaction and electron flow balance. The system allows electrons from the anode to the cathode without resistance movement, improving the electron transfer efficiency. In the defect area, supplement electron donors and electron acceptors adjust the surrounding redox reactions, thus affecting the activity of microorganisms [114].

Microbial Enhancement Technology

Microbial enhancement technology refers to the addition of microorganisms, nutrients, and substrates with specific functions to constructed wetlands and the inoculation of natural or genetically modified microorganisms to enhance the efficacy of water purification in constructed wetlands [115, 116]. Microbial enhanced nitrogen removal, which can be achieved by directly adding microbial bacterial agents with nitrogen removal function. For example, the addition of heterotrophic nitrification-aerobic denitrifying bacteria to tidal flow-constructed wetlands can improve nitrification and denitrification-related genes (such as; *nirS*, *amABC*, *napA*, and *hao* genes). The addition of heterotrophic nitrification-aerobic denitrifying bacteria, which enhances the removal of NO_3^--N by the denitrification pathway by increasing the *napA* and *nirS* genes, can also stimulate the *gltD* and *nirA* genes of the nitrogen assimilation process, which may lead to direct removal of NH_4^+-N [117]. Chen et al. [118] added denitrifying polyphosphate bacteria liquid to the subsurface flow constructed wetland, and the study found that, in the hypoxic environment, the denitrifying phosphorus-accumulating bacteria can use the polyhydroxyalkanoates in the body as the electron donor through nitrate reductase and the nitrate as the electron acceptor to provide energy for its growth. At the same time, the nitrate is finally reduced to nitrogen for removal through denitrification. It not only saves the carbon source, but also improves the nitrogen removal efficiency of the subsurface flow-constructed wetland in treating the tailwater of the sewage plant.

The dosing method can also be through immobilized microbial dosing, which is a way to fix the nitrogen removal bacteria on a suitable carrier to ensure their activity and make the nitrogen removal bacteria grow rapidly and multiply in large quantities under suitable conditions. Wang et al. [119] used a polyvinyl alcohol immobilized nitrification unit to treat ammonia-rich wastewater, and the immobilized nitrification unit could promote nitrification and improve ammonia nitrogen removal. Biochar is an excellent bacterial immobilization vector, and biochar can promote the expression levels of denitrification functional genes (*napA* and *nirK*) and electron-transferring genes (*napB* and *napC*) related to denitrification [120]. Zhao et al. [121] also found that biochar bacterial agents can substantially increase the abundance of genes associated with nitrogen removal (*drsA*, *drsB*, *nirK,* and *nirS*), thus improving the nitrogen removal efficiency of tidal flow-constructed wetland treatment of saline wastewater.

To ensure the nitrogen removal efficacy of constructed wetlands under certain extreme environments (such as low temperature and high salt), nitrogen removal microorganisms isolated and screened from the environment with the function of low temperature or salt tolerance can be added [122]. Low-temperature microorganisms can still maintain high activity in low-temperature environments, so the water purification efficiency and stability of wetland systems can be ensured. Zhao et al. [123] added compound microbial inoculants to the subsurface flow-constructed wetland to treat campus wastewater under low-temperature conditions, and the study showed that the removal rate of ammonia nitrogen and total nitrogen was increased by microbial inoculation, and the soil microbial community structure and nitrogen-related bacterial species balance were changed, and a new bacterial community balance was created in the original place. Combined with the growth characteristics of low-temperature microorganisms in low-temperature environments, it can be widely used for wastewater treatment in constructed wetlands in cold areas.

The addition of exogenous microorganisms can improve plant performance by enhancing nutrient utilization and decreasing plant stress responses to contaminants, and it can also facilitate plant uptake of contaminants by producing solubilizers or facilitate rhizosphere degradation of organic contaminants by secreting biosurfactants [124, 125]. Lingua et al. [126] inoculated arbuscular mycorrhizal fungi into reeds, which improved nitrate removal efficiency and promoted the growth of plant roots and stems, resulting in larger plants and more developed root systems.

Aeration

Aeration can be divided into continuous aeration and intermittent aeration. Although continuous aeration can significantly raise dissolved oxygen levels, it consumes too much energy. When intermittent aeration is used, dissolved oxygen levels rise as aeration progresses, and aerobic microorganisms use oxygen for the removal of organic matter, which is favorable for nitrification, when the aeration stops, the dissolved oxygen is gradually consumed by microorganisms, and the concentration decreases, which is conducive to the denitrification process. Intermittent aeration makes the dissolved oxygen alternately rise and fall, thus forming an alternating aerobic and anaerobic environment [127]. However, there were significant differences between aerated and non-aerated wetlands. Some studies showed that the nitrification intensity and denitrification intensification of the aerated wetland were 5.3 times and 1.3 times higher than those of the control wetland, and the copy density of the *nosZ* gene in the aeration wetland was higher, about 3.8 times that of the control wetland [128]. In addition, the aeration volume, aeration intensity, and frequency also influence nitrification and denitrification by generating different dissolved oxygen concentrations [129]. The aeration location can influence the spatial distribution of dissolved oxygen by affecting the diffusive path of oxygen [130]. Liu et al. [131] studied the treatment of polluted urban river water in horizontal subsurface flow-constructed wetlands with no aeration, continuous aeration, and intermittent aeration and demonstrated that the removal effectiveness of COD and

Table 1. Nitrogen removal efficiency and power generation in the microbial fuel cell system of constructed wetland.

Reactor volume and HRT	Type of wastewater	Substrate	Plant	Anode material	Cathode material	Nitrogen removal rate	Maximum power density	References
Total volume 15.7 L,working volume 5.5 L, HRT 72 h	Antibiotic wastewater	Coke, gravel, cobblestone	*Calamus*	Carbon fiber felt	Carbon fiber felt	79.89% for NH_4^+-N, 82.67% for NO_3^- -N,70.29% for TN	1.33 mW/m^2	[182]
Total volume 39 L, working volume 13.5 L, HRT 48 h	Low carbon wastewater	Sand, gravel	*Calamus*	Graphite plates	Graphite plates	97.6% for TN	12.5 mW/m^2	[183]
HRT is 4 d	Synthetic wastewater	Sand, zeolite, volcanic cinders, gravel	*Typha latifolia* L.	Graphite	Magnesium	93.2±7.01% for NH_4^+, 81.1±19.8% for NO_3^-	15.1 mw/m^2	[184]
HRT is 1 d	Synthetic wastewater	Gravel	*Typha latifolia*	Activated carbon	Activated carbon	96% for NH_4^+, 46% for NO_3^--N	93 mW/m^3	[185]
Total volume 39 L, working volume approx 13.5 L, HRT 48 h	Carbon constraint wastewater	Gravel, sand	*E.crassipes*	Graphite plates	Graphite plates	TN removal >85% when C/N >2.6	1.17 mW/m^2	[186]
Total volume 11.62L, working volume 4.08 L, HRT 24h.	Domestic sewage	Cobblestone, granular activated carbon, alabaster, quartz sand	*Canna*	Granular activated carbon	Carbon felt	82.72±10.2 for NH_4^+-N, 90±1.27% for NO_3^--N	107.54 mW/m^3	[187]
The total volume was 7.95 L, of which the liquid volume was 3.75 L. The HRT was 6 h	Simulated sewage	Pyrite, quartz sand	*Canna*	Carbon fiber felt	Carbon fiber felt	70.1% for NO_3^--N, 63.2% for TIN	6.74 mW/m^2	[188]

Table 2. Nitrogen removal efficiency in the coupled system of biofilm electrode reactor and constructed wetland.

Type of wastewater	Substrate	Plant	HRT	Electric current	Anode material	Cathode material	Nitrogen removal effect	References
Synthetic wastewater with different COD/N	Gravel	*Canna*	24 h	15 mA	Granular active carbon	Granular active carbon	91.3±7.2% for NO_3^--N,68.8±7.9% for TN	[189]
Low C/N wastewater	Quartz sand	*Canna*	12 h	15 mA	Carbon rod	Foamed nicke	63.03% for NO_3-N,98.11% for TN	[84]
Synthetic domestic sewage	Quartz sand	*Canna*	2 d	10 mA	Graphite robs	Carbon fiber felt	89.6% for NO_3-N,39.6% for NH_3-N,63.6% for TIN	[88]
Nitrate-rich wastewater	Gravel	*Canna*		15 mA	Granular active carbon	Graphite felt	NO_3^--N for 78.92±3.12%	[190]
Synthetic wastewater	Quartz sand	*Arundo donax*	24 h		Graphite felt	Graphite felt	Applied voltage did not enhance nitrogen removal	[191]

Table 3. The main mechanisms of nitrogen removal by microorganisms.

Nitrogen removal mechanisms	Reaction equation	Main microorganisms	Main functional genes
Ammonification reaction [192]	$RCH(NH_2)COOH+O_2 \rightarrow COOH+CO_2+NH_3$	*Bacillus, Proteus, Serratia, Micrococcus* [193], *Brevundimonas diminuta, Alcaligenes faecalis, Enterobacter aerogenes* [194]	*NrfA* [195]
Total nitrification reaction [196]	$NH_4^+ +2O_2 \rightarrow NO_3^- +2H^+ +H_2O$	*Candidatus_ Nitrosopumilus, Comammox, Nitrosomonas, Nitrospira*	*AmoCAB, Hao, NxrAB* [197]
Denitrification reaction [198]	$6(CH_2O)+4NO_3^- \rightarrow 6CO_2+2N_2+6H_2O$	*Bacillus, Halomonas, Mycobacterium, Nocardioides, Sulfurovum, Truepera, Pontibacter*	*Nar-GHI, NirK/NirS, norBC, nosZ* [197]

NH_4^+-N was improved by aeration, and the intermittent aeration method had the best effect, with a removal rate of up to 91.9% for TN. Therefore, intermittent aeration is one of the effective methods to increase the nitrogen removal effect.

Nitrogen Removal Mechanisms

The mechanisms of nitrogen removal in constructed wetlands mainly include ammonification, nitrification, and denitrification by microorganisms, as well as substrate adsorption and plant uptake [132, 133]. Regarding microbial nitrogen removal as in Table 3.

Microbial Degradation

Ammonification

Ammonification is the process by which ammoniated bacteria convert organic nitrogen to ammonia nitrogen [134]. Ammonia nitrogen includes ammonium ions (NH_4^+) and free ammonia (NH_3) and mainly comes from nitrogen-containing organic matter in domestic wastewater [135]. The ammonification rate is related to physicochemical parameters. The ammonification ability of organic nitrogen is different under different parameter conditions; for instance, for temperature and pH values, the optimal reaction conditions are 40-60 °C and 6.5-8.5, respectively [136], but the microbial populations involved in the ammonification process are not very different [137]. Ammonification is the first step in the removal of organic nitrogen, which is immediately linked to the follow-up nitrogen removal process and plays a significant part in the whole nitrogen removal process [138].

Nitrification and Denitrification

The process of nitrification involves the oxidation of ammonia nitrogen to nitrite nitrogen by ammonia-oxidizing bacteria in aerobic conditions. Afterward, nitrite nitrogen undergoes oxidation to nitrate nitrogen by nitrite-oxidizing bacteria. Denitrification is the use of nitrification-produced nitrate nitrogen, and under anaerobic conditions, denitrifying bacteria convert nitrate nitrogen into nitrogen [139]. Bacteria can complete the nitrogen cycle through a variety of pathways [140]. However, when oxygen transfer is restricted, nitrification is inhibited, and when an aerobic environment is generated, it is conducive to the nitrification reaction [141]. Denitrification is normally regarded as the primary and permanent process for nitrate removal, and organic carbon is a major factor limiting nitrate removal; organic carbon is an electron donor in denitrification, and its concentration affects the growth and activity of denitrifying bacteria, so insufficient organic carbon affects the effect of denitrification [142, 143]. In addition, the dissolved oxygen concentration has a large effect on the denitrification process, and the high oxygen conversion capacity will make the denitrification reaction difficult to occur [144].

Substrate Adsorption

The adsorption of the substrate can be divided into physical adsorption and chemical adsorption; physical adsorption is primarily caused by electrostatic and capillary forces on the substrate surface, and chemical adsorption is generated due to the action of chemical bonds. Compared to physical adsorption, chemisorption is more durable. Different substrates have different adsorption capacities. For example, the theoretical adsorption amount of ammonia nitrogen by volcanic rock, ceramic sand, biochar, and zeolite is 1.7 mg/g, 1.62 mg/g, 1.353 mg/g, and 1.350 mg/g, respectively, which are suitable for the adsorption of ammonia nitrogen in constructed wetlands [145]. Zheng et al. [146] found in a tidal flow-constructed wetland utilizing macroporous zeolite as a substrate that the average removal of NH_4^+-N was up to 94.09% and that NH_4^+-N was mainly removed by adsorption in the early stage. Furthermore, the mechanism and efficiency of nitrogen removal vary among substrates, and in addition to adsorption, nitrogen can be removed by ion exchange and filtration [147].

Plant Uptake

The main forms of nitrogen that plants take up are nitrate and ammonia, as well as smaller organic nitrogen compounds such as urea and amino acids. The absorbed nitrogen can be used for its growth and converted into part of its tissue through assimilation [148]. When plants grow to maturity and plant metabolism slows down and begins to die, nitrogen assimilation in constructed wetlands reaches the highest level, and finally, the plants are harvested so that pollutants are removed from the constructed wetland [149]. Different plants have different effects on nitrogen absorption. The absorption of TN by *M. aquaticum* was 1.89 $g \cdot m^{-2} \cdot d^{-1}$ [150]. The rate of nitrogen absorption by *M. elatinoides* was 1.24 $gm^{-2}d^{-1}$ [2]. The absorption rates of NH_4^+-N and NO_3^--N by *P. crispus* were 1.59 and 0.62 $mgNmin^{-1}gDW^{-1}$, respectively [151]. Zhang et al. [152] showed that plant uptake accounted for 29.3% of the total nitrogen removal in a surface flow-constructed wetland with *Iris pseudacorus* planting. However, the amount of nitrogen uptake by plants is affected by the season and temperature, such as in summer, when the temperature is suitable, the light is strong, and the photosynthetic rate is high, which is favorable for the growth of plants and the uptake of nitrogen; on the contrary, in winter, when the temperature is low, the light and photosynthetic rate are relatively weak, and the nitrogen removal effectiveness is not as good as in summer [153, 154].

Microbe-Substrate-Plant Coupling

There is a complex relationship between microorganisms, substrates, and plants in the constructed wetland, and they play different roles in the process of nitrogen removal, as shown in Fig. 4. However, nitrogen was removed mainly by microbial action, and therefore, the microbe-substrate-plant coupling was mainly reflected in the substrate-microbe and plant-microbe coupling.

Substrate-Microbe Coupling

The substrate can provide a carrier for the microbes to attach, support the development of the biofilm, and boost the removal of contaminants by providing electrons to the microbes [155]. For example, biochar, with its large surface area and porous structure, can provide rich attachment sites for microorganisms, creating a favorable environment for denitrifying bacteria to survive, and can release carbon sources to enhance denitrification. In addition, the high oxygen content and various electron-donor chemical structures (e.g., hydroxyl, carboxyl, and phenolic hydroxyl groups) in biochar can provide electrons to promote the activity of denitrifying bacteria [156, 157]. Biochar is a new multifunctional adsorption material extracted from waste biomass that has been widely used in sewage treatment in recent years and can be used as a new alternative material for constructed wetlands, which can remove pollutants more effectively than conventional substrates, modified biochar is also commonly used in sewage treatment of constructed wetlands [158]. The addition of biochar can enhance the diversity of wetland microbial communities and improve the performance of wastewater purification in constructed wetland ecosystems [159]. In addition, natural carbon source substrates such as wheat straw, apricot pits, and walnut shells can also improve the removal rate of total nitrogen by supplying a good environment for microorganisms to form biofilms and providing more electron donors for denitrification [160]. Inorganic substrates such as ceramics, gravel, and quartz sand have a large specific surface area and pore volume, which is also favorable for the survival

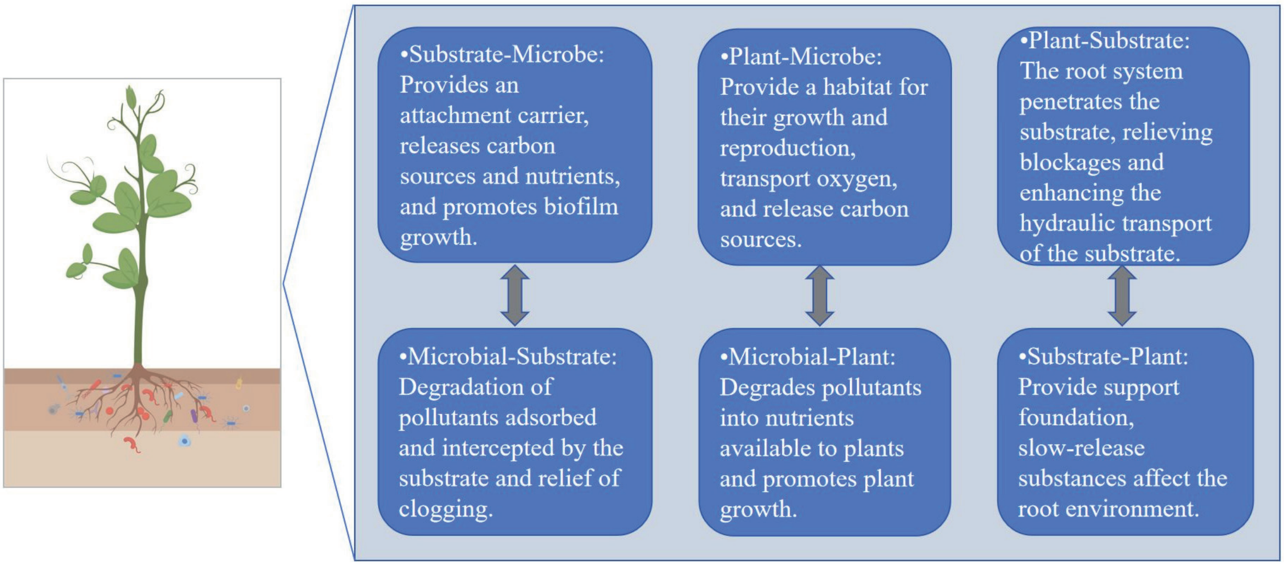


Fig. 4. Coupling between microorganisms, substrates and plants.

of microorganisms [161]. Wan et al. [162] used ceramic grains as the substrate to treat domestic wastewater. The findings indicated that ceramic grains can help increase the percentage of beneficial microorganisms (nitrogen, sulfur, and phosphorus) in the wastewater treatment process, stimulate key microorganisms, and improve the efficiency and abundance of nitrifying bacteria.

The nature of the substrate has a great impact on the microbial community structure, mainly reflected in the particle size, pore structure, and specific surface area of the substrate [19]. The smaller the substrate particle size, the larger the specific surface area per unit volume of substrate, the more microorganisms can be accommodated, and the better the purification efficiency of wastewater, but if the substrate particle size is too small, the porosity and hydraulic conductivity are low, and it is easy to block and affect the wastewater purification effect [163]. The larger the substrate particle size, the more oxygen enters the substrate pores, which is favorable for the growth of aerobic microorganisms, thereby enhancing the nitrification strength of the substrate. On the contrary, when the substrate particle size is small, the oxygen content in the substrate pore is less, which promotes the growth of anaerobic microorganisms and then strengthens the denitrification strength [20]. It has been found that the substrate with a larger pore volume or specific surface area generally has higher adsorption and removal performance for pollutants in constructed wetlands, and the porous structure of the substrate can improve the supply of dissolved oxygen, and promote the formation of biofilm and the propagation of microbial communities.

Significant differences in dissolved oxygen distribution and microbial community structure in different wetland depths [65]. In deep-constructed wetlands ($d > 0.6$ m), an alternating anaerobic-anoxic environment can be formed to create a favorable living environment for anaerobic ammonia-oxidizing bacteria. For the deeper bed, the oxygen transfer potential is low, and the anaerobic process will occupy a dominant position at the bottom of the bed [42]. When the concentration of nitrogen and organic matter is high, the constructed wetland can promote the heterotrophic nitrification of the upper aerobic zone and the denitrification and anaerobic ammonia oxidation processes in the lower anaerobic zone [136]. Chen et al. [164] found that when vertical flow-constructed wetlands were used to treat domestic sewage at a depth of 0-20 cm, the number of microorganisms was the largest and decreased with the increase of substrate depth, with ammonia-oxidizing bacteria and nitrifying bacteria primarily found at a depth of 0-40 cm and denitrifying bacteria primarily found at a depth of 60-80 cm.

Plant-Microbe Coupling

Plants ensure the stability of rhizosphere microorganisms, and their roots provide a good habitat environment for microorganisms [165]. Plants can form well-defined bacterial communities around the rhizosphere by inducing and stimulating the growth of specific bacterial communities [166], by influencing the distribution of microorganisms, thus affecting the nitrogen removal capability of constructed wetlands [167].

The rhizosphere of plants is a multifaceted and dynamic environment, and root exudates are good at attracting specific microorganisms. Plants can actively select specific key microbial species under spatiotemporal conditions, and the spatiotemporal dynamic changes of root exudates are accompanied by dynamic changes in the composition and function of rhizosphere microbial communities. As the rhizotrophic environment evolves, the composition of the microbial community will also generate corresponding dynamics [168]. Plant changes can lead to changes in sediment dissolved oxygen levels, nutrient availability, and organic matter content, all of which can affect microbial communities [169]. The root system of plants mainly affects the surrounding microorganisms through the role of oxygen delivery and secretion, and the root secretion can provide reducing carbon, nitrogen, and other nutrients for microorganisms, different plant root systems secrete different secretions, so there are differences in the types and numbers of microorganisms around the root system of different plants [170, 171]. For example, *Phragmites australis* can secrete glucose, sucrose, acetic acid, and succinic acid, and *Cyperus alternifolius* can secrete glucose, sucrose, malic acid, and malonic acid [172]. These can be utilized as a source of carbon for the denitrification process. Xu et al. [173] found that when using macrophytes to treat pretreated pig wastewater, root exudates had a positive effect on microorganisms, and the removal rate of ammonium nitrogen with root exudates treatment (90.4% and 69.3% on average) was significantly higher than that without root exudates treatment (87.3% and 62.0% on average).

In vertical-flow constructed wetlands, microorganisms in plant seedling roots preferentially utilize simple amino acids, while those in mature plant roots utilize more complex carbohydrates [174]. Prokaryotic and eukaryotic microorganisms are able to use organic pollutants as sources of carbon and energy, and organic pollutants can act as electron donors oxidized in aerobic and anaerobic environments, where various electron acceptors other than oxygen can achieve anaerobic respiration processes [175]. Plant litter can also be utilized as a solid carbon source to provide electrons for denitrification, which plays a role in mitigating carbon sources [176]. The growth and metabolism of microorganisms are also linked to pH; the optimal pH range for nitrification is 7.5-8.6, while for denitrification it is 7-8 [177]. However, studies have shown that plant roots can secrete hydrogen ions or hydroxide into the rhizosphere environment, such as hydrogen ions brought by organic acids secreted by some plants, thus playing a role in regulating the pH of constructed wetlands [178, 179].

The effect of plants on microorganisms is also reflected in different seasons. In winter, the light time is shorter, the temperature is lower, the enzyme activity in the plant is reduced, and the metabolic capacity of the plant is decreased. It had a significant effect on rhizosphere microbial diversity and significantly reduced denitrifying enzyme activity and functional microbial diversity [180]. Wang et al. [181] found in a research of subsurface flow-constructed wetland planted with *phragmites*: In summer, the appropriate temperature is favorable for the growing of plants and microorganisms, the respiration rate of plant roots is high, the level of metabolic flora is high, and the constructed wetland ecosystem is rich in functions, which can maintain a stable and good removal rate of nitrogen. In winter, although plant and microbial activity is affected by low temperatures and the release of plant oxygen is reduced, the existence of plants still has a positive impact on microbial communities and abundance.

Conclusion and Outlook

Traditional nitrogen removal nitrification-denitrification is still the main path of nitrogen removal, although the new path of nitrogen removal can save energy consumption and have higher nitrogen removal efficiency, it requires strict control over the conditions and operating parameters. Configuration optimization measures (improving the water intake mode, optimization of substrate combinations, optimization of plant species configuration, and novel constructed wetland coupling process) and improved operating conditions (additional carbon source, improved redox conditions, and aeration) can promote nitrification and denitrification by improving the concentration and distribution of dissolved oxygen or carbon sources, while microbial enhancement technology can directly improve nitrogen removal. In order to make the measures of enhanced nitrogen removal of constructed wetlands more detailed, comprehensive, and effective, the following prospects for future research are proposed:

1. Novel nitrogen removal pathways such as simultaneous nitrification and denitrification, partial nitrification-denitrification, and anaerobic ammonia oxidation are difficult to achieve, and the specific conditions under which they can occur are not yet known, making them a key area for future research.

2. Substrate clogging is one of the main factors impacting the nitrogen removal capability of constructed wetlands, but currently, the factors leading to substrate clogging are complex and uncertain, so further research is necessary.

3. The specific effects of interactions between different species on nitrogen removal microorganisms under mixed planting conditions are still unclear, and more detailed studies are needed.

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Conflict of Interest

The authors declare no conflict of interest.

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