



Article Environmental Factors Drive Periphytic Algal Community Assembly in the Largest Long-Distance Water Diversion Channel

Yuxuan Zhu^{1,2}, Wujuan Mi¹, Xiaojie Tu¹, Gaofei Song¹ and Yonghong Bi^{1,*}

- State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China; zhuyuxuan1991@126.com (Y.Z.); miwj@ihb.ac.cn (W.M.); tuxj@ihb.ac.cn (X.T.); song@ihb.ac.cn (G.S.)
- ² University of Chinese Academy of Sciences, Beijing 100049, China
- * Correspondence: biyh@ihb.ac.cn; Tel.: +86-27-687-800-16

Abstract: Periphytic algae exist widely in different waters. However, little is known about periphytic algae in long-distance water diversion channels across watersheds. We investigated the periphytic algae and the environmental factors at twenty sampling sites in the middle route of the South-to-North Water Diversion Project (MRP). The dominant species were Desmodesmus intermedius (Hegewald), Calothrix thermalis (Bornet & Flahault), Calothrix parietina (Bornet & Flahault) and Leptolyngbya benthonica (Anagnostidis) (dominance > 0.02) as measured in a whole year. Habitat heterogeneity in the MRP led to lower spatial heterogeneity and higher temporal heterogeneity of the periphytic algal community. Stochastic processes are the major process in periphytic community assembly. In deterministic processes, homogeneous selection had the major role in structuring the periphytic community, whereas the role of heterogeneous selection was less significant. In stochastic processes, dispersal limitations had the major role in structuring the periphytic community, whereas the role of homogenizing dispersal and drift were less significant. The variation in total nitrogen and total phosphorus promoted more stochastic processes ($-1.96 < \beta NTI < 1.96$). The variations in water temperature and water velocity promoted more heterogeneous selection (β NTI > 1.96). In integrating all of this empirical evidence, we explore the role of environmental factors in the action of ecological processes shaping the community assembly of the periphytic algal community.

Keywords: artificial diversion channel; periphytic algal community; environmental heterogeneity; stochastic process; deterministic process

1. Introduction

The definition of periphytic algae is algae living attached to any substrate under water like submerged plants or plant parts, rocks, and sediment [1,2]. Periphytic algae is an important part of an aquatic ecosystem and participates in the material cycle, energy flow and food web of the ecosystem [3–5]. As the food source of aquatic organisms, it sometimes provides more resources than phytoplankton and higher aquatic plants [6–8]. Moreover, periphytic algae can provide habitats for the growth, development, survival and reproduction of a large number of aquatic animals [9,10]. The dynamics of periphytic algae can be mainly affected by biotic and abiotic factors, such as interspecies competition, grazing, nutrient concentration and nutrient availability, water velocity, substrate type, light and temperature [11–13]. Periphytic algae grow by attaching to the substrate, but can't obtain a favorable living environment and avoid adverse environmental changes through motion like planktonic algae, so periphytic algae are more sensitive to environmental dynamics [14,15]. Therefore, changes in the periphytic algae community can be used to indicate the variation in aquatic ecosystems [16]. In response to the spatiotemporal dynamics of the habitat, the communities of periphytic algae show corresponding spatiotemporal



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). dynamics [17,18]. It can be confirmed that periphytic algae play an important role in the maintenance of ecosystems, and research in periphytic algal community ecology is helpful to deepen the understanding of aquatic ecosystems.

Understanding the mechanisms of assembly can effectively help us understand the ecology of the periphytic community [19,20]. It is well known that both deterministic and stochastic processes play pivotal roles in regulating the assembly of ecological communities [21–23]. Community assembly is driven by deterministic processes, stochastic processes, or the interaction between the two types of ecological processes [24–26]. Deterministic processes include "homogeneous selection" and "heterogeneous selection", and they lead to more similar or dissimilar structures among communities under the homogeneous or heterogeneous environment [22,26]. Stochastic processes include "homogenizing dispersal", "dispersal limitation" and "drift and others"; homogenizing dispersal lead a low turnover within communities, dispersal limitations increase community turnover, drift and others influences changes in communities due to random birth, death, and reproduction, independently of species fitness [26–28]. The interaction between deterministic and stochastic processes may arise when the timing of the arrival of species in the community, an intrinsically stochastic type of event, leads to multiple equilibria through more deterministic succession or priority effects [24,29]. Environmental factors are important drivers for shaping diversity and community structures and driving community assembly [23]. Most studies on the periphytic algal community include the relationship between environmental factors and algae, but few involve the relationship between environmental factors and community assembly [11–13]. Interspecies interaction is a key point in the study of periphytic algae and can affect the succession process through facilitation, tolerance and inhibition effects, but few involve the relationship between interspecies interaction and periphytic community assembly. [30,31]. Therefore, elucidating the relative impacts of stochastic and deterministic processes on the community and underlying factors is essential to reach a comprehensive understanding of periphytic algal community assembly.

Due to artificial disturbance, the spatiotemporal characteristics of habitats in manmade waterbodies (such as water temperature, nutrition and hydraulic condition, etc.) are often different from those in natural waterbodies, and always lead to different periphytic algal communities [32,33]. The long-distance water diversion channel is a kind of man-made waterbody that transfers clean drinking water for a large number of people [34,35]. As an important member of aquatic ecosystems, periphytic algae is closely related to the safety of water transportation [36]. The middle route of the south-to-north water diversion project is the world's largest long-distance water diversion channel; this project has a total length of 1432 km, with the function of transferring drinking water from the Danjiangkou reservoir to Henan, Hebei, Beijing and Tianjin, which could service nearly 200 million people [37]. As a drinking water provider, $TN \le 1.8 \text{ mg/L}$ and $TP \le 0.02 \text{ mg/L}$ in MRP [38,39]. Construction of MRP began in 2003, and the drinking water supply began in 2014, with periphytic algae thriving in the channel over the next four years in MRP [37,40]. Dead periphytic algae and planktonic algae accumulate at the end outlets. The silted algal biomass increased the severity of the drinking water pretreatment, which caused the management departments to regularly remove silt. If dredging could not be done in time, it might also bring the risk of odorous compounds, black and odorous water, and even algal toxins [41–43]. On the other hand, there are few data on algae in MRP, especially periphytic algae, which makes it difficult to reveal periphytic algal community assembly. To ensure the safety of water conveyance and understand the changes in MRP, exploration of the relative impacts of assembly processes on the periphytic algal community and the relationship with underlying factors is necessary.

In this study, we hypothesize that the influence of different ecological processes shaping periphytic algal communities will depend mainly on the system's environmental factors. To test this hypothesis, we investigated the community structure, spatial and temporal patterns of periphytic algae in the MRP, and the spatiotemporal changes in the community were demonstrated. We analyzed the processes shaping periphytic algal community structure using the approach proposed by Stegen et al. [22]. The effects of stochastic and deterministic processes on the periphytic algal community were elucidated, and their driving factors were identified. Then, we constructed co-occurrence networks to explore how ecological processes affect putative interactions between taxa. The aim of this study was: (i) to identify important periphytic algae, and (ii) to determine the relative importance of the assembly processes structuring the periphytic algal community.

2. Materials and Methods

2.1. Site Stations and Samples Collection

Periphytic algal samples were collected in the first half (May 2019) and the second half (October 2018) of the year at 20 sampling sites along the main route of the MRP (Figure 1 and Supplementary Table S1). Sampling sites P1–P20, 'H1' and 'H2' were added after the names of the sampling sites to denote samples of the first half of the year and the second half of the year, respectively.



Figure 1. Sketch map of sampling sites in the main route of the middle route of the South-to-North Water Diversion Project in China.

Periphytic algal samples were collected from the side slope below the water surface area at 0.35 m depth. The algal sample collection procedure was to remove large algae pieces from the substrate with a spade, divide the large pieces into small pieces of 10 cm² with a knife, put each small piece into a white porcelain dish, and to stir it evenly with 100 mL of pure water. At each sampling site, four pieces of a 10 cm² algal sample were collected, of which three pieces of algal samples were stored at -80 °C. DNA extraction and a piece of algal sample was stored at 4 °C for determination of chlorophyll a. In addition, subsurface (0.35 m depth) water samples (1.5 L) were collected synchronously for chemical analysis in 20 sampling sites (Figure 1), water samples were filtered by GF/C glass microfiber filters (Whatman, Germany), and the filtrate was then stored in sterilized collection bottles at 4 °C until analyses.

2.2. Environmental Parameters Determination

When collecting algae in the 20 sampling sites (Figure 1), water temperature (WT), pH, turbidity (Tur), specific conductivity (SPC), and dissolved oxygen (DO) were monitored

by YSI (ProPlus, Yellow Springs, OH, USA), and water velocity (V) was monitored by a flowmeter (Flowatch, Yverdon-les-Bains, Switzerland). Periphytic algal chlorophyll a was determined according to the standard practices for measurement of chlorophyll content of algae in surface waters (ASTM D3731-87 (2012)) [44]. Other environmental parameters of the water sample, such as total nitrogen (TN), nitrate nitrogen ($NO^{3-}-N$), ammonium nitrogen (NH_3-N), total phosphorus (TP), orthophosphate ($PO_4^{3-}-P$), and potassium permanganate index (COD_{Mn}), were determined according to APHA [45].

2.3. Molecular Analyses

In the laboratory, periphytic algal DNA was extracted with an E.Z.N.A.® Soil/Stool DNA Kit (Omega Biotek, Norcross, GA, USA) according to the manufacturer's instructions. DNA concentrations and quality levels were measured with an ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). PCRs were performed in triplicate in a 20 μ L mixture containing 4 μ L of 5 \times FastPfu Buffer, 2 μ L of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu Polymerase, 0.2 µL of bovine serum albumin (BSA) and 10 ng of template DNA. The PCR parameter was 95 °C for 3 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s and a final extension at 72 °C for 10 min. Cyanobacteria V3-V4 hypervariable region of the 16S rRNA were amplified by PCR using primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and eukaryotic algae V4 hypervariable region of the 18S rRNA were amplified by PCR using primers 3NDF (5'-GGCAAGTCTGGTGCCAG-3') and V4_euk_R2 (5'-ACGGTATCT(AG)ATC(AG)TCTTCG-3'), where the barcode was an eight-base sequence unique to each sample. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions, and quantified using QuantiFluorTM-ST (Promega, Madison, WI, USA). Paired-end sequencing for PCR products was conducted on Illumina MiSeq (Illumina, San Diego, CA, USA) as previously described [46]. The overlapping paired-end reads were joined with FLASH using the default setting [47]. The merged sequences were subsequently quality filtered and analyzed with QIIME 1.9.1 [48]. Chimera sequences were removed using USEARCH 7.0 [49]. The remaining sequences were clustered into operational taxonomic units (OTUs, 97% similarity level) using UPARSE 7.0 [50]. The representative sequence of each OTU was taxonomically annotated based on the SILVA 128 database. After taxonomies had been assigned, OTUs that were affiliated with archaea, chloroplasts, unclassified (not affiliated with bacteria), and singleton OTUs were removed from the dataset. In H1, 151,259 reads of cyanobacteria were produced, with an average of 7961 reads per sample, and 131 species of cyanobacteria were identified. In H2, 121,087 reads of cyanobacteria were produced, with an average of 6373 reads per sample, and 140 species of cyanobacteria were identified. In H1, 148,390 reads of eukaryotic algae were produced, with an average of 7810 reads per sample, and 82 species of eukaryotic algae were identified. In H2, 109,098 reads of eukaryotic algae were produced, with an average of 5742 reads per sample, and 81 species of eukaryotic algae were identified.

2.4. Environmental Heterogeneity

Environmental heterogeneity was estimated by computing the average dissimilarity between sites based on environmental factors [51]. For each sampling phase, we computed a Euclidean distance matrix (Vegan package, R) and calculated the dissimilarity between sites (*Ed*) as follows:

$$Ed = \left(\frac{Euc}{Euc_{max}}\right) + 0.001\tag{1}$$

where *Euc* is the Euclidean distance between two sites and *Euc_{max}* corresponds to the maximum Euclidean distance considering all the pairwise distances in the overall dataset. 0.001 was added to account for zero similarity between sites [52]. Then, we calculated the mean Ed of each computed similarity matrix and used it as an index of environmental hetero-

geneity in H1, H2 and for the whole year (Supplementary Table S2). We compared the mean of ranked dissimilarities between phases (H1 and H2) to the mean of ranked dissimilarities within phases with analysis of similarities (ANOSIM) (Supplementary Table S2). In addition, we calculated the coefficient of variation (CV%) for each variable and hydrological phase, as the standard deviation divided by the mean of each variable (Supplementary Table S3).

To test whether the environmental conditions in the sampling phases and sampling sites differed significantly, we performed permutational multivariate analysis of variance (PERMANOVA) (Supplementary Table S4). We first tested for homogeneity of multivariate dispersion (PERMDISP) with the betadisper function (Vegan package, R), which compares the within-group spread among groups using the average value of the individual observation distances to the centroid of the own group. Differences in environmental conditions were also analyzed with performed permutational multivariate analysis of variance (PERMANOVA) for testing the effects of phases (adonis function in Vegan package, R) [52].

2.5. Periphytic Algal Community Structure

The effect of environmental factors on periphytic algal community structures was assessed by calculating the community turnover in each sampling phase. We computed a Bray-Curtis matrix on the basis of OTU normalized abundances for each sampling phase to calculate the dissimilarity (Xd) between the local communities as follows:

$$Xd = \left(\frac{Bray}{Bray_{max}}\right) + 0.001\tag{2}$$

where *Bray* is the Bray-Curtis dissimilarity between two communities and *Bray*_{max} corresponds to the maximum Bray-Curtis dissimilarity considering the overall dataset. Next, we calculated the mean Xd of each matrix computed, which was used as a value of community turnover.

To test whether the community structure in the sampling phases and sampling sites differed significantly, we performed permutational multivariate analysis of variance (PER-MANOVA) (Supplementary Table S4). Since PERMDISP was significant, we ran the PER-MANOVA with betadisper function (Vegan package, R). We additionally calculated the Whittaker index to explore the β -diversity (Past software V4). To visualize the taxonomic similarity across local communities, a Principal Co-ordinates Analysis (PCoA) was performed using the Bray-Curtis metric (Vegan package, R).

2.6. Networks Analysis

Network analysis represents an approach for exploring and identifying patterns in large, complex datasets and patterns that may be more difficult to detect using the standard alpha/beta diversity metrics widely used in microbial ecology. To screen the interspecies interaction of periphytic algae, network analysis was used to explore cooccurrence patterns in the algal community. To describe the topology of the resulting network, the average path length and modularity were calculated.

The average path length $C_B(v)$ was calculated as follows:

$$\sigma_{st}(v) = \begin{cases} 0 & if \ d_G(s,t) < d_G(s,v) + d_G(v,t) \\ \sigma_{sv} \cdot \sigma_{vt} & otherwise \end{cases}$$
(3)

$$\delta_{st}(v) = \sigma_{st}(v) / \sigma_{st} \tag{4}$$

$$C_B(v) = \sum_{s \neq v \neq t \in V} \delta_{st}(v) \tag{5}$$

Networks are conveniently described as a graph G = (V, E), where the set V of vertices represents actors, and the set E of edges represents links between actors. A path was defined from $s \in V$ to $t \in V$ as an alternating sequence of vertices and edges, beginning with s and ending with t, such that each edge connects its preceding with its succeeding vertex.

The modularity *Q* was calculated as follows:

$$Q = \frac{1}{2m} \sum_{i,j} \left[A_{ij} - \frac{k_i k_j}{2m} \right] \delta(c_i, c_j)$$
(6)

where A_{ij} represents the weight of the edge between *i* and *j*, $k_i = \sum_j A_{ij}$ is the sum of the weights of the edges attached to vertex *i*, c_i is the community to which vertex *i* is assigned, the δ function $\delta(u, v)$ is 1 if u = v and 0 otherwise and $m = \frac{1}{2} \sum_{ij} A_{ij}$.

Network analysis was conducted based on Spearman's correlations calculated using SPSS 24.0 and visualized using Gephi 0.9.2.

2.7. Analysis of Periphytic Algal Community Assembly

Null-modelling based approaches were preferentially performed in this study to infer community assembly mechanisms. The null model analysis was conducted following the framework described by previous research. The phylogenetic β -diversity was quantified using beta mean nearest taxon distance (BMNTD) and beta nearest taxon index (BNTI) by using the 'picante' package [22,53,54]. β MNTD represents the phylogenetic distance between each OTU in one community and its closest relative in a second community, and β NTI quantifies the difference between the observed β MNTD and the null distribution of β MNTD. A value of $|\beta$ NTI| > 1.96 indicates that the community assembly is governed primarily by deterministic processes, which can be divided into homogeneous selection (β NTI < -1.96, leading to similar community structures in similar environments) and heterogeneous selection (β NTI > 1.96, leading to dissimilar community structures in heterogeneous conditions). When $|\beta NTI| < 1.96$, the value suggests that the community compositions are the result of stochastic processes. Additionally, The Bray-Curtis based Raup-Crick (RC_{brav}), the deviation between the observed Bray–Curtis and the null distribution was calculated to further partition the stochastic processes [22]. A value of |RC_{bray}| > 0.95 indicates that homogenizing dispersal ($RC_{bray} < -0.95$) or dispersal limitation ($RC_{bray} > 0.95$) drives compositional turnover. If $|\beta NTI| < 1.96$ and $|RC_{bray}| < 0.95$, this estimates the influence of 'drift and others' assembly, such as weak selection, weak dispersal, diversification, and/or drift [21,55]. Furthermore, a modified framework to quantitatively infer community assembly mechanisms by phylogenetic-bin-based null model analysis (iCAMP), based on the turnovers of individual bins across communities, was used to quantify the relative importance of ecological processes in controlling microbial community diversity by using the 'iCAMP' package [56].

The relevant calculation was as follows:

$$MNTD = \sum_{ik=1}^{nk} fikmin(\Delta ikjk)$$
(7)

$$NTI = (mean(MNTD_{null}) - MNTD_{obs})/sd(MNTD_{null})$$
(8)

$$\beta \text{MNTD} = 0.5 \left[\sum_{ik=1}^{nk} \min(\Delta i k j m) + \sum_{im=1}^{nm} \min(\Delta i m j k) \right]$$
(9)

$$\beta NTI = (\beta MNTD_{obs} - mean(\beta MNTD_{null})) / sd(\beta MNTD_{null})$$
(10)

where *fik* represents the relative abundance of OTU_i in community *k*, *nk* is the number of OTUs in community *k*, $\Delta ikjk$ is the minimum phylogenetic distance between OTU_i and all other OTU_j in community *k*, and min($\Delta ikjm$) is the minimum phylogenetic distance between OTU_i in community *k* and all OTU_j in community m.

The normalized stochasticity ratio (NST) was further applied to evaluate the microbial community assembly. The relative importance of deterministic and stochastic processes can be quantified by the index NST with 50% as the boundary point between more deterministic (NST < 50%) and more stochastic (NST > 50%). The NST estimated the relative importance of stochastic processes in community structure and was tested with simulated communities

by considering abiotic filtering, competition, environmental noise, and spatial scales. NST values based on Bray–Curtis (tNST_{bray}) dissimilarity were calculated by using the 'iCAMP' package [56].

2.8. Statistical Analysis

The sampling diagram was drawn using ArcGIS 10.4. Dominance and niche analysis was performed with the package "spaa" in R 4.0.5. Principal Coordinate Analysis (PCoA) was used to measure the similarity between the samples based on OTU level with the package "Vegan" in R 4.0.0. The Wilcoxon test (nonparametric method) was used to compare the physicochemical temporal variations of the water and alpha diversity indices. Alpha diversity indices were calculated using Past 3. A redundancy analysis (RDA) was performed using CANOCO 4.5. Raw data were uploaded as FASTQ files registered to the National Center for Biotechnology Information (NCBI) under bioproject number PRJNA706557.

3. Results

3.1. Characteristics of Periphytic Algal Community

The relative abundances of *Calothrix parietina* (Bornet & Flahault), *Calothrix thermalis* (Bornet & Flahault), *Leptolyngbya benthonica* (Anagnostidis), *Cyanobium distomicola* (Rippka & Cohen-Bazire.), *Nodosilinea epilithica* (Perkerson & Casamatta) and *Microseira minor* (Geng & Yu) were higher in cyanobacteria (Figure 2a,b). In eukaryotic algae, *Desmodesmus intermedius* (Hegewald), *Ulnaria ulna* (Compère), *Monactinus sturmii* (Jena & Bock), *Navicula phyllepta* (Kützing), *Ulothrix zonata* (Kützing) and *Spirogyra crassispina* (Jao) had higher relative abundance (Figure 2c,d).



Figure 2. Periphytic algal relative abundance ((**a**) H1 cyanobacteria; (**b**) H2 cyanobacteria; (**c**) H1 eukaryotic algae; (**d**) H2 eukaryotic algae).

There were 15 dominant periphytic algal species in the community (Dominance > 0.02) (Table 1). The niche width of these dominant species was larger than that of nondominant species (Table 1). Filamentous algae accounted for a higher proportion of dominant species, such as *Spirogyra crassispina*, *Calothrix thermalis*, and *Leptolyngbya benthonica*.

	H1			H2	
Dominant Species	Dominance	Niche Width	Dominant Species	Dominance	Niche Width
Desmodesmus intermedius	0.257	5.317	Desmodesmus intermedius	0.204	5.666
Calothrix thermalis	0.179	2.900	Calothrix thermalis	0.157	2.301
Calothrix parietina	0.168	7.481	Leptolyngbya benthonica	0.130	14.845
Ulnaria ulna	0.107	2.711	Cyanobium distomicola	0.129	13.066
Leptolyngbya benthonica	0.046	11.096	Calothrix parietina	0.053	4.220
Spirogyra crassispina	0.045	4.732	Microseira minor	0.037	4.182
Monactinus sturmii	0.035	5.618	Leptolyngbya valderiana	0.035	2.807
Microseira minor	0.026	6.393	Nodosilinea epilithica	0.031	10.410
Navicula phyllepta	0.026	10.880	Ulothrix zonata	0.027	3.437
			Microcoleus amoenus	0.023	11.109
			Leptolyngbya foveolarum	0.022	10.958
nondominant species	average = 0.001	average = 6.194	nondominant species	average = 0.004	average = 4.287

Table 1. Dominant species of periphytic algal communities.

Community richness (Chao1) and community coverage (Coverage) of algal communities showed upward trends along the MRP, while community evenness (Pielou) and community diversity (Shannon) showed downward trends along the MRP (Supplementary Table S2).

The relative abundance of periphytic algae has a spatial difference: *Calothrix parietina, Calothrix thermalis, Microseira minor, Spirogyra crassispina* and *Ulothrix zonata* preferentially distributed at the lower latitude sample sites, while *Leptolyngbya benthonica, Cyanobium distomicola, Nodosilinea epilithica* and *Monactinus sturmii* preferentially distributed at the higher latitude sample sites. The dominance of periphytic algae has a temporal difference: dissimilarity tests confirmed that dominant species between the first half year (H1) and the second half year (H2) were significantly different (Figures 3 and S2); *Calothrix parietina, Ulnaria ulna, Desmodesmus intermedius, Monactinus sturmii, Spirogyra crassispina, Calothrix thermalis* and *Navicula phyllepta* were the dominant species in H1, whereas H2 was dominated by *Microseira minor, Nodosilinea epilithica, Leptolyngbya foveolarum* (Anagnostidis & Komárek), *Microcoleus amoenus* (Komárek & Johansen), *Leptolyngbya valderiana* (Anagnostidis & Komárek), *Leptolyngbya benthonica* and *Cyanobium distomicola* (Figures 3 and S2).



Figure 3. Comparison of differences in the temporal distribution of dominant species (**a**) relative abundance (**b**) difference in relative abundance.

The community structure according to species abundance varied with significant differences between H1 and H2 (PERMANOVA, F = 5.5657, p = 0.001, Supplementary Table S4). In PCoA, local communities were more similarly grouped in a distinct cluster in H2. In contrast, those from H1 were more spread (Supplementary Figure S1). The community turnover (*Xd*) varied markedly between H1 and H2, being significantly lower (PERMANOVA, p < 0.005) in H1 (*Xd* = 0.71) than in H2 (*Xd* = 0.81) (Supplementary Tables S2 and S4).

The alpha diversity of algal communities had spatial heterogeneity: the community richness in P15–P20, the community coverage in P5–P15, the community evenness in P10–P15 and the community diversity in P10–P15 were significantly different from those of other samples (p < 0.05). The alpha diversity of algal communities also had temporal difference: community richness and community coverage were higher in H1 than in H2, and community evenness and community diversity were higher in H2 than in H1 (Supplementary Table S5).

3.2. Periphytic Algal Community Associations

In Figure 4a, species in positive correlations were generated into some relatively independent groups; the average path length was 1.636 and the modularity was 0.684. This result suggested that the network had a modular structure. In the negative correlation network, the average path length was 3.379, and the modularity was 0.018. There were some key nodes shown in the negative correlations (Figure 4b). This result suggested that the network had no modular structure, and nodes tend to disperse in the negative correlation network.



Figure 4. Co-occurrence network of the periphytic algal communities in H1 and H2 (**a**) positive correlation network (**b**) negative correlation network. Each node represents an individual species, and the species belonging to the same phylum are displayed in the same color. The sizes of nodes reflected their connecting degrees. The higher the degree, the more connections there were between this species and other neighboring species. Each edge between two nodes represents positive or negative interactions between the two species. A connection stands for a significant (|r| > 0.7, p < 0.05).

Compared with positive associations, the negative associations among species in periphytic algal communities showed a more complex and tighter network structure with a higher average connectivity and clustering coefficient, shorter harmonic geodesic distance and smaller modularity (Figure 4). Moreover, the negative interactions in periphytic algal communities were clustered between different species, and the positive interactions in periphytic algal communities were clustered within modules possessing the same taxonomic affiliations (Figure 4). In both positive and negative associations, dominant species occupy key positions with higher centrality than nondominant species, and the difference in niche width between dominant and nondominant species also supports this view (Figure 4 and Table 1).

3.3. Periphytic Algal Community Assembly

The large proportion of stochasticity in dominant species and nondominant species communities (79.32% and 68.08%) suggested that stochastic processes exerted a greater influence on the community (Figure 5a). In deterministic processes, homogeneous selection had a major role in structuring the periphytic community, whereas the role of heterogeneous selection was less significant (Figure 5a). In stochastic processes, dispersal limitations had the major role in structuring the periphytic community, whereas the role of homogenizing dispersal and drift were less significant (Figure 5a). Compared with dominant species, determinism processes exerted a greater influence on nondominant species, and the proportion of homogeneous selection in dominant species (14.77%) was lower than that in nondominant species (29.97%) (Figure 5a).

The average environmental dissimilarity between sites (*Ed*) as well as the CV% of the 12 environmental factors indicated a clear trend to higher spatial homogenization from H1 to H2 (Supplementary Tables S2 and S3). The H1 and H2 were significantly different according to their environmental characteristics (PERMANOVA F = 341.95, p = 0.001, Supplementary Figure S2 and Tables S2 and S4). The Monte Carlo unrestricted permutation test identified 11 environmental factors (Supplementary Figure S3) as significant influencing factors for the periphytic algal communities (p < 0.05). Total nitrogen and total phosphorus were positively related to chlorophyll a, community evenness and community diversity, but were negatively related to chlorophyll related to community richness and community richness and community coverage but negatively related to chlorophyll a, community evenness and community diversity (Figure 5b).

Based on RDA, a subset of environmental factors (total nitrogen, total phosphorus, water temperature and water velocity) was selected to identify the relationship between β NTI and the variation in environmental factors (Figure 5b and Supplementary Figure S4). β NTI was significantly negatively correlated with the variation in total nitrogen and total phosphorus (Figure 5c,d), indicating that the variation in total nitrogen and total phosphorus led to a shift from heterogeneous selection (β NTI > 1.96) to stochastic processes ($-1.96 < \beta$ NTI < 1.96). β NTI was significantly positively correlated with variations in water temperature and water velocity (Figure 5e,f), indicating that variations in water temperature and water velocity led to a shift from stochastic processes ($-1.96 < \beta$ NTI < 1.96) to heterogeneous selection (β NTI > 1.96).



Figure 5. Cont.



Figure 5. Relative influences of deterministic and stochastic processes in structuring the periphytic algal community. (**a**) The fraction of ecological processes (deterministic: homogeneous and heterogeneous selection; stochastic: dispersal limitations and homogenizing dispersal; drift and others) governing the community assembly in the dominant species and nondominant species. (**b**) Redundancy analysis ordination plot for the first two principal dimensions. (**c**) Relationships between β NTI and differences in total nitrogen. (**d**) Relationships between β NTI and differences in total phosphorus. (**e**) Relationships between β NTI and differences in water velocity. The value of $|\beta$ NTI| > 1.96 indicated that the community assembly was governed primarily by deterministic processes, which could be divided into homogeneous selection (β NTI < -1.96, leading to similar community structures in similar environments) and heterogeneous selection (β NTI < 1.96, the value suggested that the community compositions were the result of stochastic processes. Linear regression models (shown as red lines) and associated correlation coefficients and *p* values are provided in each panel.

4. Discussion

4.1. Periphytic Algal Communities in the MRP

The cyanobacteria in MRP were also commonly found in rivers, e.g., *Calothrix* were observed in 80% USA rivers, *Calothrix, Cyanobium, Leptolyngbya* were observed in the upper Paraná River, *Calothrix, Leptolyngbya* were observed in seven rivers of different continents [10,57,58]. The eukaryotic algae in MRP were generally found in rivers or streams, e.g., *Navicula, Fragilaria, Achnanthes* were observed in Weihe RiverBasin, *Ulnaria, Navicula, Cymbella* were observed in six rivers below the Dujiangyan irrigation project, *Desmodesmus, Ulothrix, Ulnaria, Navicula, Spirogyra* were observed in various streams [59–61]. Several species of periphytic algae in MRP prefer to grow on rock surfaces in rivers or streams rather than lakes, e.g., *Calothrix thermalis, Leptolyngbya valderiana*, and *Gleocapsa gigas* [62,63]. That is to say, the habitat provided by MRP for periphytic algae was similar to rivers or streams. Previous studies have shown that the structure of algal communities in different

habitats is different [64,65]. It could be judged that the MRP is a river-type waterbody based on the periphytic algae community.

ANOSIM and PERMDISP tests that were performed on the community structure spatial heterogeneity found that different regions of MRP had no significant difference in community structure (Supplementary Tables S4 and S5); this is different from the spatial heterogeneity of community structure in natural rivers [66,67]. Several studies have found evidence of the pivotal role of the variation of environmental factors in determining community structure, and an increase in its relative importance as environmental heterogeneity increases and the community structures become more heterogeneous [21,68,69]. Furthermore, ANOSIM and PERMDISP tests on the environmental spatial heterogeneity found that no significant difference of environmental factors between different regions of MRP (Supplementary Tables S4 and S5). The relatively small environmental spatial heterogeneity of MRP leads to the fact that the spatial heterogeneity of the periphytic algal community structure is smaller than that of natural rivers.

The temporal heterogeneity of the community structure was tested with ANOSIM and PERMDISP, community structure of different phases (H1 and H2) with significant difference (Supplementary Figure S1 and Tables S4 and S5), this was similar to natural rivers [70–72]. Previous studies have found evidence of the pivotal role of environmental temporal heterogeneity in determining biofilm community structure, environmental temporal heterogeneity increase and its relative importance increase, and temporal heterogeneity of biofilm community structures increase [71,73,74]. Next, we tested environmental temporal heterogeneity with ANOSIM and PERMDISP and found a significant difference of environmental factors between different phases (Supplementary Figure S1 and Tables S4 and S5). The environmental temporal heterogeneity of MRP leads to the temporal heterogeneity of the periphytic algal community.

4.2. Periphytic Algal Community Assembly Processes

Stochastic processes include weak selection, dispersal limitation, diversification, and drift, and are not the consequence of environmentally determined fitness [21]. Dispersal limitation signifies the circumstance that low dispersal rates could increase community richness variations [22,26]. When the environmental conditions were suitable for the coexistence of more species, the community suffered less selection pressure, and the assembly was mainly governed by stochastic processes [75,76]. The results of this study showed that the variation in total nitrogen and total phosphorus promoted more stochastic processes, the periphytic algal community assembly was mainly affected by dispersal limitation, and community richness increased, suggesting that the MRP provided a relatively suitable growth environment for periphytic algae, and a low dispersal rate increased the community richness.

The deterministic process was the result of ecological filtering (environmental filtering and biological filtering) [29,77]. Existing research results showed that environmental filtering drove the community to be more similar under homogeneous environmental conditions or more dissimilar if the environment was heterogeneous, while biological filtration generally drove the communities to be more dissimilar [78,79]. The results of this study showed that deterministic processes had a greater impact on nondominant species than dominant species, and homogenous selection had a greater impact on nondominant species than heterogeneous selection.

Environmental factors affect communities through environmental filtering [80]. Environmental filtering exerts more selective pressure on algae and affects niches, interspecies interactions and community assembly [81,82]. The MRP ecosystem has stronger selective pressure than natural aquatic ecosystems, as confirmed by narrower niche widths of periphytic algae than those in natural aquatic environments [83,84]. Stronger selective pressure led to interspecies interactions of communities tending to be negative interactions rather than positive ones [80,83]. Community assembly was also affected by stronger selective pressure in MRP ecosystems, as confirmed by higher NTI values (Supplementary Table S6)

in MRP ecosystems compared to NTI < 1.8 in streams and NTI < 1 in wetlands [85,86]. This result indicated that periphytic algae in environments with relative lower nutrient availability were undergoing stronger selection pressure than those in normal aquatic environments, and selection pressure influenced the assembly of the algal community.

Temperature was a key factor affecting periphytic algae [87–89]. Temperature could make a direct difference to the metabolism and growth of algae [3,90] and enhance the dissolution of mineral elements and accelerate the rate of redox reactions, which could influence the algal community [89,91]. In the MRP, when the variation in water temperature was less than 5 °C, deterministic processes dominated the community assembly, and the existing species in the community competed for ecological resources, which manifested as homogenous selection and heterogeneous selection [3]. When the variation in water temperature was more than 5 °C, stochastic processes dominated the community assembly, and many species could not adapt when the temperature change was drastic, and the vacant niche was randomly filled by new species which manifested as dispersal and drift [31]. Therefore, temperature could directly and indirectly affect the physiological activities of periphytic algae, thus mediating the balance of stochastic and deterministic assembly for algal communities in the MRP.

Water velocity exerts selective pressures on cyanobacteria and eukaryotic algae, primarily because different algae need different water velocities to complete colonization and suitable growth [92,93]. In the MRP, when the variation in water velocity change was less than 0.04 m/s, deterministic processes dominated the assembly, species were less affected by current scour, and homogenous selection and heterogeneous selection had a greater impact on community assembly [94,95]. When the variation in water velocity was more than 0.04 m/s, stochastic processes dominated the community assembly, large amounts of algae were stripped when the water velocity changed drastically, the vacant niche was randomly filled by new species, and dispersal and drift had a greater impact on the assembly of the algal community [31,95]. Water velocity mediated the balance of the stochastic and deterministic assembly by affecting the attachment stability of periphytic algae.

In this study, with the increase of environmental heterogeneity, the impact of stochastic process on community increased, and community structure heterogeneity, community turnover and network connectedness all increased accordingly (Supplementary Figure S5). There is a general view that deterministic process is weakened by environmental homogenization [96–98]. We predict that with the further increase of environmental heterogeneity, especially spatial heterogeneity, the dynamics of periphytic communities in MRP will become more rapid and diversified, and the prediction and management of periphytic algae will become more difficult. Death of algae during community changes was a potential threat to drinking water delivery, which is not conducive to maintaining stable water quality in an artificial channel [99,100]. Therefore, maintaining the stability of MRP's environment is what its managers need to do.

5. Conclusions

The MRP provided a habitat for periphytic algae similar to rivers or streams. The dominant species were *Desmodesmus intermedius*, *Calothrix thermalis*, *Calothrix parietina* and *Leptolyngbya benthonica*. Environmental heterogeneity in the MRP led to lower spatial heterogeneity and higher temporal heterogeneity of the periphytic algal community. Stochastic processes were the major process community assembly. Deterministic processes mainly affected the nondominant periphytic algae through the homogenous process of environmental filtration in the MRP. It was identified that nutrient availability; water temperature and water velocity were important factors affecting the assembly. With the increase of environmental heterogeneity, the impact of stochastic process on community increased, and community structure heterogeneity, community turnover and network connectedness all increased accordingly.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/w14060914/s1. Figure S1: Principal coordinate analysis (PCoA) of periphytic algal communities. Figure S2: The values and trend of environmental parameters. Figure S3: Co-occurrence network of the periphytic algal communities. Figure S4: Redundancy analysis in H1 and H2. Figure S5: Environmental heterogeneity determines the action of the ecological processes assembling the periphytic algal community. Table S1: Latitude and longitude of sampling sites. Table S2: Environmental heterogeneity and community structure heterogeneity. Table S3: Coefficient of variation (CV%) of each environmental parameter. Table S4: Result of PERMDISP test and PERMANOVA test. Table S5: Alpha diversity index of periphytic algal communities. Table S6: Mean-nearest-taxon-distance and nearest-taxon-index.

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