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Response of Nitrogen Removal Performance and Microbial Distribution to Seasonal Shock Nutrients Load in a Lakeshore Multicell Constructed Wetland

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Abstract: Multicell constructed wetlands (MCWs) on lakeshores are a prospective treatment technique. However, the factors affecting the nutrient removal performance of lakeshore MCWs at the field scale are unclear. This study chose a field-scale lakeshore MCW with the highest mass removal efficiency (approximately 49,175.12 mg m^{-2} day^{-1}) for total nitrogen removal in the wet season to investigate the response of nitrogen removal and microbial distribution to seasonal shock nutrients load. The mass loading rates in the wet season were as high as 43~72 times over those in the dry season. Hence, a storage pond (SP), as a forebay retention cell, was necessary to mitigate the shock loads of the influent, which is beneficial to nitrogen removal of the MCW system. The two major genera in the sediments are heterotrophic nitrification–aerobic denitrification bacteria, and the abundance and species of the nitrogen-related functional genera were higher in the wet season than the dry season. According to the results of redundancy analysis, the hydraulic residence time (29.4%, F = 2.2, $p < 0.1$) and hydraulic loading rate (85.9, F = 36.5, $p < 0.05$) were the major factors explaining microbial community variation, instead of environmental factors (temperature, pH, and dissolved oxygen). The shock loads of influent and the periodic saturation in sediments contributed to a complicated oxygen and nitrogen nutrient exchange environment resulting in higher abundance and species of nitrogen-related microbes, which is beneficial to nitrogen removal in lakeshore MCWs. The results provided a scientific basis for the optimal design of constructed wetlands on lakeshores.

Keywords: lakeshore wetlands; nitrogen removal; nonpoint source pollution; hydraulic characters; microbial community; spatial and temporal regulation

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1. Introduction

Nonpoint source (NPS) pollution is one of the crucial sources resulting in surface water pollution, especially agricultural runoff, as the primary form in rural nonpoint sources and contains excessive nitrogenous and phosphorus compounds that cause eutrophication in recipient water bodies, in which the total nitrogen concentration could be as high as nearly 50 mg L^{-1} [1–3]. Lakeshores are transition zones between terrestrial and aquatic environments and are vital to mitigate diffuse NPS pollution before entering a lake [4]. To control NPS pollution, constructed wetlands (CWs) in lakeshores have become a popular eco-friendly and cost-effective method to capture and purify parts of nutrient-rich runoff water before they reach lakes, reducing nutrient loads discharging into lakes [5].

In contrast to common CWs, multicell constructed wetlands (MCWs) in lakeshores increase the nutrient purification process through biodegradation, adsorption, and assimilation and consist of various kinds of treatment cells, such as vertical flow, horizontal flow, and eco-floating beds [3,6,7]. Studies have shown that the combination of vertical and horizontal flow increases the removal of nitrogen, which is applied to purify NPS pollution [8].

In addition, the greater water depths in the cells of MCWs contribute to storing and purifying wastewater because there is more space without increasing the land source of MCW systems [9]. Li et al. [10] studied 63 MCWs grouped into four kinds depending on various water depths and treatment processes, and the maximum nitrogen removal efficiency was 63.7%. Wang et al. [11] constructed two pilot-scale MCW systems to treat micro-polluted river water with a low C/N ratio, and the total nitrogen removal rates were between 19.56% and 34.84%. The design and operational parameters were vital factors in contaminant removal efficiency, including hydraulic residence time (HRT), hydraulic loading rate (HLR), mass loading rate (MLR), water depth, influent contamination, etc. [12,13]. However, in previous studies and reviews, most empirical design and operational parameters were highly related to lab-scale/pilot-scale experiments [11,14]. Moreover, those at the field scale have rarely been reported, which are easily affected by environmental factors, such as climate, rainfall, and season, which are uncontrollable but crucial to the operational performance of MCWs in engineering practice [15]. Therefore, the hydraulic character of the MCW system, characterized by the seasonal variation in the design and operational parameters, is one of the critical factors affecting nutrient removal.

Numerous studies have demonstrated that assimilation and storage by plants and microbes serve as the primary nitrogen removal pathways in MCWs [5,16–18]. The microbial nitrogen cycle within CWs is driven by nitrogen-related microorganisms, such as heterotrophic denitrification genera, facultative autotrophic denitrification genera, autotrophic denitrification genera, anaerobic ammonium oxidation genera, and nitrification genera [9,19]. Environmental conditions in MCW systems influence nitrogen conversions, while the wet and dry seasons, with variations in precipitation volume, are essential in the microbial nitrogen cycle. For example, surface runoff caused by rainfall brings more contaminants into MCWs, and nitrogen-converting microbes fluctuate in microbial communities during the wet and dry seasons along with changes in nitrogen contents [20]. Lakeshore zones are regarded as biochemical hotspots of nitrogen cycling because of the fluctuating hydrological regime and intensive material exchange between inland areas and water, particularly oxygen and nitrogen nutrients [21]. However, our knowledge about nitrogen contaminants carried by NPS pollution and the effect of design parameters in lakeshore MCWs on microbial community distribution is limited.

In this study, we aimed to investigate the response of nitrogen removal and microbial community structure to the design and operational parameters (HRT, HLR, and MLR) in a field-scale lakeshore MCW. We hypothesized that the microbial community related to the fluctuating hydrology and nitrogen nutrients of the influent in the wet and dry seasons was beneficial to nitrogen removal and purification of the lakeshore wetlands. Therefore, the primary objectives of this study were (1) to characterize the hydraulic conditions of the MCW system depending on the seasonal variation of the design and operational parameters, (2) to identify the spatial and temporal characteristics of the microbial structure, and (3) to disclose the effects of seasonal parameters on the removal performance and the microbial community distribution.

2. Materials and Methods

2.1. Sites and MCW System Description

Lake Erhai is a subtropical plateau lake in the city of Dali, Yunnan Province, Southwest China. The mean annual precipitation of Lake Erhai is approximately 1000 mm, and more than 50% of the yearly precipitation concentrated in the summer months is attributed to the monsoon climate. It is evident in Figure S1 that most of the precipitation in 2021 was concentrated from May to October, which was regarded as the wet season in this study, while the other months were the dry season, with precipitation being less than 50 mm. The maximum and minimum temperatures of 2021 were 23.65 °C (in June) and 3.26 °C (in January), respectively, and the average annual temperature was 16.14 °C.

Lake Erhai is in the mesotrophic status and has been at risk of changing to the eutrophic stage in recent years due to NPS pollution [22]. To control the water quality, many MCWs are applied to detain stormwater runoff and purify the discharge before entering Lake Erhai. The Lake Erhai Ecological Restoration and Constructed Wetlands Project located on the lakeshore, which is 129 km long and approximately 900 hectares surrounding the lake, broke ground in 2019 and was completed recently in succession. The MCW at Dazhuang Village was chosen as the research site because it is one of the surrounding-lake MCWs with typical design and structures (Figure 1a). The influent of the MCW system was from nonpoint source pollution, whose effluent was mixed with that of other MCWs and eventually discharged into Lake Erhai. Figure 1b shows an overview diagram, and Table S1 shows the basic information of this MCW system. The chosen MCWs consisted of four cells, including a storage pond (SP), followed by an ecological oxidation pond (EP), and then a horizontal subsurface flow constructed wetland (HSCW) and surface flow constructed wetland (SFCW). The treatment process is shown in Figure 1c, and photographs of the four cells are shown in Figure S2.

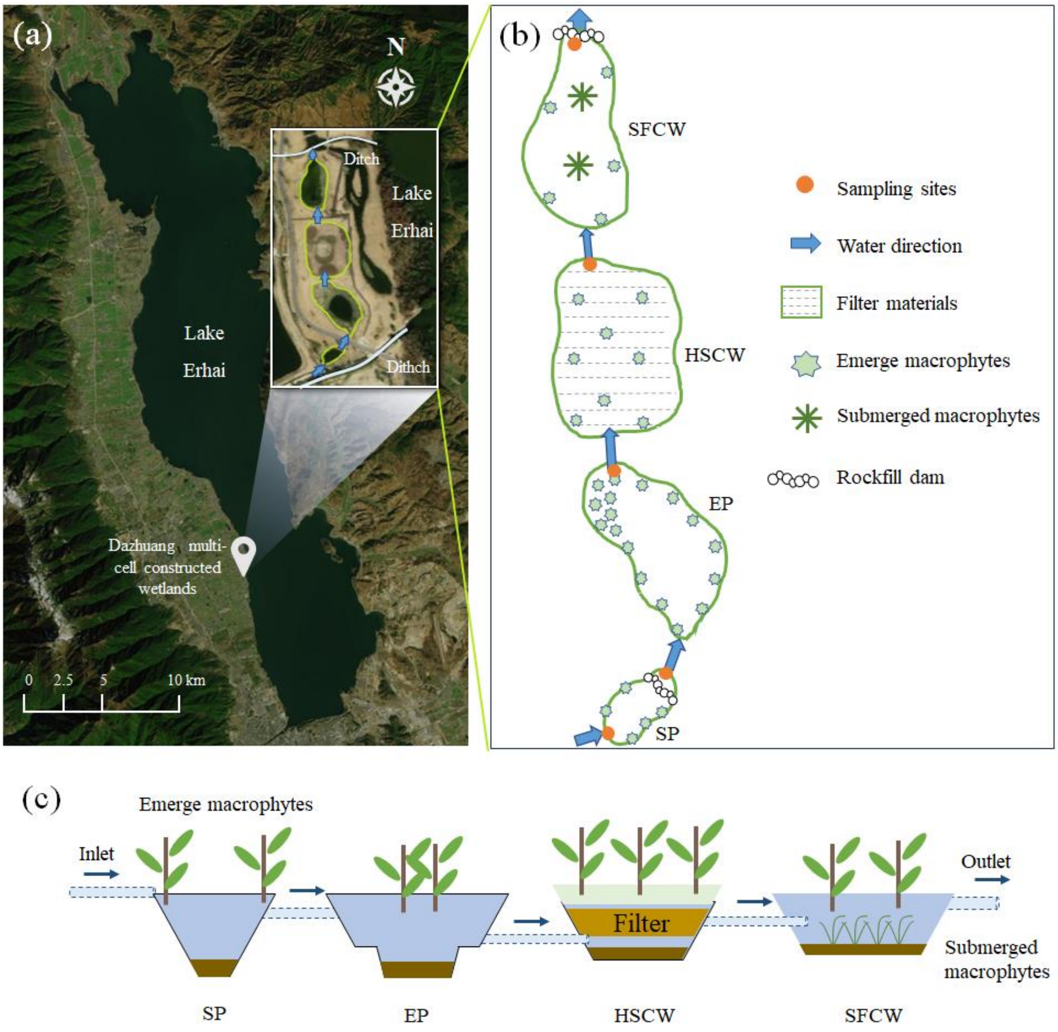


Figure 1. Basic information on the MCW system: (**a**) location of Lake Erhhai and the MCW system; (**b**) the layout of MCW system; (**c**) the treatment process. SP: storage pod; EP: ecological oxidation pond; HSCW: horizontal subsurface flow constructed wetland; SFCW: surface flow constructed wetland.

2.2. Sample Collection and Analyses

Water samples were collected with sample bottles monthly from January to December 2021 in the inlets and outlets of the MCW system (from five sites and repeated three times) at a certain depth range (0–10 cm) for further analysis. All samples were analyzed immediately after sampling. The environmental parameters, namely the temperature (T), pH, and dissolved oxygen (DO) content, were analyzed with specific portable meters (Mettler Company, Columbus, OH, USA).

To investigate the microbial community in the MCW system, surface sediment samples (0–10 cm) were collected using the sediment column sampler device at four cells. Three parallel samples in the device were mixed thoroughly as one composite sample and repeated at two random points beside the sample site per cell. The sediment samples were collected in the dry season in January 2021 and in the wet season in June 2021. All the fresh samples were kept in polyethylene plastic zip lock bags and refrigerated at −60 °C.

Total nitrogen (TN), nitrate (NO_3^--N), and ammonium (NH_4^+-N) concentrations were measured according to standard methods [23]. TN concentrations were determined by the alkaline potassium persulfate digestion UV spectrophotometric method. NO_3^--N concentrations were detected with an ultraviolet–visible spectrophotometer at a wavelength of 410 nm. NH_4^+-N concentrations were assayed using Nessler's reagent colorimetry method.

2.3. Microbial Analyses

The DNA from the MCW system's sediment samples was extracted using a PowerSoil™ DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA). A NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to quantify and evaluate the genomic DNA. Universal primers 515FmodF (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806RmodR (5′-GGAC TACNVGGGTWTCTAAT-3′) were used for polymerase chain reaction (PCR) [9]. PCRs were performed as described in a previous study [9]. The purified amplicons were pooled in equimolar and paired-end (2 × 250 bp) sequencing and conducted using an Illumina MiSeq PE300 platform (Illu-mina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The raw data were processed and analyzed following the guidelines described by Li et al. [9]. The process results were conducted to further analyze the OUT-level species richness and alpha diversity index values (Shannon, ACE, Chao1, and Simpson indices).

2.4. Statistical Analyses

The mass loading rate per square meter (MLR, mg m^{-2} day^{-1}), hydraulic loading rate (HLR, m day^{-1}), and hydraulic residence time (HRT, day) were calculated using Equations (1)–(3), respectively. Equations (4)–(6) were used to determine the removal efficiency (RE, %), mass removal rate per square meter (MRR, mg m^{-2} day^{-1}), and mass removal rate per cubic meter (MRR_V, mg m^{-3} day^{-1}), respectively.

$$\mathrm{MLR} = C_{in} \times Q_{in}/A \tag{1}$$

$$\mathrm{HLR} = Q_{in}/A \tag{2}$$

$$\mathrm{HRT} = V/Q_{in} \tag{3}$$

$$\mathrm{RE} = (C_{in} \times Q_{in} - C_{out} \times Q_{out})/(C_{in} \times Q_{in}) \times 100\% \tag{4}$$

$$\mathrm{MRR} = (C_{in} \times Q_{in} - C_{out} \times Q_{out})/A \times 1000 \tag{5}$$

$$\mathrm{MRR_V} = (C_{in} \times Q_{in} - C_{out} \times Q_{out})/V \times 100 \tag{6}$$

Here, C_{in} and C_{out} represent the nitrogen concentrations in influent and effluent (mg L^{-1}), respectively; Q_{in} and Q_{out} represent the daily flow water quantity of influent and effluent (m^3 day^{-1}), respectively; V is the water capacity of each cell (m^3); and A is the water surface area of each cell (m^2).

The constrained ordination of redundancy analysis (RDA) was performed by Canoco 5.0. Furthermore, linear and nonlinear regression simulations and Spearman's rank correlation coefficients were calculated and performed using Origin 2021 and Microsoft Excel 365.

3. Results and Discussion

3.1. Characteristics of Water Quantity and Nitrogen Concentrations in Influent and Effluent

3.1.1. Water Quantity

The water quantity of the influent varied greatly between the dry and wet seasons. The amount of influent in the dry season was below 10 × 10^3 m^3 $month^{-1}$, except January (18.66 × 10^3 m^3 $month^{-1}$), while most of that in the wet season was more than 100 × 10^3 m^3 $month^{-1}$, and the highest water quantity was in August, which was as high as 643 × 10^3 m^3 $month^{-1}$. The amounts of influent water in the wet season were approximately 20–60 times greater than those in the dry season, attributed to the concentrated precipitation in summer, and the rainfall amount decreased sharply from November. The characteristics of the water quantity of the influent were consistent with the local rainfall patterns, as shown in Figure S1. The influent water from the lakeshore wetlands' ditches mostly consisted of irrigation drainage and wastewater from the catchment. In the wet season, the amount of the influent rises gradually along with the increasing surface runoff for rainy reasons and agricultural runoff during the growing season [3].

3.1.2. Nitrogen Concentration

The nitrogen concentrations of the influent and effluent are shown in Figure 2, and the seasonal variation in water quality is presented in Table 1. Compared to the influent, the nitrogen concentrations in the effluent decreased significantly, suggesting effective nitrogen interception in the lakeshore MCW. Overall, the average TN, NO_3^--N, and NH_4^+-N concentrations in the influent over the year were 21.47 ± 12.23 mg L^{-1}, 18.58 ± 12.04 mg L^{-1}, and 0.45 ± 0.39 mg L^{-1}, respectively. The average nitrogen concentrations in the wet season were higher than in the dry season, indicating that nutrients carried by rain runoff were the primary reason for the water quality fluctuations. In the wet season, the TN concentrations were 14.75–52.90 mg L^{-1}, while those in the dry season were all below 24.05 mg L^{-1}. Thus, the lakeshore MCW needs to cope with the high nutrient load of stormwater runoff, especially in the flood season. Considering the nitrogen composition in the influent, the higher concentration of NO_3^--N (2.40–47.57 mg L^{-1}) than that of NH_4^+-N (0.08–1.53 mg L^{-1}) resulted from the high percentage of agricultural lands (65.07%) in the catchment (Table S2), and NO_3^--N was the primary form of nitrogen fertilizer in the Lake Erhai basin, which is evidence that agricultural nonpoint sources were the primary pollution source for the lakeshore MCW [3].

Table 1. Nitrogen concentration and removal performance of the lakeshore MCW.

		c_{in} (mg L^{-1})	c_{out} (mg L^{-1})	RE (%)	MRR (mg m^{-2} day^{-1})	MRR_v (mg m^{-3} day^{-1})	MLR (mg m^{-2} day^{-1})	HLR (m day^{-1})	HRT (day)
Wet (*n* = 6)	TN	27.30 ± 14.36	10.11 ± 8.83	65.95 ± 25.01	15,741.45 ± 17,419.21	21680.63 ± 23991.40	846.65 ± 1173.06	0.84 ± 0.90	2.42 ± 3.20
	NO_3^--N	24.43 ± 13.89	8.54 ± 8.43	68.61 ± 26.41	15,088.87 ± 17,337.83	20,781.84 ± 23,879.31	791.85 ± 1135.34		
	NH_4^+-N	0.62 ± 0.49	0.21 ± 0.19	53.64 ± 30.57	295.09 ± 339.01	406.43 ± 466.91	13.53 ± 14.57		
Dry (*n* = 6)	TN	15.64 ± 4.99	9.21 ± 7.63	46.22 ± 38.60	180.03 ± 108.41	247.96 ± 149.32	15.60 ± 7.29	0.04 ± 0.02	24.53 ± 9.71
	NO_3^--N	12.72 ± 5.31	6.86 ± 7.36	49.66 ± 42.61	154.41 ± 127.25	212.67 ± 175.26	11.30 ± 5.35		
	NH_4^+-N	0.29 ± 0.10	0.15 ± 0.07	45.11 ± 25.16	5.77 ± 6.33	7.95 ± 8.72	0.33 ± 0.31		
Year (*n* = 12)	TN	21.47 ± 12.23	9.66 ± 8.26	56.09 ± 32.68	7960.74 ± 14,281.83	10,964.30 ± 19,670.30	431.12 ± 902.14	0.44 ± 0.74	13.47 ± 13.45
	NO_3^--N	18.58 ± 12.04	7.70 ± 7.96	59.13 ± 35.22	7621.64 ± 14,052.49	10,497.25 ± 19,354.43	401.57 ± 867.22		
	NH_4^+-N	0.45 ± 0.39	0.18 ± 0.15	49.37 ± 27.06	150.43 ± 274.02	207.19 ± 377.40	6.93 ± 12.00		

Data are presented as means ± standard deviation (S.D.) with *n* = 3 or *n* = 6.

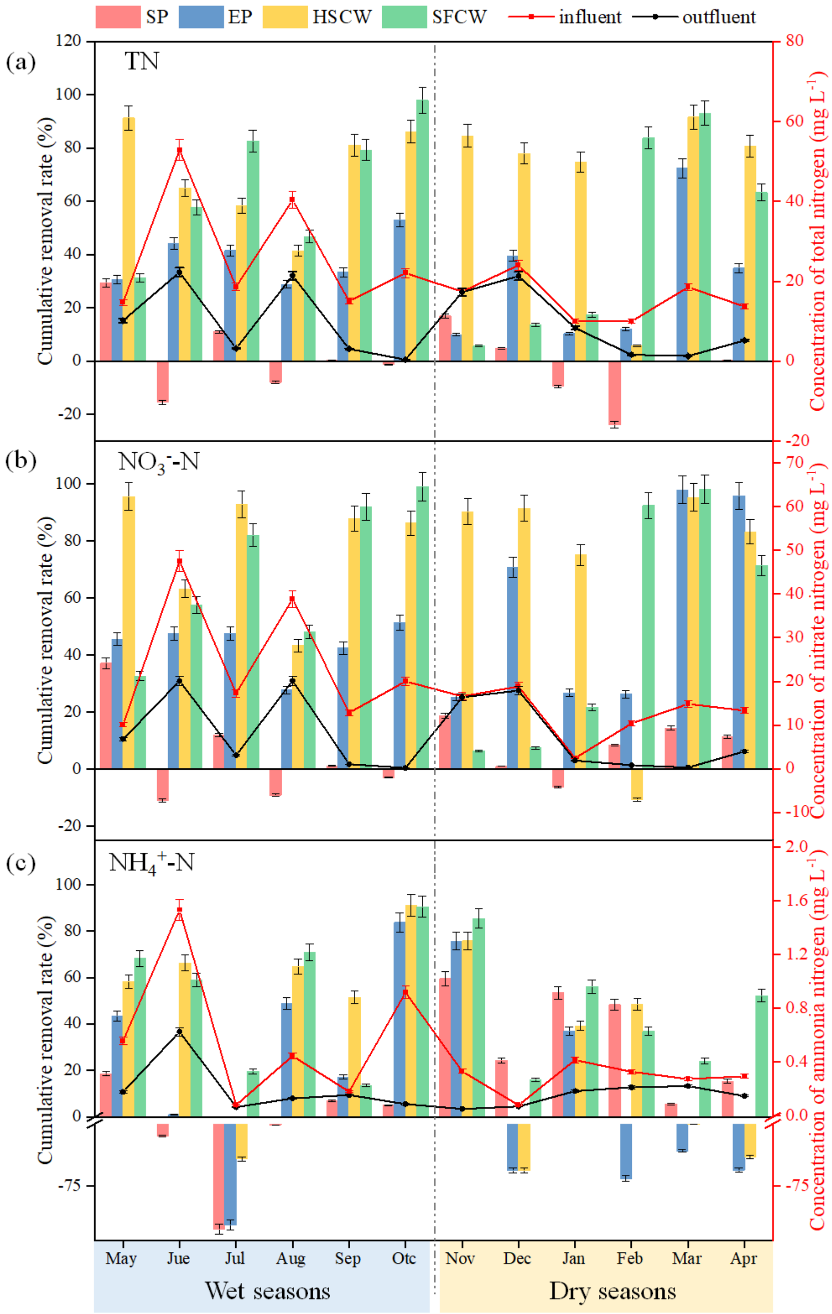


Figure 2. Removal rates of the lakeshore MCW and nitrogen concentrations in the influent and effluent. (**a**) TN; (**b**) NO_3^--N; (**c**) NH_4^+-N. SP: storage pod; EP: ecological oxidation pond; HSCW: horizontal subsurface flow constructed wetland; SFCW: surface flow constructed wetland.

3.2. Seasonal Variation in Design and Operational Parameters

The significant differences in water quantity and quality of the influent between various seasons can greatly influence the operational parameters of the lakeshore MCW, such as the mass loading rate (MLR), hydraulic loading rate (HLR), and hydraulic residence time (HRT) [13]. Seasonal variations in MLR, HLR, and HRT were investigated as follows, and monthly data are provided in Table S3.

3.2.1. MLR

MLR is a comprehensive result of the water quantity and quality of the influent. As shown in Table 1, the average MLRs of TN, NO_3^--N, and NH_4^+-N in the wet season were 846.65 ± 1173.06, 791.85 ± 1135.34, and 13.53 ± 14.57 mg·m^{-2} day^{-1}, respectively, which were higher than those in the dry season (15.60 ± 7.29, 11.30 ± 5.35, and 0.33 ± 0.31 mg m^{-2} day^{-1}, respectively). The higher MLRs in the wet season were attributed to the large water quantity and high nitrogen concentration caused by concentrated rainfall. It is a common feature of the surface runoff in the basin of Lake Erhai that the nutrient loading fluctuates seasonally [9,10]. The seasonal variation was more evident in the lakeshore MCW for the average nitrogen MLRs in the wet season, which were as high as 43~72 times over those in the dry season, while the storing multipond CWs further than 10 km away from the lake were only 2~3 times in early research [24]. Therefore, the seasonal shock loads of the lakeshore MCW are characterized by intermittent and impulse-type discharges into the receiving waters. Interestingly, the average nitrogen concentration in the wet season was only 1.75–2.16 times that in the dry season, so the water quantity of the influent was affected more than the nitrogen concentration on the MLRs in the wet season, while the opposite trend was observed in the dry season. This is another piece of evidence illustrating that the large water quantity caused by concentrated rainfall was one of the critical factors for fluctuating loads in the wet season. In addition, intermittent desiccations of EP were observed in the lakeshore MCW in the dry season, as shown in Figure S3, which were attributed to less rainfall and rapid evaporation in the dry season.

3.2.2. HLR

HLR is one of the vital parameters related to the storage capacity or amount of influent [13,24]. As shown in Table 1, the HLRs of the lakeshore MCW were usually below 0.05 m day^{-1} in the dry season, while those in the wet season were almost beyond 0.05 m day^{-1}, especially the HLR as high as 2.60 m day^{-1} in August. As shown in Figure S1, the precipitation in August was the highest over the year, and the evaporation was lower than that of other months in the wet season, such as July and September. The influent amount was in accordance with the precipitation trend, which was highly related to HLR, so HLRs in August were highest over the year. Because of the high response between the precipitation and HLR, the rainfall fluctuations in both the wet and dry seasons contributed to the fluctuating HLR. Ruan et al. [25] investigated the influent factors on the purification of 75 field-scale CWs in rural and urban areas, and the results showed that the HLRs of 45 CWs were below 0.5 m day^{-1}, while those of only 5 CWs exceeded 1 m day^{-1}. Overall, the HLRs of the lakeshore MCW were slightly higher than those of other constructed wetlands. Comparing the HLRs in various MCW cells, as shown in Figure 3, the HLRs of the SP cell in the wet season (1.93–61.67 m day^{-1}) were significantly higher than those of other cells. The HLR sharply decreased after flowing through the SP cell, and the highest HLR of the next cell (the EP) in the wet season was only 10.72 m day^{-1}, far from that of SP (61.67 m day^{-1}). The value gap between the two cells was the biggest in August, and the HLR decreased by as much as 82.50% after flowing through the SP. Therefore, it was such an efficient way to mitigate the HLR fluctuation and shock loads in the wet season that the storage pond was set as the first cell of the lakeshore MCW.

3.2.3. HRT

HRT dominates the reaction time, directly affects the biochemical process, and leads to fluctuations in the removal efficiency [13]. The HRTs of the lakeshore MCW were also different between the wet and dry seasons, as shown in Table 1. The HRTs in the wet season were shorter (0.28–8.87 days) than those in the dry season (9.63–38.32 days), attributed to the concentrated rainfall, which increased the water quantity in the influent. It was reported that the HRT and HLR were highly related in MCW purification, and the HRT was negatively related to the HLR of the influent [13], as shown in Figure 3. The unusual decrease in the HRT in January may be related to the manual cleaning of sludge of the MCWs during the low water level. The HRTs for the four MCW cells are shown in Figure 3, and the shortest HRT (the annual average value was approximately 0.79 days) in all the MCW cells appeared in the SP, which was related to the highest HLR in the SP among the four MCW cells. The relatively long HRTs in the other three MCW cells benefited from adequate storage in the SP, which provided sufficient reaction time for biochemical remediation during nitrogen purification in the MCW system. This was evidence that the forebay SP was essential in the lakeshore MCW to cope with the water quantity and quality fluctuations before entering the lake.

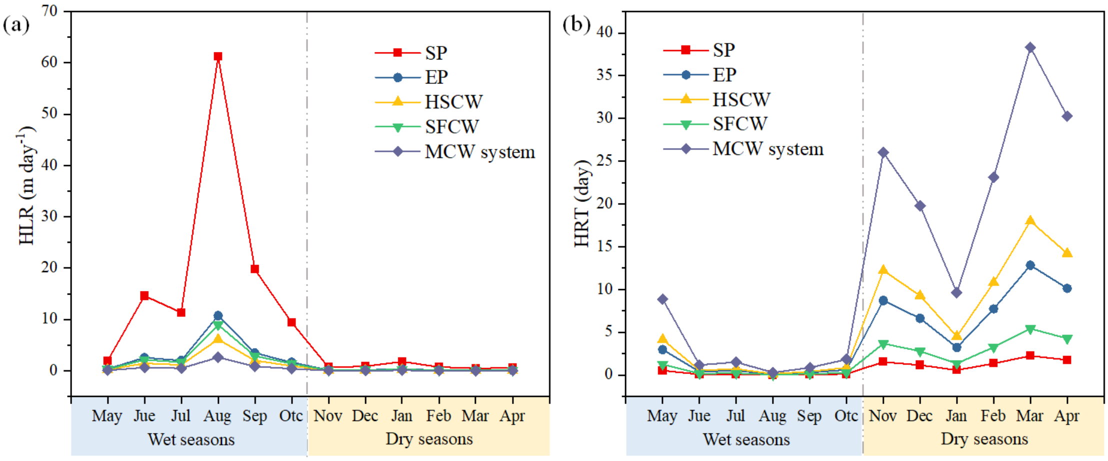


Figure 3. HLRs (**a**) and HRTs (**b**) of various cells of the lakeshore MCW system. SP: storage pod; EP: ecological oxidation pond; HSCW: horizontal subsurface flow constructed wetland; SFCW: surface flow constructed wetland.

3.3. Comparison of Nitrogen Removal Performances

3.3.1. Seasonal nitrogen removal performance of the lakeshore MCW system

The REs of the wetland in various seasons are shown in Table 1 and Table S3. The differences in N removal observed under various seasons and treatment processes are statistically significant ($p < 0.05$), as shown by one-way ANOVA in Table S4. Overall, the nitrogen REs of the wetland in the wet season were higher than those in the dry season because the average TN, NH_4^+-N, and NO_3^--N REs of the wetland in the wet season were 65.95 ± 25.01%, 53.64 ± 30.57%, and 68.61 ± 26.41%, respectively. In comparison, those in the dry season were 46.22 ± 38.60%, 45.11 ± 25.16%, and 49.66 ± 42.61%, respectively. Similarly, the MRRs and MRR_Vs in the wet season were higher than those in the dry season. A higher nitrogen concentration and water quantity of the influent were positively related to a higher nitrogen removal effect in the wet season, illustrating a high potential purification and operation effectiveness of the lakeshore MCW in the wet season. Compared to TN and NO_3^--N, the lower NH_4^+-N REs were related to the relatively low NH_4^+-N concentrations in the influent. The average value over the year was 0.45 ± 0.39 mg L^{-1} (as shown in Table 1), which was far from the surface water quality standard class III (1 mg L^{-1}) and the first grade A standard of the municipal sewage discharge standard (5 mg L^{-1}) in China [26]. This was also the main reason for the significantly lower MRR and MRR_v of NH_4^+-N than those of TN and NO_3^--N.

3.3.2. Nitrogen Removal Performances of the Various Cells

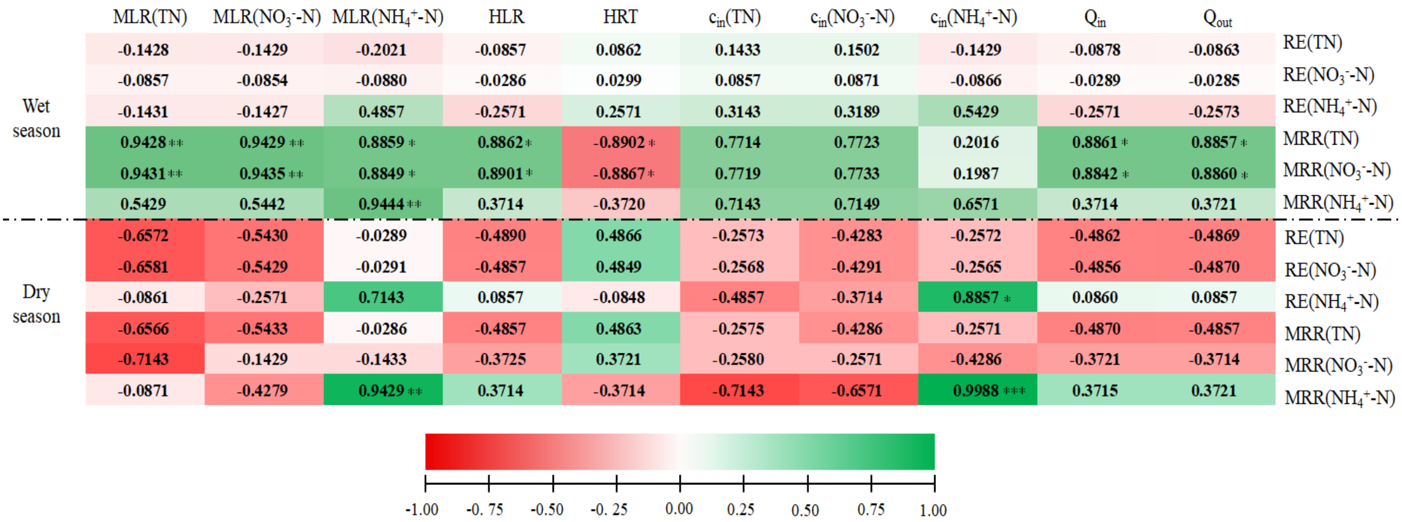
The nitrogen concentrations of the influent along the flowing route in the lakeshore MCW are shown in Figure S4, directly showing the purification effectiveness. The nitrogen concentrations of the influent were significantly decreased after flowing through each wetland cell in general, although those of certain cells in several months slightly fluctuated. To further analyze the contribution of the RE of each cell to those of the MCW system, the cumulative REs from the influent of the first cell (EP) to the outlet of the other three cells were sequentially calculated, as shown in Figure 2, that is, the cumulative RE of the SFCW precisely showed the nitrogen purification ability of the MCW system.

The SP was the first cell in the lakeshore MCW, and the maximum design water depth (1 m) was higher than that in the other cells, as shown in Table S1. As the above results showed, it functioned as a pre-retention pond and was essential in water quantity regulation. The REs of nitrogen in the SP were not as satisfactory as those in other cells, and the nitrogen concentrations in the outlet of the SP were even slightly increased, especially in June and August. It was a sacrifice for purification effectiveness caused by nitrogen resuspension carried by the particles in the influent. In the EP, the RE of NO_3^--N was the best (the average value was 46.42 ± 28.00%), and that of TN was the second best (the average value was 32.60 ± 20.93%). This was attributed to the extensive plant coverage and varying water depth in the EP, contributing to the creation of different aerobic, facultative, and anaerobic oxygen zones in the sediments, which had an advantage for denitrification by microorganisms. For the HSCW, the REs of TN and NH_4^+-N were the highest (the average values were 55.46 ± 28.01% and 29.43 ± 22.61%, respectively) among the four cells. The differences between the REs in the two seasons of the HSCW were not as noticeable, indicating that the primary mechanism for nitrogen retention of the HSCW was pollution adsorption by the lava filters, which were barely affected by the seasons. The high nitrogen retention performance was attributed to the vertical flow path in the filtration bed of the HSCW, which provides adequate contact between the wastewater and lava fillers and is barely affected by the seasons. The adsorption mechanism for nitrogen retention in the HSCW was quicker and had more retention ability compared to the biological treatment by the plants and microbes in the wetland. In the SFCW, the nitrogen concentrations in the influent were lower than those in the effluent in other seasons, except that the NH_4^+-N REs of the SFCW in the dry season were approximately 13.32%. Nevertheless, the performance of the SFCW in nitrogen removal has potential because the nitrogen concentrations of the influent were lower than those in other kinds of MCWs [7,11].

3.4. Effects of Seasonal Parameters on the Nitrogen Removal

To further analyze the coupling effects of the seasonal variation in parameters and nitrogen removal properties, Spearman's rank correlation coefficients between the nitrogen removal performance (RE and MRR) and the design and operational parameters in the two seasons were analyzed (Figure 4). The corresponding relations in the wet season differed from those in the dry season. The RE was negatively related to the MLR and HLR in the wet season while being positively associated with the HRT. A lower MLR or HLR and a longer HRT benefited settling down the particles in the influent and for adequate biochemical degradation of nitrogen pollutants, contributing to a satisfactory removal rate [27]. However, the relationships between MRR and the design and operational parameters were reversed, and a high mass loading of pollutants in the wastewater increased the MRR because the MRR was positively correlated with the MLR or HLR. Surprisingly, the MRR was negatively related to the HRT, meaning that the longer the wastewater retention was, the lower the efficiency of contaminant mass intercepted. Therefore, to balance RE and MRR to achieve a satisfactory purification performance of wetlands, the HRT should be limited to a specific range that is not very high [24]. In addition, a higher nitrogen concentration in the influent was related to a higher RE and MRR, meaning that the potential nitrogen retention capacity was not achieved in CWs in the wet season.

In the dry season, REs of TN and NO_3^--N were slightly negatively related to HLR, MLR, nitrogen concentrations, water quantity, and MRR, which were significantly different from those in the wet season and inconsistent with the previously reported storing MCW [24]. This was attributed to the excessively shallow water depth of lakeshore MCWs in the dry season, and even the bottoms of certain ponds were exposed (Figure S3). Hence, the biochemical purification process of the MCW was not typical, which was also why the nitrogen REs were lower than those in the wet season. The nitrogen concentrations and water quality were lower in the dry season than the wet season, so the purification demand was lower. The primary ecosystem service functions of the lakeshore MCWs were not retaining and purifying the contaminants in this period.



		MLR(TN)	MLR(NO_3^--N)	MLR(NH_4^+-N)	HLR	HRT	c_{in}(TN)	c_{in}(NO_3^--N)	c_{in}(NH_4^+-N)	Q_{in}	Q_{out}
Wet season	RE(TN)	-0.1428	-0.1429	-0.2021	-0.0857	0.0862	0.1433	0.1502	-0.1429	-0.0878	-0.0863
	RE(NO_3^--N)	-0.0857	-0.0854	-0.0880	-0.0286	0.0299	0.0857	0.0871	-0.0866	-0.0289	-0.0285
	RE(NH_4^+-N)	-0.1431	-0.1427	0.4857	-0.2571	0.2571	0.3143	0.3189	0.5429	-0.2571	-0.2573
	MRR(TN)	0.9428 **	0.9429 **	0.8859 *	0.8862 *	-0.8902 *	0.7714	0.7723	0.2016	0.8861 *	0.8857 *
	MRR(NO_3^--N)	0.9431 **	0.9435 **	0.8849 *	0.8901 *	-0.8867 *	0.7719	0.7733	0.1987	0.8842 *	0.8860 *
	MRR(NH_4^+-N)	0.5429	0.5442	0.9444 **	0.3714	-0.3720	0.7143	0.7149	0.6571	0.3714	0.3721
Dry season	RE(TN)	-0.6572	-0.5430	-0.0289	-0.4890	0.4866	-0.2573	-0.4283	-0.2572	-0.4862	-0.4869
	RE(NO_3^--N)	-0.6581	-0.5429	-0.0291	-0.4857	0.4849	-0.2568	-0.4291	-0.2565	-0.4856	-0.4870
	RE(NH_4^+-N)	-0.0861	-0.2571	0.7143	0.0857	-0.0848	-0.4857	-0.3714	0.8857 *	0.0860	0.0857
	MRR(TN)	-0.6566	-0.5433	-0.0286	-0.4857	0.4863	-0.2575	-0.4286	-0.2571	-0.4870	-0.4857
	MRR(NO_3^--N)	-0.7143	-0.1429	-0.1433	-0.3725	0.3721	-0.2580	-0.2571	-0.4286	-0.3721	-0.3714
	MRR(NH_4^+-N)	-0.0871	-0.4279	0.9429 **	0.3714	-0.3714	-0.7143	-0.6571	0.9988 ***	0.3715	0.3721

Figure 4. Heatmap of Spearman's rank correlation coefficients. * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$.

3.5. Spatial and Temporal Variations in Microbial Community

The functions and behaviors of bacteria are one of the major factors affecting the performance of lakeshore MCWs. The response of the microbial community in various cells to fluctuating nitrogen loads was investigated to promote the possible reason for the seasonal variability in the nitrogen removal performance of lakeshore MCWs. The Illumina sequencing statistics of different sediment samples from the four cells in the two seasons are compared in Tables S5 and S6. To ensure sequencing uniformity [28], the species richness and alpha diversity index values were calculated based on 5845 operational taxonomic units (OTUs) (ranging from 1065 to 5334 per sample). The ACE and Chao1 indices reflect the microbial community richness, and Sobs is the observed richness. The Shannon and Simpson indices indicate bacterial community diversity. Therefore, the microbial community richness and diversity in the sediments of the EP and SFCW were relatively higher than those in the other cells, while those of the HSCW were the lowest. This was attributed to the high-density planting in the SFCW and EP, which was essential in rhizosphere bacterial activity, community richness, diversity, and function [7,15]. A complicated oxygen environment with different aerobic, facultative, and anaerobic oxygen zones easily occurs in the rhizoplane and water for the oxygen released from the root, which is beneficial for increasing the microbial community richness [29]. As shown in Table S1, the HSCW and SFCW had a high plant coverage (more than 90%), followed by EP. The amphibious plants (*Iris pseudacorus* L. and *Cyperus involucratus*) in the HSCW, as shown in Figure S2, covered the surface soil layer upon the filter layer through which the water fluid passes. The sediment moisture and oxygen concentrations in the HSCW differed from the root microenvironments underwater in other cells, resulting in significant variations in the microbial community richness [30]. Similarly, the microbial richness and diversity in the sediment were lower than those in the dry season. Because of the deeper depth of the ponds in the wet season for the concentrated precipitation, the oxygen in the rhizoplane and water was lower in the wet season than in the dry season, which was not friendly to the aerobic microorganisms.

To investigate the difference in the microbial community between the two seasons in the four cells, PCA based on Bray–Curtis dissimilarity was performed, and the result is shown in Figure 5a. Microbial communities are grouped by cells resulting from water quality and rhizosphere environmental changes in the four cells. For the SFCW samples, the differences within groups were more significant than those between groups, as shown in Figure S5. This was attributed to the substantial changes in sediment moisture between the two seasons, and the oxygen content and substrate availability were consequently changed after SFCW rewetting [31]. Interestingly, the samples in the wet season clustered together while those in the dry season could be separated, indicating that the treatment technologies in each cell and spatial heterogeneity had more significant potential impacts on microbial community distribution in the dry season than in the wet season.

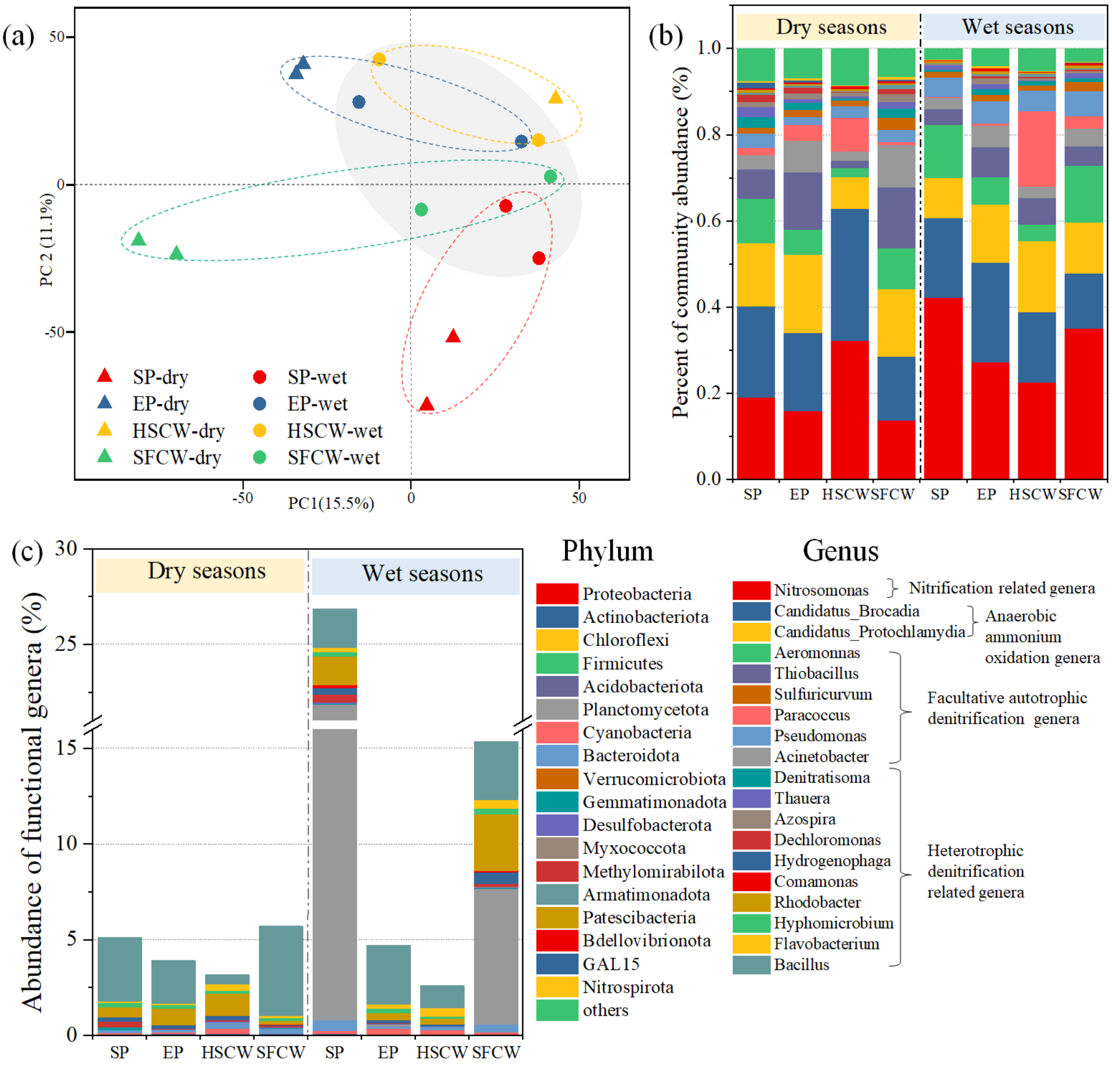


Figure 5. PCA and relative abundance of bacteria at the phylum and genus levels. (**a**) PCA analysis of bacterial communities from different sediments of four cells in various seasons (R = 0.1338, $p < 0.05$); (**b**) relative abundances of bacteria at the phylum level; (**c**) relative abundances of nitrogen-related functional genera. SP: storage pod; EP: ecological oxidation pond; HSCW: horizontal subsurface flow constructed wetland; SFCW: surface flow constructed wetland.

The microbial community composition and bacterial relative abundances at the phylum level are shown in Figure 5b. The most abundant phylum detected in the lakeshore MCW was *Proteobacteria*, ranging from 13.75% to 42.21%, followed by *Actinobacteriota* (12.73–30.67%), *Chloroflexi* (7.37–18.18%), *Firmicutes* (2.12–13.12%), *Acidobacteriota* (1.64–13.35%), *Planctomycetota* (2.12–9.71%), and *Cyanobacteria* (0.17–17.52%). *Proteobacteria* is identified as the most abundant phylum in the sediments of constructed wetlands for wastewater treatment [32]. Most of the nitrifying bacteria belong to *Proteobacteria* and *Nitrospirota* [33]. The high relative abundance of *Proteobacteria* but incredibly low *Nitrospirota* indicated that *Proteobacteria* executed NH_4^+-N conversion in the lakeshore MCWs. In the wet season, the total relative abundance of denitrificans, such as *Proteobacteria*, *Chloroflexi*, *Firmicutes*, and *Nitrospirota*, was higher than that in the dry season, which was the result of differences in the water temperatures and the operating parameters of the lakeshore CWs between the two seasons [34].

The structural evolution of the known bacterial communities at the genus level is shown in Figure S6, and nitrogen-related functional genera were abundant in the lakeshore MCW, as shown in Figure 5c, which demonstrated the complex biological nitrogen removal pathways in the lakeshore MCW, such as nitrification, denitrification, and anammox. *Bacillus* and *Rhodobacter* are the two major genera in the lakeshore MCW, and both are heterotrophic nitrification–aerobic denitrification (HN-AD) bacteria. The decrease in *Bacillus* was accompanied by the increase in *Rhodobacter*, which may be attributed to the competitive relationship. Research on HN-AD bacteria has been popular at home and abroad for altering the traditional theory suggesting that nitrification can be performed only by autotrophic bacteria and denitrification can be carried out only under anaerobic conditions, which is evidence for simultaneous nitrification and denitrification (SND), and *Pseudomonas* was one of the typical SND bacteria [35]. The greater number of nitrifying bacteria in the wet season explained the higher nitrogen removal in the lakeshore MCW. The abundance and species of the nitrogen-related functional genera were higher in the wet season than the dry season. This was the reason for the better nitrogen removal of the lakeshore MCWs in the wet season. Interestingly, the relative abundance of *Acinetobacter* in the SP and SFCW in the wet season was higher than that in the dry season. *Acinetobacter* is the primary bacterium related to nitrogen and phosphorus in enhanced biological phosphorus removal (EBPR) sludge, for which the high temperature of 22.5 °C during the wet season was most conducive to growth and reproduction [36,37]. In addition, a high phosphate concentration and an extensive phosphorus loading of the influent in the wet season resulted in phosphorus-accumulating bacteria growth. The high relative abundance of *Acinetobacter* showed potential phosphorus removal of the lakeshore MCW, which needs further investigation.

3.6. Effects of Seasonal Parameters on the Microbial Community

The activity of microorganisms was directly affected by environmental conditions such as pH, DO, and temperature, and the nitrogen cycle in sediments of the lakeshore MCW was affected by the nitrogen concentrations and the design and operational parameters, such as the MLR, HLR, and HRT [9,20,38]. To explore the effects of seasonal shock nutrient loads on microbial communities, RDA was used to identify the relationships between the microbial community (based on abundances at the phylum and genus levels), design and operational parameters (based on nitrogen concentrations, MLR, HLR, and HRT), and environmental conditions (based on pH, DO, and temperature). The interactive-forward-selection analysis method was chosen to select the best subset of the variables to summarize the variation in species composition. Figure 6 displays the structural evolutions of the phylum and nitrogen-related genera in various cells. The RDA results showed that the major variables to the variation in species composition were HRT, HLR, MLR, TN and NO_3^--N concentrations, DO, and pH.

As shown in Figure 6a, the RDA between phyla distributions in each MCW cell and seasonal factors indicated that the HRT (29.4%, F = 2.2, $p < 0.1$) was the main factor explaining variation in the microbial community composition at the phylum level (Figure 6a). A longer HRT value supports adequate reaction time for biotic processes, which affects the microbial community [24] and explains the excellent nitrogen removal rate in the lakeshore MCW. In addition, the design and operational parameters related to the shock loadings (such as MLR and HLR) also affected the microbial community, especially the MLR of NO_3^--N, because nitrate level was one of the key factors that control the structure of denitrifying communities [39]. In addition, the microbial communities at the phylum level were clustered by season in the SP, EP, and SFCW, demonstrating that the influence of seasonal changes in surface runoff on the biochemical purification of the lakeshore MCW is significant. However, the microbial communities of the HSCW in the two seasons were similar and separated from the other cells for the various nitrogen retention mechanisms dependent on the adsorption of the filler, which is the reason for the higher nitrogen RE in the HSCW.

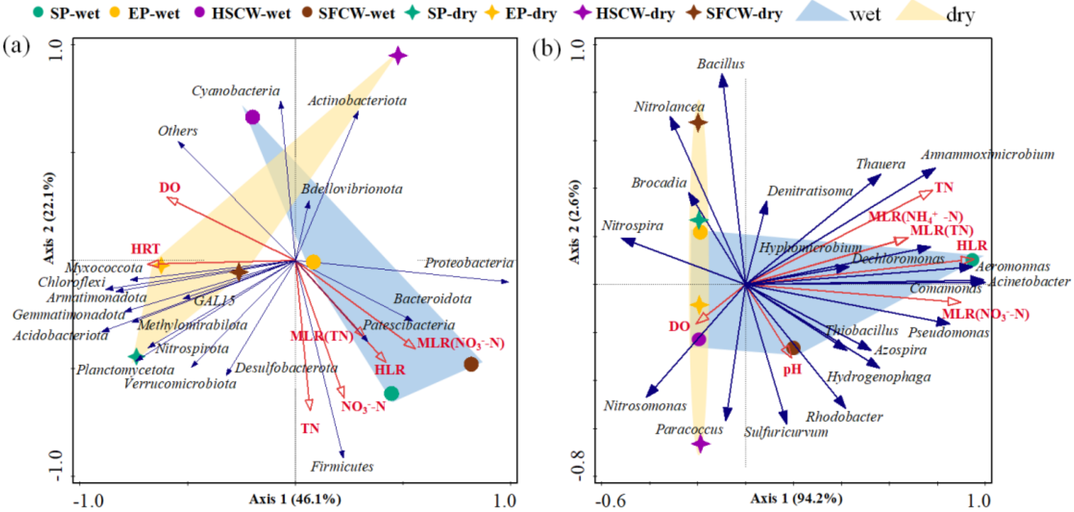


Figure 6. RDA between microbial community, operational parameters, and environemntal conditions in the lakeshore MCW: (**a**) at the phylum level; (**b**) at the nitrogen-related functional guera level. SP: storage pod; EP: ecological oxidation pond; HSCW: horizontal subsurface flow constructed wetland; SFCW: surface flow constructed wetland.

As shown in Figure 6b, HLR, the design and operational parameter, was the significant factor explaining variation in the nitrogen-related functional genera composition (85.9, F = 36.5, $p < 0.05$). The typical character of shock loads carried by the surface runoff in the Lake Erhai basin by intermittent and impulse-type discharges into the wetlands resulted in large HLRs, bringing enormous disturbances and water exchange to enrich oxygen in the water column by changing hydrological and hydraulic performance [9], which was beneficial to create complicated environmental conditions for the biochemical process. In addition, anaerobic ammonium oxidation genera and facultative autotrophic denitrification-related genera (such as *Aeromonnas*, *Acinetobacter*, and *Pseudomonas*) were positively correlated with nitrogen concentration, and heterotrophic denitrification related genera (such as *Comamonas*, *Hydrogenophaga*, *Azospira*, and *Rhodobacter*) were positively correlated with NO_3^--N MLR. A high nitrogen concentration in the influent was conducive to the domestication and aggregation of nitrogen-related functional genera. Additionally, nitrification-related genera (such as *Nitrosomonas* and *Nitrospira*) were positively correlated with DO. Periodic saturation of wetland sediments due to significant changes in seasonal streamflow would create fluctuating oxic/anoxic conditions for ammonium nitrification by *Nitrosomonas* and subsequent nitrite oxidation by *Nitrospira*, which is vital for nitrogen retention [40]. Therefore, the seasonal shock loads contributed to a unique community of the nitrogen-related microbes, which benefited the favorable nitrogen removal in the lakeshore MCW.

3.7. Regulation Suggestion for Lakeshore Multicell Constructed Wetlands

The lakeshore MCW is a prospective technique to control the nonpoint sources of water pollution before the surface runoff enters the lake. Compared to the traditional CWs, the seasonal shock loads were a key factor affecting the nitrogen removal performance. So, a forebay retention cell was necessary to mitigate the shock loads. To improve the efficiency of the practice engineering program, a combination of the storing pond/wetland project with the adjacent eco-purification CW project was a good idea for the best management practice (BMP). In addition, the methods contributed to a complicated oxygen environment, which is friendly to the rhizosphere bacterial community diversity and nitrogen-related microbe richness, which were suggested to improve the nitrogen removal performance. For example, various treatment processes, including the horizontal subsurface flow constructed wetland with filters and the surface flow constructed wetland, showed good nitrogen retention properties in this study. Finally, this study allowed us to gain more insight into how nitrogen and phosphorus collaborative purification of lakeshore MCWs will be necessary in the future.

4. Conclusions

In summary, this study investigated the response of nitrogen removal and microbial community structure to the design and operational parameters (HRT, HLR, and MLR) in a field-scale lakeshore MCW. The surface runoff by intermittent and impulse-type discharges into the wetlands resulted in the seasonal shock loads of the influent. The fluctuating hydrology and nitrogen nutrients of the influent in the wet and dry seasons were so evident that the MLRs in the wet season were as high as 43~72 times over those in the dry season. The high MLRs in the wet season were one of the major factors affecting the nitrogen retention rates of the lakeshore wetland, which resulted in higher average removal efficiencies of TN, NH_4^+-N, and NO_3^--N in the wet season than in the dry season. The sufficient effective storage in SP contributed to the adequate HRTs in the MCW system under the shock loads. Therefore, a forebay retention cell is crucial to alleviate the shock loads in the lakeshore MCW system. High-density planting in the SFCW and EP contributed to a complicated oxygen environment with different aerobic, facultative, and anaerobic oxygen zones that easily occur in the rhizoplane and water, which played an essential role in rhizosphere bacterial community richness and diversity. The HRT and HLR were the major factors explaining the phylum and nitrogen-related genera variations. More nitrifying and denitrifying bacteria appeared in the wet season, and alternating aerobic and anaerobic conditions occur in the water quantity fluctuation in the wet and dry seasons, resulting in coupled nitrification and denitrification, which explains the higher nitrogen removal in the lakeshore CWs. The results provide a scientific basis for the optimal design of constructed wetlands in lakeshores.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11092781/s1. Figure S1: Rainfall, evaporation and influent amount in 2021; Figure S2: Photographs of the cells in the lakeshore of MCW; Figure S3: Comparisons of EP in wet and dry seasons; Figure S4: The nitrogen concentrations of the influent along the flowing rate. (**a**) TN; (**b**) NO_3^--N; (**c**) NH_4^+-N; Figure S5: The visualization of beta diversity and analysis of similarities (ANOSIM); Figure S6: Relative abundances of the top 100 genera in various sediments of cells; Table S1: Basic information of the multi-cell constructed wetlands; Table S2: Land use in the catchment around the lakeshore MCW; Table S3: Monthly nitrogen concentration and removal performance of the lakeshore MCW; Table S4: Sequencing statistics for the sediment samples of lakeshore MCW; Table S5: Microbial diversity indices for the cells of lakeshore MCW; Table S6: Microbial diversity indices for the cells of lakeshore MCW.

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