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Dynamic Characteristics of Periphytic Algae Communities on Different Substrates and the Host Response in Subtropical-Urban-Landscape Lakes

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Abstract: Outbreaks of periphytic algae, including filamentous algae, have been observed after submerged macrophyte restoration and are common in early stages. Dynamic changes in the periphytic algae community on *Vallisneria natans* and artificial *V. natans* were investigated in situ, and their characteristics were compared on the two substrates. The results showed that more periphytic algae species occurred on *V. natans* (77 taxa) than on artificial *V. natans* (66 taxa) (F = 2.089, $p = 0.047$). The cell density and chlorophyll a (Chl. a) content of periphytic algae were 3.42–202.62-fold and 2.07–15.50-fold higher on the artificial substrate than on *V. natans*, respectively. Except for *Lyngbya perelagans* (i.e., the only common dominant periphytic algae species on the two substrates), the dominant species on *V. natans* were *Cocconeis placentula* and *Ulothrix tenerrima*, while those on the artificial substrate were *Stigeoclonium aestrivale*, *Oscillatoria tenuis* and *Achnanthes minutissima*. The cell density of periphytic algae was significantly affected by the total phosphorus (TP) and NO_3^--N and electric conductivity on *V. natans*, and by TP and NH_4^+-N on artificial *V. natans*. The malondialdehyde content of *V. natans* was significantly correlated with the periphytic algae biomass. *V. natans* was more affected by periphytic algae during its slow-growing period, and the contribution order of stress to *V. natans* was diatoms > cyanobacteria > green algae. Our findings might contribute to the understanding the effect of substrate specificity on periphytic algae communities, and have important implications for the restoration of submerged plants in eutrophic lakes.

Keywords: periphytic algae; *Vallisneria natans*; artificial substrates; dynamic changes; subtropical-urban-landscape lakes

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1. Introduction

Urban-landscape lakes are major freshwater resources in cities and have high ecological value for maintaining biodiversity, regulating climate, and providing leisure activities [1,2]. With socioeconomic development and accelerated urbanization, a series of ecological problems related to urban-landscape-lake ecosystems have appeared, such as water eutrophication, catastrophic declines in aquatic biodiversity, and toxic-cyanobacteria blooms [3–5]. In recent years, the reconstruction of submerged macrophytes has been a widely used measure to control lake eutrophication and improve water quality due to various macrophyte functions, e.g., reducing the nutrition load, inhibiting the release of nitrogen and phosphorus in the sediment, and suppressing harmful algal growth by allelopathy [6,7]. However, outbreaks of periphytic algae (including filamentous algae) have frequently been observed following submerged macrophyte reconstruction, and have been reported to be common in early stages [8,9]. Excessive periphytic algae proliferation on submerged leaves results in the reduction in or loss of submerged macrophytes, which may have been the cause of a noticeable shift from a clear-water, macrophyte-dominated state to an algae-dominated turbid state again [10].

Periphytic algae is a kind of autotrophic and extremely small algae community, which mainly includes Bacillariophyta, Chlorophyta, Cyanobacteria, Euglenophyta, etc., and grow on the surface of various underwater substrates (e.g., submerged macrophytes, sediments, and rocks) [11]. Periphytic algae can jointly regulate the material cycling and energy flow in an aquatic ecosystem with submerged plants, and the presence of periphytic algae can also protect submerged plants from host grazers [12]. However, the overgrown periphytic algae in eutrophic lakes can form a physical barrier on the leaf surface of plants to affect their nutrient uptake, gas exchange, and release of harmful secondary metabolites, which can have adverse effects on submerged plants [13,14]. For instance, periphytic diatoms can produce copious extracellular polymeric substances (EPS) which may form a variety of structures that are crucial for attachment (e.g., stalks, pads, and adhering films); however, these substances are harmful to submerged macrophytes due to their photosynthetic toxicity [15]. Periphytic green algae often lead to mechanical damage and compete with submerged macrophytes for space, light, nutrients, and other resources [10]. Furthermore, submerged macrophytes are potentially impaired by noxious toxins released by periphytic cyanobacteria [16]. Malondialdehyde (MDA), an important parameter reflecting the potential antioxidant capacity of the cell, can indirectly display the degree of peroxidation damage to the host [17]. Submerged macrophytes (as a living substrate) can release oxygen, carbon dioxide, organic nutrients, and allelochemical to form a specific microenvironment on the surface of leaves, which might significantly affect the community composition and abundance of periphytic algae [18]. In order to successfully re-establish a macrophyte-dominated state in a eutrophic lake, it is necessary to understand the complex interactions between periphytic algae and submerged macrophytes. Artificial substrates (non-living) were introduced to compare whether there were differences of periphytic algae between the submerged plants and artificial submerged plants. An artificial substrate has the advantages of high sampling precision, low sampling workload and cost, and is often used to study the growth and community composition of periphytic algae in aquatic ecosystems.

Periphytic algae are significantly affected by the substrate types, nutrient levels, and other factors, which may result in unique periphytic algae communities [19,20], whereas, comparatively, little information is available regarding the dynamic characteristics of periphytic algae or the relationships with environmental factors on natural and artificial submerged macrophytes with similar morphology in the process of periphytic algae community construction. Such information may contribute to improving our understanding of living substrate specificity, reveal the reasons for the decline in submerged macrophytes, and guide future lake restoration. Regardless of their types, the growth status (i.e., fast or slow growth) of submerged macrophytes is intimately linked to the periphytic algae community structure due to the apparently seasonal growth features of these plants [15,21]. However, little attention has been given to the variations in periphytic algae communities on living hosts with different growth statuses. Moreover, according to Olsen et al. (2015), periphytic algae become a dominant factor in winter [22]. To the best of our knowledge, there have been no previous reports on the characteristics of periphytic algae community dynamics in the continuous growth process of host plants, during which the host plant growth changes gradually from slow growth to fast growth; however, this period is related to the recovery of submerged macrophytes in the following year.

We hypothesized that the community composition, biomass, and species diversity of periphytic algae are affected by the submerged plants, and the extent of this effect depends largely on the growth state of submerged plants. To verify these hypotheses, the artificial *Vallisneria natans* (referring to an artificial macrophyte with a similar leaf shape and composed of non-toxic plastic materials) were introduced as a control to compare the difference in the periphytic algae community between their and natural *Vallisneria natans*. This study was carried out in a subtropical shallow-lake ecosystem in which there was a significant interaction between macrophytes and periphytic algae. The aims of this study were: (1) to compare the dynamic changes and host specificity of the periphytic algae communities on natural and artificial substrates; and (2) to evaluate the stress response of *V. natans* to periphytic algae.

2. Materials and Methods

2.1. Study Areas

The Hangzhou West Lake (120°08′, 30°15′) is located in the west of Hangzhou City, Zhejiang Province, China. It is famous for its cultural history and beautiful scenery, and is a UNESCO world heritage site. The West Lake consists of seven sub-lakes. The surface area is approximately 6.50 km^2 and the mean water depth is approximately 2.27 m. Maojiabu Lake and Xiaonanhu Lake are two of the main sub-lakes and are connected to the main lake. To avoid the disturbances from cruise ships and tourists, the Maojiabu and Xiaonan Lakes were selected as the experimental sites. Additionally, the surface areas of Maojiabu Lake (120°07′, 30°14′) and Xiaonanhu Lake (120°08′, 30°13′) are 0.48 km^2 and 0.09 km^2, respectively.

2.2. Experimental Setup

Uniform-size *V. natans* seedlings were collected from Maojiabu Lake and the attachment on leaf surface was washed off with distilled water in laboratory. Artificial *V. natans* were purchased from the market. Twelve *V. natans* seedlings or artificial *V. natans* were each planted in plastic polyethylene pots (height: 75 cm, diameter: 37.5 cm) containing 7 cm sediments, respectively. Seven pots of *V. natans* (MV) and seven pots of artificial *V. natans* (MA) were placed in the littoral zone in Maojiabu Lake, and the sediments in those pots were from Maojiabu Lake (Figure 1). Seven pots of *V. natans* (XV) and seven pots of artificial *V. natans* (XA) were placed in the littoral zone in Xiaonanhu Lake, and the sediments in those pots were from Xiaonanhu Lake (Figure 1). Those pots' locations were marked with float balls. The experiments were conducted from 1 October 2014 to 30 April 2015. Samples of periphytic algae, *V. natans*, and water were collected every month.

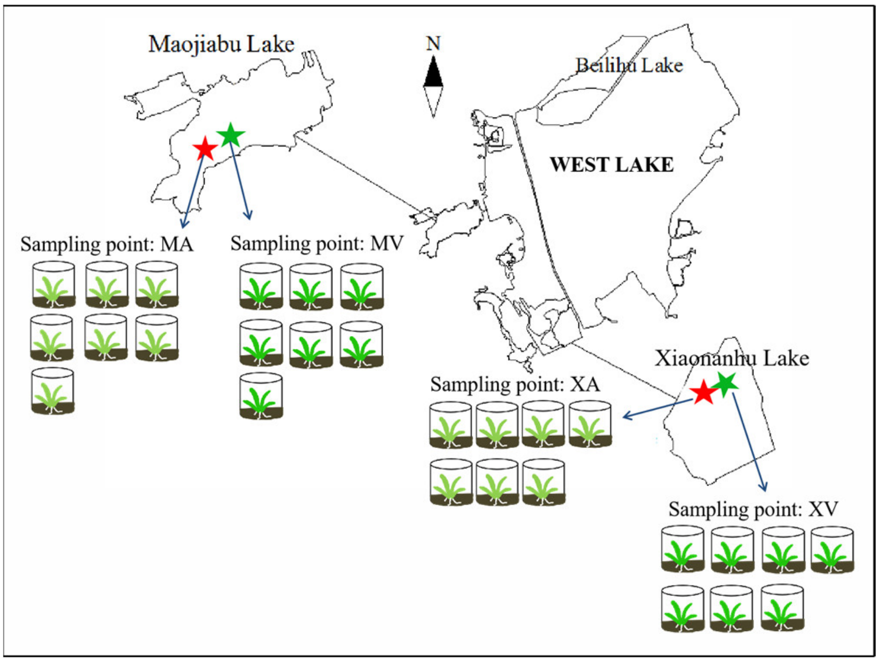

Figure 1. The experiment sampling sites in Maojiabu Lake and Xiaonanhu Lake.

2.3. Periphytic Algae Sampling and Treatment

We randomly chose three plastic pots planted with *V. natans* and artificial *V. natans* from the two lakes to collect leaves' samples for the analysis of periphytic algae on the 25th day of each month. After each sampling, plastic pots with plants were still put back. After the sediment attached to the roots was removed by washing, *V. natans* and artificial *V. natans* were placed in sealed pockets and transported to the laboratory on ice within 2 h. Leaves (with an area of approximately 100 cm^2) from the roots of 5 cm to 25 cm were cut, and periphytic algae were subsequently detached in 300 mL distilled water using soft brushes. Finally, the periphytic algae samples were divided into two parts: the 200 mL samples were used for the analysis of the chlorophyll a (Chl. a) content, and the remaining 100 mL samples were fixed with Lugol iodine liquid for further quantification and classification using an optical microscope [23]. Moreover, the Shannon–Wiener index and Jaccard similarity coefficient were also calculated [24].

2.4. Determination of Malondialdehyde (MDA) Content

After the periphytic algae were detached, about 0.2 g of fresh plant leaves was ground with 5 mL of 1% trichloroacetic acid (TCA) to measure the content of MDA according to the previously reported methods [25].

2.5. Determination of Physio-Chemical Parameters of Water

The pH, temperature (WT), dissolved oxygen (DO), electric conductivity (EC), total dissolved matter (TDS), pH, and oxidation–reduction potential (ORP) of water were monitored in situ using an online instrument (HQ-30d, HACH) at the time of periphytic-algae sampling. The water depth at each site was measured using a Secchi disc. Turbidity (NTU) determined using a turbidimeter (WGZ-20B, Shanghai Xinrui Instrument & Meters Comapany, Shanghai, China). After online monitoring, 2 L of water was sampled and stored in iceboxes. All samples were immediately transported to the laboratory for analyses. Total nitrogen (TN) was measured using the alkaline potassium persulfate digestion; total phosphorus (TP) was measured with the ammonium molybdate spectrophotometric method. Nitrate nitrogen (NO_3^--N) was evaluated using phenol disulfonic acid, nitrite nitrogen (NO_2^--N) was analyzed by applying the *N*-(1-naphthyl)-1,2-ethylenediamine spectrophotometric method, ammonium nitrogen (NH_4^+-N) was measured using a Nessler's reagent spectrophotometric method, and the phytoplankton Chl.a content was assayed spectrophotometrically with 90% acetone [26]. Additionally, local water samples' collection was synchronous with that of the periphytic algae sampling.

2.6. Statistical Analyses

All assays were conducted in triplicate. The histograms were drawn using Origin software. Spearman's correlation coefficient was used to measure the degree of relationship between MDA and the biomass of periphytic algae (including cell density, Chl.a, the cell densities of Bacillariophyta, the cell densities of Chlorophyta, and the cell densities of Cyanobacteria). The relation between cell density and Chl.a content of the periphytic algae was tested using linear regression. T tests were carried out to compare the differences in environment parameters between Maojiabu Lake and Xiaonanhu Lake. The differences in the community parameters of periphytic algae (including species richness, cell density, Chl.a, Shannon–Wiener diversity index, and Jaccard similarity coefficient) between the two substrates were tested using a *t* test, and the differences in time were evaluated by one-way analysis of variance. The interaction between substrates and time on the parameters of periphytic algae community was compared using two-way analysis of variance. Data were tested for normality and homogeneity of variance prior to ANOVA tests, and were appropriately transformed when required. A significance level of $p < 0.05$ was considered significant, and $p < 0.01$ was considered extremely significant. All statistical analyses were performed using SPSS version 19.0 (SPSS Inc. Chicago, IL, USA).

The relative abundances of the dominant taxa of periphytic algae community (>1% total cell density) were considered in non-metric multidimensional scaling (NMDS) and redundancy analysis (RDA) to minimize the influence of rare taxa. NMDS was performed to present the differences visually in dominant taxa of periphytic algae in different substrates and lakes. Detrended correspondence analysis (DCA) was employed to detect the gradient length of biological data. RDA (i.e., a linear model) was performed on environmental variables and the periphytic-algae cell density to determine their relationship when the gradient length of DCA was <3 [27]. The environmental predictor variables were assessed for statistical significance using 999 restricted Monte Carlo permutations tests, and the relationship between each species and significant environmental factors was tested by a T-value test. The NMDS, DCA, and RDA were carried out using the computer program Canoco version 5 for Windows.

3. Results

3.1. Physio-Chemical Characteristics

The mean ± standard deviation (SD) and the minimum and maximum values of the physio-chemical parameters of water at different lakes are summarized in Table 1. A significant difference was found in the TP concentration between the two lakes, which was lower in Maojiabu Lake (0.02–0.06 mg/L) than in Xiaonanhu Lake (0.01–0.09 mg/L) ($F = -3.395$, $p = 0.003$). The TN concentration was slightly higher in Maojiabu Lake (2.27 ± 0.95 mg/L) than in Xiaonanhu Lake (2.09 ± 0.57 mg/L); however, this difference was not significant ($F = 0.672$, $p = 0.507$). NO_3^--N, the main N form, was present at mean concentrations of 2.01 ± 0.71 mg/L in Maojiabu Lake and 1.79 ± 0.40 mg/L in Xiaonanhu Lake, and there was no significant difference between the two lakes ($F = 1.152$, $p = 0.260$). Minimal differences were found in the concentrations of NO_2^--N, NH_4^+-N, and phytoplankton Chl. a concentrations between the two lakes, and the concentrations of all these parameters were low (Table 1). Additionally, there were no significant differences in the pH, DO, EC, TDS, ORP, NTU, or WT between the two lakes (Table 1).

Table 1. Mean (± standard deviation), minimum, and maximum values of environment parameters at Maojiabu Lake and Xiaonanhu Lake, and T test results.

Parameters	**Maojiabu Lake**		**Xiaonanhu Lake**			
	Min–Max Value	**Mean ± SD**	**Min-Max Value**	**Mean ± SD**	**F Values between Lakes**	***p* Values between Lakes**
TP (mg/L)	0.02–0.06	0.03 ± 0.02	0.01–0.09	0.05 ± 0.03	−3.395	0.003
TN (mg/L)	1.48–4.34	2.27 ± 0.95	1.28–2.95	2.09 ± 0.57	0.672	0.507
NO_3^--N (mg/L)	1.01–3.56	2.01 ± 0.71	1.18–2.55	1.79 ± 0.40	1.152	0.260
NO_2^--N (mg/L)	0.03–0.07	0.04 ± 0.01	0.02–0.09	0.05 ± 0.02	−1.249	0.223
NH_4^+-N (mg/L)	0.15–0.53	0.31 ± 0.12	0.09–0.78	0.29 ± 0.17	−0.826	0.416
Chl.a (µg/L)	0.18–2.61	1.36 ± 0.77	0.18–2.95	1.01 ± 0.79	−0.377	0.709
DO (mg/L)	5.74–6.80	6.40 ± 1.04	5.42–6.13	5.91 ± 2.19	0.417	0.680
pH	6.94–9.25	8.23 ± 0.72	7.47–8.22	7.89 ± 0.22	0.399	0.693
EC (µs/cm)	152.10–265.00	201.29 ± 27.66	146.10–221.00	184.57± 20.67	0.415	0.681
ORP	56.80–218.60	138.77 ± 49.23	78.40–228.90	156.86 ± 47.96	0.869	0.393
TDS (mg/L)	94.20–132.00	115.33 ± 11.00	84.00–134.20	106.83 ± 14.89	0.013	0.99
WT (°C)	7.80–26.60	17.46 ± 6.43	8.30–25.60	17.16 ± 5.81	−0.004	0.997
Water depth (m)	0.70–0.90	0.78 ± 0.06	0.70–0.90	0.80 ± 0.06	0.322	0.750
Turbidity (NTU)	0.80–7.20	2.91 ± 1.79	0.70–11.40	3.08 2.89	−0.100	0.921

3.2. Periphytic-Algae-Community Compositions on *V. natans* and Artificial *V. natans*

3.2.1. Periphytic-Algae-Community Species Richness

The periphytic-algae-community species richness (>1% of total cell density) is shown in Figure 2a. We observed 66 (45 genera) and 60 (43 genera) species on *V. natans*, and 57 (41 genera) and 50 (34 genera) species on artificial *V. natans* in Maojiabu Lake and Xiaonanhu

Lake, respectively. More species were found on *V. natans* (77 taxa) than on artificial *V. natans* (66 taxa) (Table 2; F = 2.089, *p* = 0.047). These periphytic algae species mainly belonged to Bacillariophyta, Chlorophyta, Cyanobacteria, Cryptophyta, Pyrrophyta, Chrysophyta, Xanthophyta, and Euglenophyta. Bacillariophyta was the most abundant group on both *V. natans* and artificial *V. natans*, comprising 56.06% and 56.67% of the total taxa, respectively, followed by Chlorophyta which represented 19.70% and 23.33% of the overall composition on *V. natans* and artificial *V. natans*, respectively. Cyanobacteria was the third most abundant periphytic algae component, which occupied 12.12% on *V. natans* and 11.67% on artificial *V. natans*.

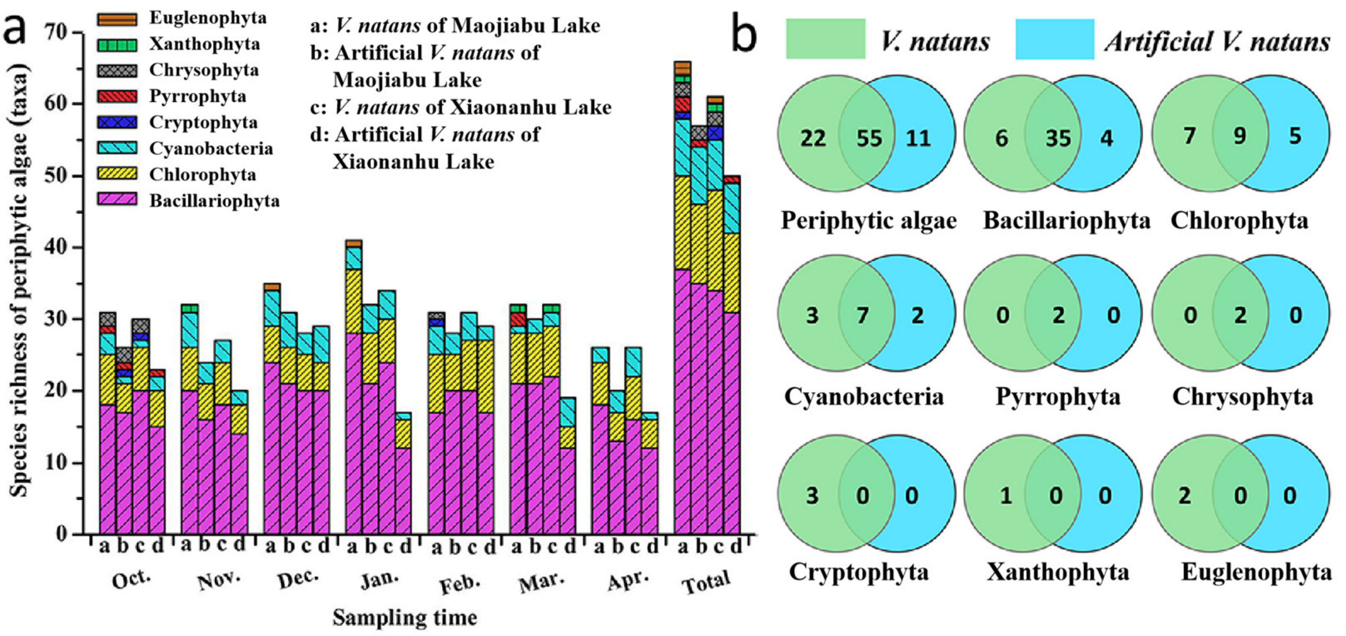

Figure 2. Periphytic-algae species richness: (**a**) At different lakes; and (**b**) on different substrates.

Table 2. The effects of time and substrate on periphytic algae species richness and biomass.

	Substrates		**Time**		**Substrates × Time**	
Parameters	**F Value**	***p* Value**	**F Value**	***p* Value**	**F Value**	***p* Value**
Species richness	2.089	0.047	1.277	0.310	0.795	0.579
Cell density	−4.943	0.000	2.600	0.048	7.226	0.000
Chl.a	−2.514	0.025	3.955	0.008	4.898	0.001
Shannon–Wiener diversity index	1.387	0.181	0.885	0.523	25.273	0.000
Jaccard similarity coefficient	2.544	0.021	0.403	0.869	0.521	0.783

The highest species richness on artificial *V. natans* in Xiaonanhu Lake appeared in December 2014 and February 2015. Species richness increased from October 2014, reached a peak in January 2015, and showed a downward trend before reaching the lowest levels in April 2015. The two-way ANOVA (Table 2) indicated that there were no significant differences in the sampling time and the second-order interactions of time and substrate (F = 0.795, *p* = 0.579).

3.2.2. Host Preferences of Periphytic Algae on Different Substrates

A total of 88 taxa (77 taxa on *V. natans* and 66 taxa on artificial *V. natans*) were identified as periphytic algae. A total of 55 taxa were the same on the two substrates (35 taxa of Bacillariophyta; 9 taxa of Chlorophyta; 7 taxa of Cyanobacteria; 2 taxa of Pyrrophyta; and

2 taxa of Chrysophyta) (Figure 2b). Only Xanthophyta, Cryptophyta, and Euglenophyta appeared on *V. natans* (Figure 2b). The taxa are listed in Table S1. Most species did not demonstrate host preferences, whereas *Pinnularia gibba*, *Fragilaria granulata*, *Cyclotella stelligera*, *Surirella linearis*, *Cladophora oligoclada*, *Scenedesmus quadricauda*, *Raphidonema nivale*, *Tetrastrum hastiferum*, *Spirulina platensis*, *Rhabdoderma lineare*, *Cryptomons ovata*, *Chroomonas acuta*, *Ophiocytium capitatum*, and *Euglena gasterosteus* only occurred on *V. natans*. Differing from *V. natan*, artificial *V. natans* was inhabited by *Melosira varians*, *Surirella robusta*, *Scenedesmus dimorphus*, *Dictyosphaerium pulchellum*, and *Oscillatoria tenuis*. In addition, there were some organisms (for which only the genus could be determined) that exhibited species specificity on *V. natan* and artificial *V. natan*, such as *Surirella* sp.

3.2.3. Dominant Periphytic-Algae Community Taxa

The relative abundances of the top ten taxa of the periphytic algae community are shown in Figure 3. *Cocconeis placentula* was the dominant taxa on *V. natans* during October–December 2014 in Maojiabu Lake, for which the relative abundance was 17.65%, 44.44%, and 36.63% (Figure 3a). However, the dominant taxon during January–April 2015 was *Lyngbya perelagans* with relative abundances of 52.20%, 29.17%, 39.78%, and 35.57% during January to April 2015, respectively (Figure 3a). *Stigeoclonium aestivale* predominated on artificial *V. natans* in October 2014 (36.42%) and January 2015 (42.93%) in Maojiabu Lake (Figure 3b). *O. tenuis* was the most representative in November 2014 (19.23%), and *L. perelagans* was the most representative in other months, with relative abundances of 34.80% in December 2014, 27.54% in February 2015, 47.05% in March 2015, and 61.19% in April 2015 (Figure 3b).

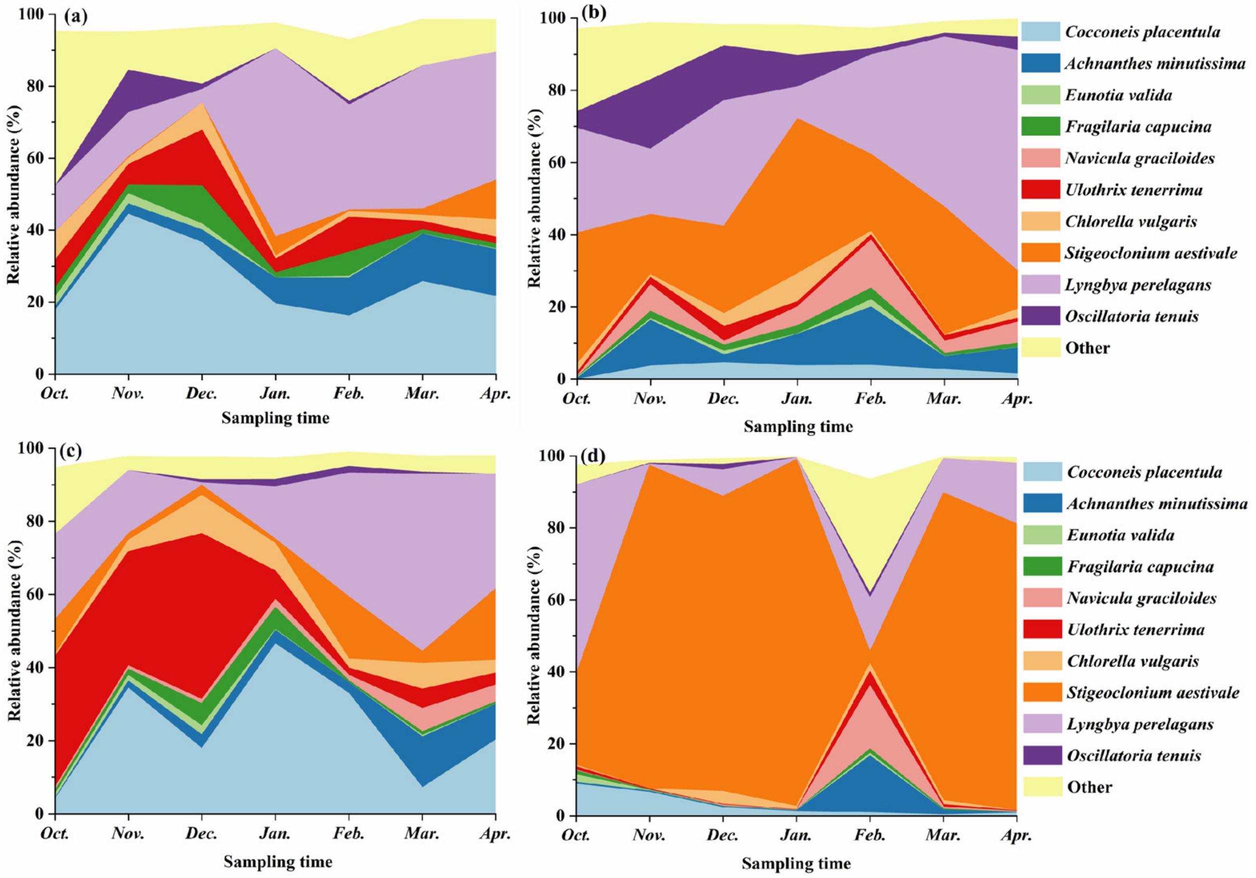

Figure 3. Relative abundance (%) of periphytic algae species on two different substrates in Maojiabu Lake and Xiaonanhu Lake: (**a**) periphytic algae on *V. natans* in Maojiabu Lake; (**b**) periphytic algae on artificial *V. natans* in Maojiabu Lake; (**c**) periphytic algae on *V. natans* in Xiaonanhu Lake; and (**d**) periphytic algae on artificial *V. natans* in Xiaonanhu Lake.

Ulothrix tenerrima had the highest abundance on *V. natans* in October (35.81%) and December 2014 (45.36%) in Xiaonanhu Lake (Figure 3c). *L. perelagans* was the dominant taxa in other months and had relative abundances of 33.88% in February 2015, 48.50% in March 2015, and 31.34% in April 2015 (Figure 3c). *L. perelagans* dominated on artificial *V. natans* in October 2014 (52.81%) in Xiaonanhu Lake, and *Achnanthes minutissima* dominated in February 2015 (15.78%). *S. aestivale* was the dominant taxa in other months, with relative abundances of 89.90% in November 2014, 81.97% in December 2014, 96.41% in January 2015, 85.53% in March 2015, and 79.65% in April 2015 (Figure 3d).

3.3. Periphytic Algae Biomass on V. natans and Artificial V. natans

As shown in Figure 4a, the periphytic-algae cell densities on *V. natans* began to accumulate in October 2014 in Maojiabu Lake and Xiaonanhu Lake, peaked in March 2015 with cell densities of 225,527 ± 18,031 cells/cm^2 in Maojiabu Lake and 394,055 ± 9821 cells/cm^2 in Xiaonanhu Lake, and subsequently declined by 1.79-fold and 1.67-fold in Maojiabu Lake and Xiaonanhu Lake, respectively, in April 2015. The change in the periphytic-algae Chl. a content was similar to that of cell density on *V. natans* (Figure 4b). The biomass, including cell density and Chl.a, on artificial *V. natans* continued to increase from October 2014 to the end of the experiment in Maojiabu Lake and Xiaonanhu Lake, i.e., by 15.89-fold and 176.87-fold in cell density, and 2.03-fold and 2.31-fold in Chl. a content, respectively (Figure 4). The main components of the cell density of periphytic algae on the two substrates were Bacillariophyta, Chlorophyta, and Cyanobacteria at both lakes (Figure 4a).

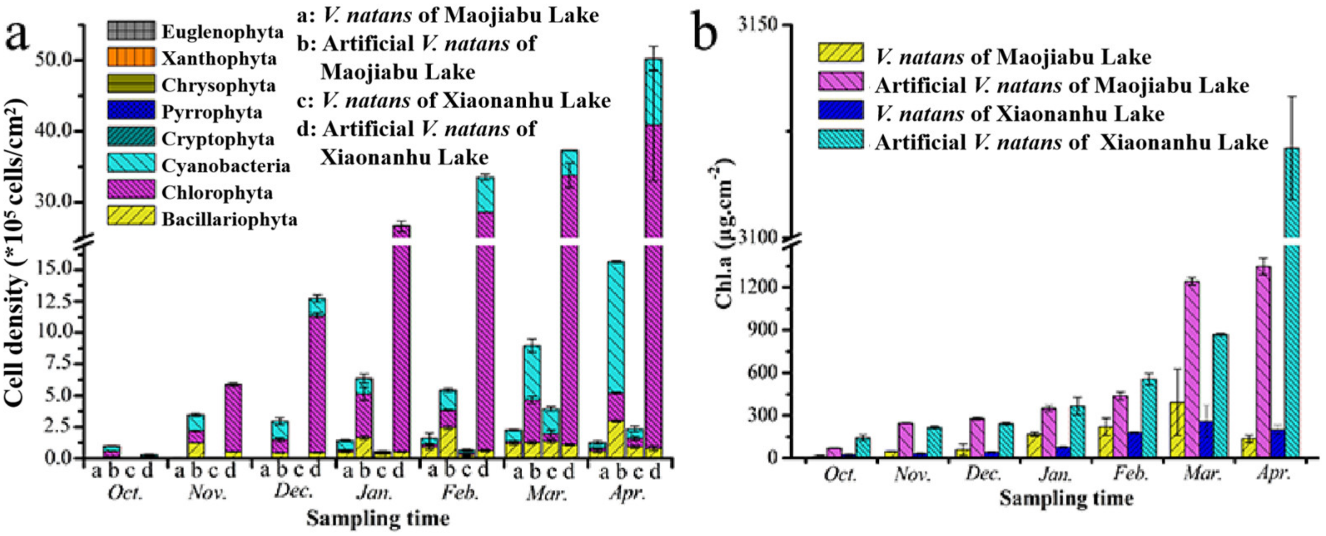

Figure 4. Periphytic-algae biomass on *V. natans* and artificial *V. natans*; (**a**) Cell density; (**b**) Chl. a content. Error bars show the standard deviations (n = 3).

There was an obvious positive linear relation between the cell density and Chl. a content of the periphytic algae community ($Y = 0.0004X + 110.5400$, $R^2 = 0.6816$). The cell density and Chl.a content were considerably influenced by the substrate, time, and their interactions (Table 2). The biomass was significantly higher on *V. natans* than on artificial *V. natans* (cell density: 3.42–202.62-fold; Chl.a content: 2.00–15.50-fold) (Table 2, $F = -4.943$, $p = 0.000$; $F = -2.515$, $p = 0.025$). Compared to cell density, the difference in the Chl. a content was relatively significant in time, and the enrichment in the initial five months was significantly lower than that in the final two months ($F = 3.955$, $p = 0.008$).

3.4. Diversity Index and Similarity Coefficient of Periphytic Algae on V. natans and Artificial V. natans

The Shannon–Wiener diversity index of the periphytic algae community on *V. natans* varied from 1.62 ± 0.07 to 2.66 ± 0.03 in Maojiabu Lake, and from 1.726 ± 0.04 to

2.18 ± 0.05 in Xiaonanhu Lake throughout the experiment, while that on artificial *V. natans* was from 1.45 ± 0.06 to 2.45 ± 0.14 and from 0.23 ± 0.12 to 2.13 ± 0.03 in Maojiabu Lake and Xiaonanhu Lake, respectively (Figure 5a). The Shannon–Wiener diversity index was significantly affected by the substrate and was higher on *V. natans* than on artificial *V. natans* (Table 2; F = 2.543, $p = 0.017$). However, no significant differences were found in the Shannon–Wiener diversity index when analyzed in terms of time and the interaction of substrate and time (Table 2; F = 0.853, $p = 0.610$).

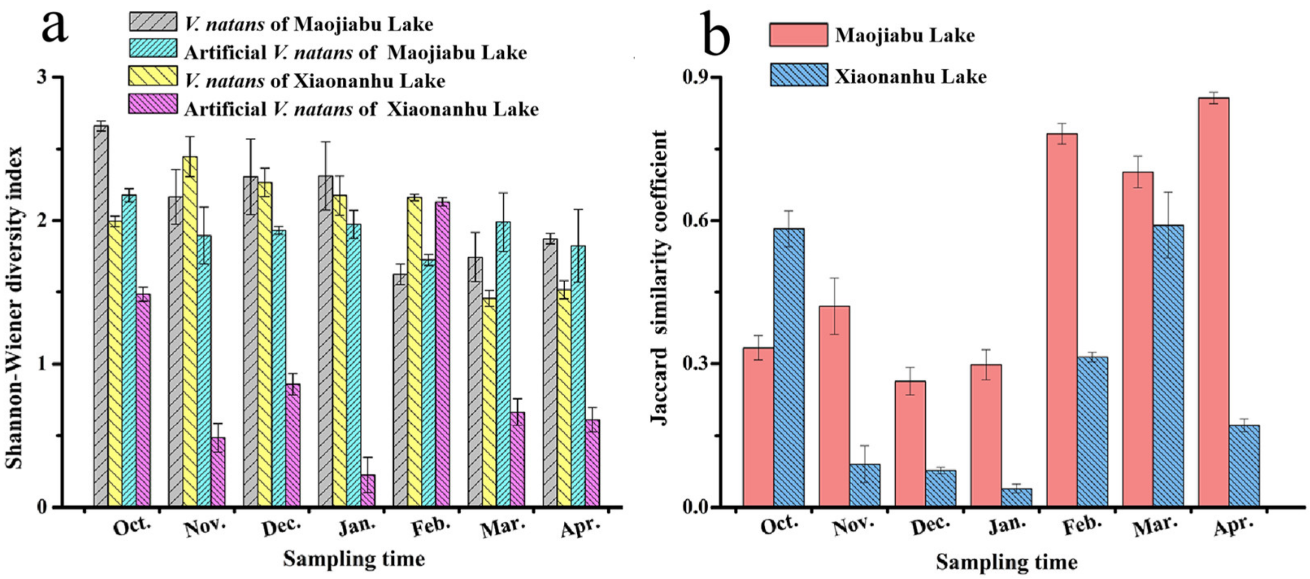

Figure 5. Shannon–Wiener diversity index (**a**) and Jaccard similarity coefficient (**b**) of periphytic algae at different times and sampling lakes. Error bars show the standard deviations (n = 3).

The Jaccard similarity coefficient of the periphytic algae community on *V. natans* and artificial *V. natans* ranged from 0.26 ± 0.03 to 0.86 ± 0.01 in Maojiabu Lake and from 0.04 ± 0.01 to 0.59 ± 0.07 in Xiaonanhu Lake, respectively (Figure 5b). This coefficient was not significantly influenced by time (F = 0.403; $p = 0.869$), which indicated that the periphytic algae community was similar over time.

3.5. Relationships between Environmental Variables and Periphytic Algae

According to the results of NMDS analysis, the dominant taxa of periphytic algae had significant differences between *V. natans* and artificial *V. natans,* and the periphytic algae on artificial *V. natans* had significant differences between two lakes (Figure 6a). The relationships between environmental variables and the cell density of dominant taxa of periphytic algae (relative abundances >1% total cell density) on *V. natans* or artificial *V. natans* were analyzed using RDA. On *V. natans*, all environmental variables accounted for 59.90% of the total variance in algae composition, and three were significant: EC, TP, and NO_3^--N (Figure 6b). EC had a positive effect on *Cryptomonas erosa* and *Dinobryon sertularia*. NO_3^--N was positively related to *C. placentula*, *A. minutissima*, *Navicula simplex*, *Navicula graciloides*, *Gomphonema constrictum*, *U. tenerrima*, *S. aestivale*, and *L. perelagans*. The TP concentrations were positively correlated for *Cyclotella meneghiniana*, *Diploneis ovalis*, *Chamydomonas ovalis*, *Pseudanabaena* sp., and *R. lineare*.

Environmental factors can explain 85.67% of the variance variation in periphytic algae on artificial *V. natans*, and TP and NH_4^+-N had significant effects on the algae community (Figure 6c). TP was significantly positively associated with *C. placentula*, *A. minutissima*, *N. graciloides*, *Melosira granulata*, *U. tenerrima*, *Cladophora oligoclona*, *Chlorella vulgaris*, *Scenedesmus bijuga*, *S. aestivale*, *Spirogyra* sp., *Zygnema* sp., *D. sertularia*, *Pseudanabaena* sp., and *L. perelagans*. NH_4^+-N was positively correlated with *A. minutissima*, *F. capucina*, *N. simplex*, *N. graciloides*, *M. granulata*, *U. tenerrima*, *C. vulgaris*, and *L. perelagans*.

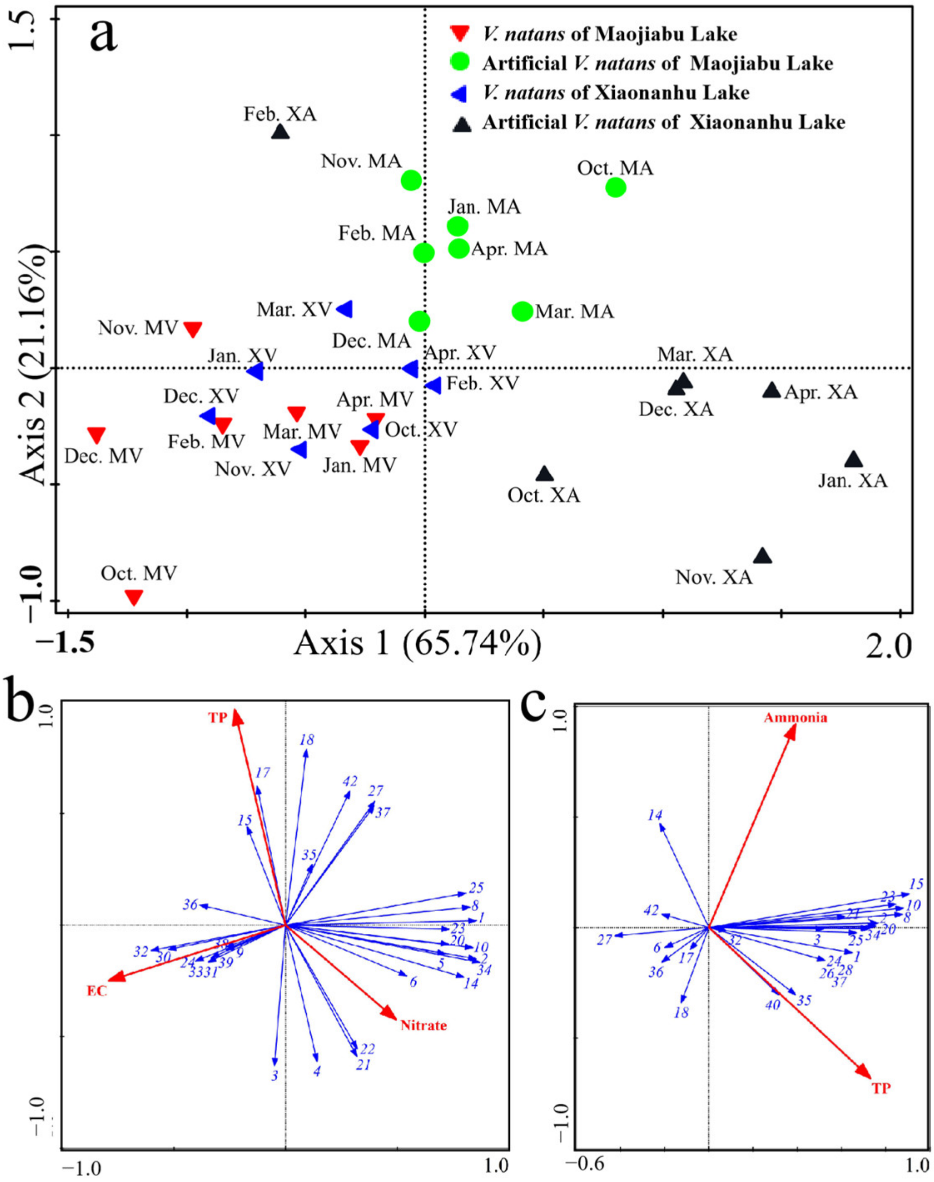

Figure 6. NMDS plot of periphytic-algae-dominant taxa in different substrates and sampling lakes (**a**). Redundancy analysis (RDA) ordination plots of physico-chemical parameters of water and periphytic algae cell density on *V. natans* (**b**) and artificial *V. natans* (**c**). 1 *Cocconeis placentula*, 2 *Achnanthes minutissima*, 3 *Eunotia valida*, 4 *Eunotia pectinalis*, 5 *Fragilaria capucina*, 6 *Synedra acus*, 7 *Synedra amphicephala*, 8 *Navicula simplex*, 9 *Navicula exigua*, 10 *Navicula graciloides*, 11 *Navicula dicephala*, 12 *Gomphonema angustatum*, 13 *Gomphonema acuminatum*, 14 *Gomphonema constrictum*, 15 *Melosira granulata*, 16 *Diatoma vulgare*, 17 *Cyclotella meneghiniana*, 18 *Diploneis ovalis*, 19 *Cymbella affinis*, 20 *Ulothrix tenerrima*, 21 *Cladophora oligoclona*, 22 *Characium strictum*, 23 *Chlorella vulgaris*, 24 *Scenedesmus bijuga*, 25 *Stigeoclonium aestivale*, 26 *Spirogyra* sp., 27 *Chamydomonas ovalis*, 28 *Zygnema* sp., 29 *Dictyosphaerium pulchellum*, 30 *Cryptomons ovata*, 31 *Chroomonas acuta*, 32 *Dinobryon sertularia*, 33 *Mallomonas* sp., 34 *Lyngbya perelagans*, 35 *Oscillatoria tenuis*, 36 *Merismopedia punctata*, 37 *Pseudanabaena* sp., 38 *Anabeana* sp., 39 *Aphanizomenon* sp., 40 *Protoderma* sp., 41 *Rivularia* sp., 42 *Rhabdoderma lineare*.

3.6. Changes in MDA Content of *V. natans*

We measured the content of MDA in leaves to evaluate the oxidative damage of periphytic algae to *V. natans*. The lowest content of MDA (Maojiabu Lake: 0.61 ± 0.04 nmol/g; Xiaonanhu Lake: 0.53 ± 0.31 nmol/g) were found in the early stage of the experiment (October 2014), and the highest content (Maojiabu Lake: 6.54 ± 0.07 nmol/g; Xiaonanhu

Lake: 6.56 ± 0.73 nmol/g) occurred in March 2015. The MDA contents in individual *V. natans* leaves increased by more than 10-fold from October 2014 to March 2015 at both lakes, and then decreased by nearly 2-fold in April 2015 (Figure 7a). In March 2015, the biomass (cell density and Chl.a) of the periphytic algae on the surface of *V. natans* also reached the highest value in the experimental period. The MDA contents in April 2015 were 3.91 ± 0.23 nmol/g and 3.29 ± 0.20 nmol/g in Maojiabu Lake and Xiaonanhu Lake, respectively. Correlation analysis indicated that the periphytic-algae biomass (including cell density and Chl. a content) and the MDA content of *V. natans* were significantly positively correlated ($p < 0.01$) (Figure 7b). In addition, the cell densities of Bacillariophyta, Chlorophyta, and Cyanobacteria were positively associated with the MDA content ($p < 0.01$). The stress contribution order to *V. natans* growth was Bacillariophyta > Cyanobacteria > Chlorophyta (Figure 7b).

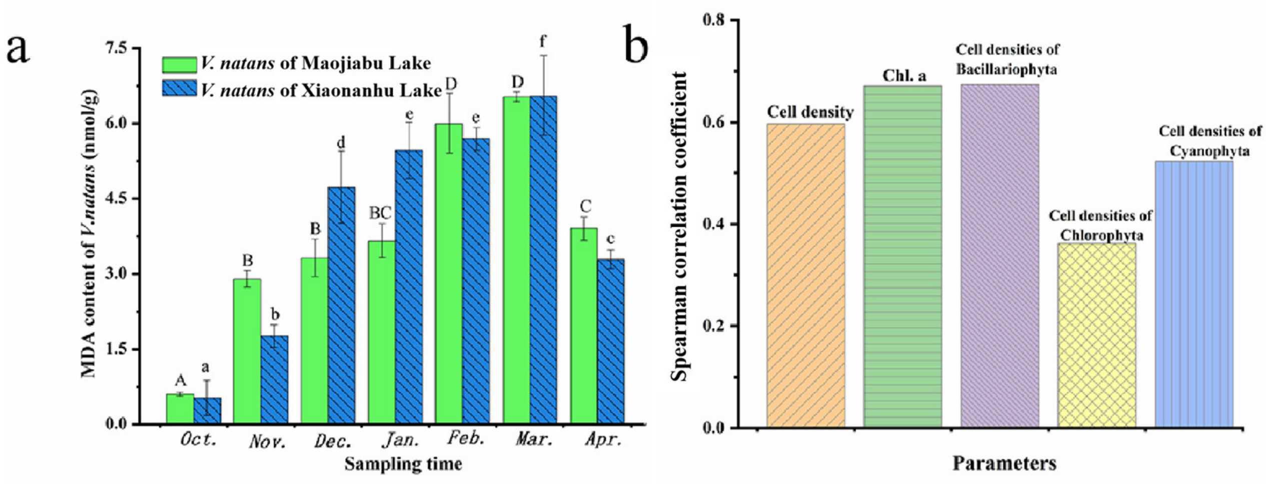

Figure 7. Malondialdehyde (MDA) contents of *V. natans* and correlations with periphytic-algae biomass: (**a**) Changes in the MDA content (mean ± standard deviation (SD)) in Maojiabu Lake and Xiaonanhu Lake; (**b**) Spearman's correlation coefficient between the MDA content and cell density, Chl. a content, Bacillariophyta cell density, Chlorophyta cell density, and Cyanobacteria cell density. The significant differences are assigned different superscript letters in Maojiabu Lake (A–D) and Xiaonanhu Lake (a–f) ($p < 0.05$). Error bars show the standard deviation (n = 3).

4. Discussion

4.1. Periphytic Algae Community on Different Substrate and Time

Species composition is the most important factor for determining the nature of a community and is also a basic feature to identify different community types. This study showed that the periphytic algae communities on *V. natans* and artificial *V. natans* were mainly Bacillariophyta, Chlorophyta, and Cyanobacteria [28,29]. These species are also the dominant groups of periphytic algae in shallow-lake ecosystems [29]. There are two reasons for this type of periphytic-algae composition. First, most species of diatoms have specialized cushion-like, neck-like, or tubular structures for attaching to substrates, such as species in the genera *Gomphonema*, *Frustulia*, and *Navicula*, which possess a competitive advantage over other species under stressful environmental conditions [16]. Therefore, diatoms are a relatively common group in periphytic algae communities. Additionally, the second is that Chlorophyta have a distinct base and filamentous cells, which can pass through substrates, for example *Stigeoclonium* [30]. Cyanobacteria (e.g., *L. perelagans*) also with a viscous structure can be directly attached to substrates [31]. In addition, the composition of periphytic algae is affected by the species of submerged plants. Periphytic algae on *Potamogeton perfoliatus* included 18 taxa, i.e., fewer taxa than in the present study, while the numbers of taxa were significantly higher on *Stratiotes aloides* (159 taxa) in spring and on *Potamogeton lucens* (135 taxa) in autumn [32]. The results of Pomazkina et al. (2012)

on six species of submerged macrophytes species are comparable to the findings in the present study [33].

The composition of periphytic algae between *V. natans* and artificial *V. natans* showed a complex pattern. The dominant taxa of periphytic algae had a significant difference between *V. natans* and artificial *V. natans*, and the composition of periphytic algae on artificial *V. natans* had significant differences between two lakes. This indicated that *V. natans* had a greater impact on the composition of periphytic algae communities than water-quality parameters, which could be the periphytic algae benefit from the nutrient exudation (e.g., N, P, and DOC) of living submerged plants [18]. Biodiversity is an important parameter to describe the community stability in an ecosystem. The higher the diversity value, the more stable the community [33]. The Shannon–Wiener diversity index was significantly higher on *V. natans* than that on artificial *V. natans*. The reason for this difference might be *V. natans* could provide nutrients, vitamins, or others to form a wide variety of microhabitats, which could enhance periphytic-algae diversity, although the significance of these excretion products remains unclear [18]. In addition, the substrate architecture plays an important role in the abundance, composition, and distribution of a periphytic algae community [33,34]. Even if the morphological architecture of artificial substrates is very similar to that of natural plants, the difference in leaf structural complexity may be one of the reasons for the differences in periphytic-algae assemblages [35]. In this study, we described the differences in periphytic algae on different substrates, but not the factors controlling these differences. There were more specific species on *V. natans* than on artificial *V. natans*. Our results revealed a high algae specificity, especially *Cryptophyta, Xanthophyta*, and *Euglenophyta* species, growing on *V. natans*. This also proved our research hypothesis that there were differences in the composition of periphytic algae communities on the surface of *V. natans* and artificial *V. natans*. Substrate preferences arise from physical, chemical, and biological influences; however, substrate types have a stronger influence on host specificity [36]. In summary, host preference is an unresolved issue, and attention should be given to the chemical and biological interactions between the host and the periphytic algae community in further investigations. The morphology (e.g., overall body architecture, leaf form, etc.) and anatomy (e.g., smoothness or roughness of surface microtopography) of host plants are the primary reason for periphytic-algae richness [19].

The biomass of periphytic algae on the artificial *V. natans* was higher than that on the *V. natans*, which was shown as a opposite trend to the Shannon–Wiener diversity index. This might be because the biologically active substances (such as polyphenols and sulfur compounds) secreted by submerged plants inhibited the growth of periphytic algae and reduced their biomass [37,38]. Some researchers have reported that the periphytic-algae biomass showed the same trend as in the present study, i.e., artificial substrates >natural plants [21,38]. *C. placentula*, *Amphora lineolata*, and *Diatoma hiemale* were the most abundant and common species on *P. perfoliatus* [39]. *Oscillatoria* sp., *Planktolyngbya limnetica*, *C. placentula*, *Komvophoron crassum*, and *Fragilaria capucina* were abundant on *Ceratophyllum demersum* [24]. In the present study, the dominant species in the periphytic algae communities were *C. placentula*, *L. perelagans*, and *U. tenerrima* on *V. natans*, and *L. perelagans*, *S. aestivale*, *O. tenuis*, and *A. minutissima* on artificial *V. natans.* All of these species may depend on specific plant traits, such as allelochemical release, competition for resources (including dissolved inorganic carbon), and the incrustation of plant surfaces by calcium carbonate, which may hinder the periphytic algae growth [18]. Furthermore, besides these traits, the effects of external environmental factors and grazing pressure cannot be ignored, which have been suggested as modifying algae–substrate associations and community composition [28,40].

The species richness and biomass of the periphytic algae community may vary in different periods due to changes in the surrounding environmental factors and the host growth status [21,41]. Periphytic-algae diversity and biomass were significantly higher during the slow-growth stage than during the fast-growth stage of *V. natans*. In the early stage of enrichment, substrates have sufficient spaces for the growth and development of the periphytic algae community, which increases the species richness [42]. With the enhanced adaptability and defense capabilities of *V. natans*, some sensitive or opportunistic species may disappear, and other species which are able to grow on substrates' leaves for a long time (e.g., *C. placentula*, *L. perelagans*, *S. aestrivale*, etc.) would continue to absorb nutrients and grow in large numbers, resulting in the observed cell density increase [43]. However, the possibility that the high periphytic-algae abundance is related to the nutrients increase released from submerged macrophytes cannot be dismissed [44], whereas active growth may enable plants to overcome periphytic-algae stress to some extent [18,45].

4.2. Relationships between the Periphytic Algae Community and Environmental Parameters

The species composition in a periphytic algae community is also affected by different local water environments (especially nutrients) [46]. The nutrient level in water is one of the key factors affecting the composition and biomass of periphytic algae [11]. N and P enrichment are usually accompanied by an increase in periphytic algae in eutrophic waters [46,47]. Song et al. (2015) demonstrated that the number of periphytic algae species and their biomass on *V. natans* increased as N and P concentrations increased, including *Aphanothece, Microcystis, Chroococcus, Anabaena, Nostoc, Melosira, Navicula, Fragilaria, Cyclotella, Cymbella*, and *Chlorella* [48]. Ray et al. (2014) suggested that P was a key factor in the seasonal changes in periphytic algae communities [49,50]. NO_3^--N is the main N form, and Andrus et al. (2013) emphasized that NO_3^--N affects the periphytic algae community structure [51]. Min et al. (2017) also found that the increase in NO_3^--N concentration in water bodies significantly stimulated the growth of the periphytic algae community on *V. natans* leaves, and that the periphytic-algae biomass reached its maximum when the NO_3^--N concentration was 2.5–5 mg/L [17,18]. Moreover, Gong et al. (2018) indicated that a high NH_4^+-N concentration enhanced biofilm (containing a periphytic algae community) growth, which may increase ammonium-induced toxicity on submerged plants [25]. Most periphytic algae cannot directly synthesize N and rely on the nitrogen-fixing bacteria in the periphytic biofilm to provide and deliver NO_3^--N or NH_4^+-N [52]. Furthermore, the difference in the N species affecting the periphytic algae community on *V. natans* and artificial *V. natans* may be associated with distinct microorganism compositions in biofilm. Denitrifying bacteria were mainly present on *V. natans*, whereas nitrifying bacteria were mainly present on artificial *V. natans* [52]. The preference of NH_4^+-N over NO_3^--N for periphytic-algae uptake, and the active nitrification transfer of NH_4^+-N to NO_3^--N, may also be reasons for the higher periphytic-algae biomass on artificial *V. natans* than on *V. natans* [53]. Consequently, enhanced management to specifically reduce nutrient outputs in eutrophic lakes may assist in the control of nuisance periphyton outbreaks downstream.

Among abiotic factors, EC is a parameter that measures the total dissolved ion contents in water bodies, and can reflect the total amount of ionic components introduced into water bodies by surface runoff [54]. EC and nutrient measures correlated well in the present study, and the increases in EC may have been accompanied by elevated dissolved nutrients [55]. Additionally, Leland (1995) observed strong correlations between EC/nutrients and algae species distributions [54]. An increase or decrease in EC may change the community diversity of periphytic algae and affect their growth and reproduction. The periphytic algae community was only significantly affected by EC on *V. natans*. Our results show that *V. natans* was more sensitive and exhibited more complex responses to environmental factors than artificial *V. natans.*

4.3. Response of V. natans to Periphytic Algae

In plants, the oxidative stress reaction will occur under external pressure, resulting in the lipid peroxidation of plant-cell membranes [56,57]. The content of MDA in plant tissues can reflect the degree of damage to plants caused by membrane lipid peroxidation [58]. We measured the content of MDA in leaves to evaluate the oxidative damage of periphytic algae to *V. natans*. In this study, the MDA content of *V. natans* increased with the increase in the biomass of periphytic algae, which indicated that the coverage of periphytic algae would cause the membrane lipid peroxidation of *V. natans* and have an adverse effect on the growth of plants [14,22]. Previous studies have shown that when the Chl.a content of periphytic algae on submerged plants exceeds 50 mg/m^2, the biomass of submerged plants would be reduced by more than 50%, which could lead to the extinction of submerged plants and the deterioration of water quality [22]. In addition, periphytic algae also can disturb the host plant growth by changing the redox conditions of the adhesion interface, and exerting adverse influences due to metabolic activities and the release of secondary metabolites [14]. The MDA content of *V.natans* and biomass of the periphytic-algae biomass decreased significantly in the final month of the experiment. It is possible that the vigorous growth of submerged macrophytes reduced the nutrients available for periphyton growth and hindered algae proliferation by increasing the antioxidant capacity and/or secreting allelochemicals. For instance, previous research indicated that increased glutathione contents which improve the glutathione/glutathione (oxidized) ratio may lead to an enhanced detoxification ability of *V. natans* to the EPS produced by periphytic algae [59]. In addition, submerged macrophytes often have strategies such as allelochemical release, rapid growth at the top, and the formation of caps to prevent shading by periphytic algae [60].

In our study, diatoms and cyanobacteria caused more oxidative stress on *V. natans* than chlorophyta according to the result of correlation analysis. Diatoms can tolerate quite low temperatures, and some studies have found that the attachment ability of diatoms was strong for their high yield of harmful EPS, which are favorable for biofilms aggregated into clusters on submerged macrophytes and is considered as one of the reasons for the ecological success of diatoms [15]. Periphytic diatoms have a short life cycle and high reproductive rates, which allow them to rapidly respond to environmental alterations [43]. Among periphytic diatom species, *Achnanthidium minutissimum* (i.e., one of the dominant species found in this study and the most frequently occurring species in various freshwater ecosystems) has a high tolerance to disturbances [44]. This species is often accompanied by diatoms that are adapted to low-light conditions. Shadow-adapted species (e.g., *Cocconeis* and *Gomphonema* in the present study) were reported as the most competitive and well-developed among periphytic algae in harsh circumstances (e.g., light limitation) [61]. Although periphytic cyanobacteria may produce toxins that damage submerged macrophytes, the cyanobacteria toxins are produced inside cells, and the amount of toxins secreted by living cyanobacteria to the outside of cells is relatively small, and are all released only during cell decomposition [16]. This may be the reason why the stress contribution of cyanobacteria to *V. natans* growth was less than that of diatoms.

This study systematically analyzed the temporal- and spatial-variation characteristics of the community composition and biomass of periphytic algae on *V. natans* and artificial *V. natans*, and the physiological response of host plants to periphytic algae. However, there were some limitations which should be considered in future studies. The influence mechanism of morphological characteristics and secretions of submerged plants on the communities' composition and biomass of periphytic algae remains to be determined. In addition, besides eukaryotic algae, there are also a large number of bacteria, fungi, and protozoa in the periphytic organisms of submerged plants. It is necessary to further clarify the interaction among these periphytic organisms and their response characteristics to different host plants.

5. Conclusions

In this study, we studied the community composition and temporal succession of periphytic algae on the surface of submerged plant *V. natans* and artificial *V. natans* in two lakes after the restoration of submerged plants. The species richness and diversity of periphytic algae on the surface of *V. natans* were higher than those of artificial *V. natans*, and the biomass of periphytic algae (total cell density and chlorophyll) showed an opposite trend, which could have been affected by the active substances secreted by submerged plants. Further RDA analysis results revealed that the periphytic algae cell density on

V. natans was affected by TP, NO_3^--N, and EC, while that on the artificial *V. natans* was affected by TP and NH_4^+-N. The growth of periphytic algae would cause oxidation pressure on the *V. natans*, which was more significant in the slow-growing period of plants. This study may have important implications for the restoration of submerged plants in eutrophic lakes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15040639/s1, Table S1. List of periphytic algae of *V. natans* and artificial *V. natans*.

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