Food Web Responses to a Cyanobacterial Bloom in a Freshwater Eutrophic Lake

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Abstract: The microbial food web is an important part in aquatic ecosystem, but studies on the microbial food web in freshwater ecosystem, especially in freshwater eutrophic lakes, still need further investigation. In the present study, using eutrophic Lake Nanhu as model, the community changes of phytoplankton, zooplankton, and bacteria between the bloom and non-bloom period were analyzed, and microzooplankton grazing experiments were also conducted to measure the grazing pressure and selectivity of microzooplankton on phytoplankton community. Phytoplankton community in Lake Nanhu was mainly dominated by Cyanophyta (49.44%), especially Anabaena circinalis and Microcystis flos-aquae, during bloom period. Rotifers were the main components of zooplankton in Lake Nanhu (44.15%), Brachionus calyciflorus and Moina macrocopa were the most dominant zooplankton in the non-bloom and bloom period, respectively. Bacteroidetes showed significantly higher mean proportion in bloom period than that in non-bloom period (p < 0.001). The growth rates of phytoplankton ranged from $-1.00 \text{ d}^{-1}$ to $1.29 \text{ d}^{-1}$, while grazing rates of microzooplankton ranged from $-1.15 \text{ d}^{-1}$ to $1.05 \text{ d}^{-1}$. Results indicated that microzooplankton could respond quickly to the increase of phytoplankton during bloom period. Meanwhile, microzooplankton showed grazing preference on Cyanophyta and Cryptophyta during bloom period and non-bloom period, respectively. The microzooplankton grazing selectivity during bloom period might depend on phytoplankton community composition.

Keywords: microbial food web; plankton community; microzooplankton grazing; algal bloom; bacteria

1. Introduction

Microbial loop plays an important role in the mater circulation and energy transfer of water ecosystem, which is an effective supplement to the classic food chain [1]. The concept of microbial food web is a further extension of the microbial food loop [2]. The main components of the microbial food web include heterotrophic bacteria, phytoplankton, microzooplankton, and planktonic viruses. According to previous studies, the microbial food web was related to the classic food chain in multiple ways instead of a single “loop” [3]. At present, there are abundant researches on the structure of microbial food webs in marine ecosystems [4–6]. In the typical marine microbial food web, the abundance ratio of heterotrophic flagellates, bacteria and viruses is $1:10^3:10^4$, and heterotrophic nanoflagellates play an important role in grazing picoplankton [7]. However, studies on the microbial food web in freshwater ecosystem, especially in freshwater eutrophic lakes, still need further investigation.

Microplankton is a general term for a kind of heterotrophic and polyculture zooplankton with a body length of less than 200 µm [8], which can serve as an important link between microbial food web and classic food web [1,9]. Previous studies have shown that microzooplankton played an important role in the grazing on phytoplankton [10–12]. Previous study has reported that microzooplankton could consume 49–77% of the phytoplankton primary productivity in the global waters, with the lowest percentage found...
in the Westerlies Southern and the highest in the Coastal Indian [12]. Therefore, microzooplankton plays a momentous role in carbon circulation and energy transfer in aquatic ecosystem. The grazing of microzooplankton can control the size of phytoplankton, and influence the reuse of nutrients and the growth of certain algae [13–15]. Nevertheless, the influence of microzooplankton grazing on bloom occurrence in freshwater eutrophic lakes has still not been clearly elucidated.

In aquatic ecosystem, bacteria are an important part of the microbial food web, which are mainly responsible for the mineralization and recycling of organic matter [16–18]. Bacteria respond rapidly to environmental changes, so the change of community structure can be used to indicate the water ecological environment [19,20]. Bacterial communities in water are affected by complex biological and abiotic processes, such as dissolved oxygen, pH, temperature, water nutritional status, and plankton interactions [21,22]. It has been found that the interaction between phytoplankton and bacteria could affect the dynamics of bacterial communities [23,24]. Bacteria can make use of secretions released by phytoplankton, as well as the debris of algal cells [23–25]. Phytoplankton also adversely affect bacterial communities through nutrient competition and antibiotic release [26]. In addition, previous study found that microzooplankton grazing in shallow eutrophic lakes eliminated 90–99% of the potential single-celled cyanobacteria production and 46% of the potential heterotrophic bacteria production [27]. Planktonic virus can cause the lysis of the bacteria, transforming particulate organic matter into dissolved organic matter utilized by bacteria. At the same time, as the hosts of viruses, bacteria have a great impact on the abundance of planktonic viruses [28]. Moreover, some studies have shown that some bacteria play an important role in the occurrence of eutrophic lake blooms [29–31]. Therefore, further studies on the changes of bacterial community structure between algal bloom and non-bloom period could help people to clarify the functional role of microbial food web in freshwater eutrophic lakes.

Lake Nanhu, located in the middle reaches of the Yangtze river, is a typical eutrophic lake with high phosphorus and chemical oxygen demand (COD) in Wuhan City, China. Due to the discharge of a large amount of domestic sewage and the introduction of aquaculture feed, the water of Lake Nanhu has long-term eutrophication, and algal blooms usually occur more than three times from May to September every year. Using Lake Nanhu as the study object, this study is intended to explore the following two questions: (1) changes in the community of phytoplankton, zooplankton, and bacteria between non-bloom and bloom period in eutrophic shallow lake; (2) variations in the grazing of phytoplankton by microzooplankton between non-bloom and bloom period. This study will help us to understand the role of microbial loop during algal blooms occurred in eutrophic lakes, and provide theoretical basis for improving water quality of eutrophic lakes according to biomanipulation and sustainable development.

2. Material and Methods

2.1. Field Observations and Sample Treatments

Lake Nanhu is located in Wuhan, Hubei Province, on the middle and lower reaches of the Yangtze River in China. It covers an area of 5.50 km², with a maximum depth of 3.2 m and an average depth of 1.6 m. There are generally two views on the definition of algal bloom: (1) the cell density of algae is $0.5 \times 10^6$–$15 \times 10^6$ cells/L [32]; (2) some algae multiply and gather in large numbers, forming algae floating on the water surface [33]. Taken together, we define algal blooms in the Lake Nanhu as algal cell density of $15 \times 10^6$ cells/L and floating algae on the water surface. Therefore, the bloom period of Lake Nanhu is from May to September each year. From October 2016 to September 2017, field surveys and sample collections were conducted at three different sites in Lake Nanhu every month (Figure 1). Physical parameters including temperature (T), pH and dissolved oxygen (DO) were measured in situ using a multi parameter water quality analyzer (HQ40d, HACH). For the measurement of other chemical parameters ($\text{NH}_4^+$, $\text{NO}_3^-$, $\text{NO}_2^-$, $\text{PO}_4^{3-}$, total nitrogen (TN) and total phosphorus (TP)), 1 L surface water was collected with 1 L sampler,
and was then brought back to the laboratory for further determination. Samples for the identification and enumeration of phytoplankton and zooplankton were collected using a 1 L sampler every month and fixed with Lugol's solution (2% final concentration). In addition, surface water samples for bacteria analysis were also collected during non-bloom (March and November) and bloom (May) period. Surface water for dilution experiments were collected by a plastic bucket and screened through a 200 µm mesh net to exclude larger grazers every two months. All the water samples were returned to the laboratory within 1 h.

Figure 1. The location of three sampling sites in Lake Nanhu, Wuhan, China.

2.2. Analysis of Plankton Community Structure

Under an optical microscope (Nikon Eclipse E100, Kobe, Japan), phytoplankton samples were identified and counted using a 0.1 mL counting chamber, and mesozooplankton and microzooplankton samples were identified and counted using a 1 mL and 0.1 mL counting chamber, respectively. The identification of phytoplankton followed the references of Hu and Wei [34]. Zooplankton was identified according to Kofoid and Campbell [35], Kofoid and Campbell [36] and Lee et al. [37]. Phytoplankton and zooplankton were identified to genus or species levels.

2.3. Analysis of Bacterial Community Structure

Water samples for bacteria community analysis were firstly filtered through a 20 µm membrane to remove impurities (Millipore, Carrigtwohill, Co, Cork, Ireland) and then filtered onto 0.22 µm polycarbonate filters (Millipore, Carrigtwohill, Co, Cork, Ireland). The 0.22 µm filters containing bacteria were placed into 2 mL sterile tubes and immediately frozen in liquid nitrogen, and transferred to a −80 °C refrigerator for storage until further procedures.

Bacterial DNA was extracted from all water samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer’s protocols. The final DNA concentration and purification were determined using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, NC, USA), and the DNA quality was checked via 1% agarose gel electrophoresis. The V3–V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGA C TACHVGGGTWTCTAAT-3′) using a thermocycler PCR system (GeneAmp 9700, ABI, Foster City, CA, USA) [38]. The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C; 27 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C; and a final extension at 72 °C for 10 min. PCR reactions were performed in triplicate in a 20 µL mixture containing 4 µL of 5 × FastPfu Buffer 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu Polymerase, and 10 ng of template DNA. The PCR products were extracted from a 2% agarose gel, further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified using QuantiFluor™-ST (Promega, Madison, WI, USA) according to the manufacturer’s protocol. According to the standard protocols by Majorbio
Bio-Pharm Technology Co. Ltd. (Shanghai, China), purified amplicons were mixed isometric and paired end sequenced (2 × 300) on Illumina MiSeq platform (Illumina, San Diego, CA, USA) [31]. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRR12968935–SRR12968943 (9 objects)).

Raw reads were demultiplexed, quality filtered by Trimmomatic, and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window. (ii) Primers were exactly matched allowing two nucleotide mismatches, and reads containing ambiguous bases were removed. (iii) Sequences with overlap longer than 10 bp were merged according to their overlap sequence. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version 7.1) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed using the RDP Classifier algorithm against the Silva (SSU123) 16S rRNA database using a confidence threshold of 70%. Nonparametric indicators (the Chao 1 estimator (Chao1), the Shannon estimator (Shannon), the Ace estimator (Ace) and the Good’s coverage (coverage)) were used to evaluate the relationships between bacterial community diversity characteristics and community coverage in Lake Nanhu. The prerequisites were fulfilled for a parametric test and One-way ANOVA was used to test for significant differences in bacteria structure among different months.

2.4. Microzooplankton Grazing Experiments

Microzooplankton grazing was studied by dilution experiments every two months according to Landry [39]. Before the experiment, the culture bottle (1.2 L, Whatman, Maidstone, UK) was soaked with 10% hydrochloric acid for more than 10 h, and then washed with the collected water. Subsequently, water samples were filtered with 0.22 µm (Millipore, Carrigtwohill, Co, Cork, Ireland) filter membrane to obtain particle-free water. Then the particle-free water was mixed with the water samples in four proportions of 0:1, 1:3, 1:1, 3:1, and divided into culture bottles with 3 parallel samples for each proportion. Taking care to fill each culture bottle with water and avoid air bubbles. All bottles were incubated at ambient light levels for 24 h at the surface (~0.5 m) in an experimental tank (~10 m diameter; ~2 m deep) cooled by running natural water from Lake Nanhu. To prevent settlement of phytoplankton, the bottles were moved up and down slowly every 8 h. Before and after culture, 3 × 300 mL water samples were taken from each proportion and filtered with 20 µm, 5 µm (Millipore, Carrigtwohill, Co, Cork, Ireland) and GF/F (Whatman, 0.7 µm pore size, 47 mm diameter) filtration membrane, respectively, to obtain three kinds of phytoplankton with different particle sizes (20–200 µm, 5–20 µm, and <5 µm). The filter membranes were stored in a refrigerator at −20 °C, and the concentration of chlorophyll a was measured by spectrophotometric method to obtain the biomass of phytoplankton [40]. In addition, 3 × 300 mL water samples were taken from each proportion before and after culture and filtered through 0.7 µm Whatman GF/F filters to analyze phytoplankton pigments.

2.5. HPLC Pigment Analysis

Filters containing phytoplankton from grazing experiment were stored at −80 °C refrigerator before performing pigment extraction. The phytoplankton-containing filters were cut into small pieces under dim light and transferred to a 15 mL centrifuge tube containing 10 mL 90% acetone (HPLC grade, J.T.Baker, Phillipsburg, NJ, USA). Wrapping the centrifuge tubes with aluminum foil and treating it with sonicated for 5 min. The samples were extracted overnight at 4 °C, and centrifuged at 4000 rpm (2325×g) for 20 min at 4 °C after extraction. To remove the filter and cell debris in the extract, the supernatants were filtered through Millipore syringe filters (Hydrophobic, 0.2 µm pore size). Aliquots of the extract were analyzed by HPLC (HP Agilent 1100 Series) for pigments within 48 h. An Agilent Eclipse XDB-C18 reversed phase column with a flow rate of 1.0 mL/min was used according to the method of Wong and Wong [41]. Phytoplankton pigments were identified
by comparing their retention times and online diode array absorption spectra with those of commercial authentic standards (DHI, Institute of Water and Environment, Århus, Denmark). Comparatively identified photosynthetic pigments including fucoxanthin, peridinin, 19′-hex-fucoxanthin, alloxanthin and chlorophyll b, and chlorophyll a. To ensure that all the pigments were retained, the HPLC column was run for 30 min. According to the peak areas in the chromatogram and the equation of the standard curve, the concentrations of each pigment were calculated.

2.6. Date Analysis

According to the experimental scheme proposed by Landry [39], apparent growth rate (AGR) of phytoplankton varies with dilution ratio (D):

\[ \text{AGR} = k - gD = \left( \frac{1}{t} \right) \ln \left( \frac{P_t}{P_0} \right) \]  

(1)

In the formula, “k” is phytoplankton growth rate, “g” is microzooplankton grazing rate, “P_t” is the density of phytoplankton at time “t”, and “P_0” is the density of phytoplankton at the beginning of culture. Linear regression analysis was performed on the apparent growth rate and dilution factor of a series of phytoplankton, and the absolute values of the intercept and slope were corresponding to k (d^{-1}) and g (d^{-1}), respectively.

The grazing pressure of microzooplankton on standing stocks (P_i) and primary production (P_p) of phytoplankton can be calculated as follows Verity et al. [42]:

\[ P_i = 1 - e^{(-g't)} \times 100\% \]  

(2)

\[ P_p = \frac{e^{kt} - e^{(k-g)t}}{e^{kt} - 1} \times 100\% \]  

(3)

The grazing preference index (\( \alpha_i \)) is used to calculate grazing selectivity of microzooplankton to different pigment groups of phytoplankton [43]. The formula is as follows:

\[ \alpha_i = \frac{r_i}{n_i} \times \frac{1}{\sum_{j=1}^{m} \frac{r_j}{n_j}} \]  

(4)

\( r_i \) and \( n_i \) are respectively expressed as the proportion of the i-th pigment group in food and environment, and \( m \) is the number of pigment groups. When \( \alpha_i > 1/m \), it means that microzooplankton has grazing preference for this group of phytoplankton; when \( \alpha_i < 1/m \), it means that microzooplankton has no grazing preference for this group of phytoplankton.

In this experiment, Excel 2019 and SPSS 19.0 software were used for data analysis. Significance levels of differences in phytoplankton growth rates, microzooplankton grazing rates, and the grazing pressure of microzooplankton on phytoplankton standing stocks and primary productivity were measured using one-way ANOVA analysis. The correlation between microplankton grazing rate and phytoplankton growth rate was tested by Spearman rank correlation. Downtrend correspondence analysis (DCA) was performed on the plankton data using Canoco 5.0, and the subsequent analysis methods of plankton and environmental factors were determined according to the sequencing axis length of the analysis results. In this study, the gradient length of the sequencing axis was less than 3.0, so redundancy analysis (RDA) was performed on environmental factors to identify the main environmental factors affecting phytoplankton community [44–46].

3. Results

3.1. Environmental Conditions

As shown in Figure 2, the surface water temperature (T) was 10.83 ± 0.20 °C in February (non-bloom period) and 32.73 ± 0.59 °C in August (bloom period), with average at 21.14 ± 8.16 °C around the whole study period (Figure 2A). The pH value is relatively stable around 8.5, reaching the highest and lowest values in bloom period (9.61) and non-bloom period (7.73), respectively (Figure 2B). The annual average value of dissolved oxygen (DO)
was 12.04 ± 3.87 mg/L, and the lowest value was 5.34 ± 1.03 mg/L in July (bloom period) (Figure 2C). The concentration range of total nitrogen (TN) was 1.86–16.45 mg/L. The concentration of TN, nitrite (NO$_2^-$) and ammonia (NH$_4^+$) in non-bloom period is higher than that in bloom period, and the nitrate (NO$_3^-$) is opposite (Figure 2D–F). Moreover, the concentration of TN is significantly positively related to the concentration of NH$_4^+$ ($r = 0.93$). Additionally, the concentration range of total phosphorus (TP) was 0.17–0.54 mg/L. The concentrations of total phosphorus (TP) and phosphate (PO$_4^{3-}$) were higher in bloom period than in non-bloom period (Figure 2D–F).

**Figure 2.** Variation of environmental conditions in Lake Nanhu during non-bloom and bloom period. Changes in temperature (A), pH (B), dissolved oxygen (C), total nitrogen (D), total phosphorus (E), nitrate (D), nitrite (E), phosphate (F) and ammonia (F) throughout the year.
RDA analysis showed that the eigenvalues of axis I and axis II were 0.367 and 0.302, respectively, and the accumulative variation of the two axes to the species community and environmental indicators reached 86.4%. As shown in Figure 3, TN, temperature and NO$_3^-$ were the main factors affecting the plankton abundance in Lake Nanhu. The abundance of Cyanophyta and Euglenophyta were positively correlated with T, NO$_2^-$, and pH, and negatively correlated with NH$_4^+$ and TN. The abundance of Cryptophyta, Bacillariophyta, and copepod (Mesocyclops leuckarti) were positively correlated with TP and negatively correlated with pH and DO, while Pyrrophyta and rotifer were positively opposite to the above three. The abundance of cladoceran was positively correlated with TP and PO$_4^{3-}$, while the abundance of protozoan was positively correlated with NO$_3^-$.

Figure 3. Redundancy analysis of plankton community composition and environment factors. T: Temperature; P1: Cyanophyta; P2: Pyrrophyta; P3: Cryptophyta; P4: Bacillariophyta; P5: Euglenophyta; P6: Chlorophyta; Z1: Cladoceran; Z2: Copepod (Mesocyclops leuckarti); Z3: Rotifer; Z4: Protozoan.

3.2. Variation in Plankton Community Composition

In general, microscopic observations showed that a total of 141 species of algae were identified from Lake Nanhu, in which Chlorophyta, Cyanophyta and Bacillariophyta contributed 83.21% of the species (Supplementary Table S1). The average abundance of phytoplankton during non-bloom period in Lake Nanhu was $8.01 \times 10^6$ cells/L, mainly dominated by Bacillariophyta ($3.47 \times 10^6$ cells/L). However, the average abundance of phytoplankton during bloom period was $2.71 \times 10^7$ cells/L, and mainly dominated by Cyanophyta ($1.64 \times 10^7$ cells/L) (Figure 4A). Moreover, the abundance of phytoplankton was highest in May ($3.37 \times 10^7$ cells/L) and lowest in December ($1.26 \times 10^6$ cells/L). *A. circinalis, Microcystis flos-aquae, Pseudoanabaena* sp., *M. aeruginosa, Asplanchna* sp., and *Pandorina* sp. were the main group of phytoplankton in the Lake Nanhu.
Figure 4. Variation of abundance on plankton in Lake Nanhu during non-bloom and bloom period. The density on phytoplankton (A) and zooplankton (B) in Lake Nanhu.

Qualitative analysis of zooplankton revealed that the main zooplankton in Lake Nanhu included protozoans (39 species), rotifers (21 species), cladocerans (8 species) and copepods (Nauplii and *Mesocyclops leuckarti*) (Supplementary Table S2). Quantitative analysis of microzooplankton showed that the density abundance of rotifers was higher in bloom period (523.89 ind./L) than that in non-bloom period (mean of 471.27 ind./L), especially *Brachionus* and *Polyarthra* (Figure 4B). Moreover, the abundance of zooplankton was highest in October ($1.86 \times 10^3$ ind./L) and lowest in February ($2.66 \times 10^2$ ind./L), both in the non-bloom period. *Brachionus calyciflorus*, *Moina macrocopa*, *B. diversicornis*, *Asplanchna sp.*, and *B. urceus* were the main group of zooplankton in the Lake Nanhu.

HPLC characteristic pigments (Figure 5) also showed differences of phytoplankton community composition between the non-bloom and bloom period in Lake Nanhu. In the non-bloom period (November and March), the phytoplankton pigment composition of Lake Nanhu accounted for more than 80% of fucoxanthin and peridinin. In June (bloom period), three pigments (alloxanthin, zeaxanthin and peridinin) were detected, and their proportions were all around 30%. In September (bloom period), Fucoxanthin (42.15%) account for a higher proportion. The results revealed that Bacillariophyta biomass was high both in non-bloom and bloom period (mean of 41.26% and 21.08%, respectively), while Pyrrophyta biomass was high in non-bloom period (mean of 47.12%), Cyanophyta biomass was high in bloom period (mean of 32.67%).

Figure 5. The relative abundance of different accessory pigments (Fucoxanthin, Peridinin, Zeaxanthin, Alloxanthin, Lutein, and Violasanthin) during non-bloom and bloom period in Lake Nanhu.

3.3. Variation in Bacterial Community Structure

A total of 298,585 reads were obtained from water samples of three sampling sites in Lake Nanhu in non-bloom period (March and November) and bloom period (May). The
rank abundance curve showed that all sequencing depths were adequate to reflect the bacterial variety (Supplementary Figure S1). As can be seen from Table 1, good’s coverage (≥99%) showed a high degree of sequence coverage. Additionally, the bacterial community had the highest Chao1 (1548.40 on average), Shannon (6.12 on average) and Ace (1519.45 on average) values in November, while it had lowest Chao1 (752.06 on average) and Ace (715.42 on average) values in March, and lowest Shannon values (4.48 on average) in May.

| Sample Time | Sample ID | Assigned Reads | OTUs | Shannon | Chao1 | Ace | Coverage |
|-------------|-----------|----------------|------|---------|-------|-----|----------|
| March       | S1        | 38,094         | 584  | 4.7549  | 757.109 | 725.66 | 0.996614 |
|             | S2        | 32,198         | 678  | 4.7303  | 826.061 | 755.73 | 0.995341 |
|             | S3        | 31,849         | 737  | 4.6504  | 673.0171 | 664.86 | 0.996326 |
| May         | S1        | 32,367         | 691  | 4.4499  | 867.581 | 858.64 | 0.994161 |
|             | S2        | 33,210         | 844  | 4.4581  | 970.068 | 910.42 | 0.993767 |
|             | S3        | 32,352         | 939  | 4.5401  | 953.432 | 944.28 | 0.993447 |
| November    | S1        | 32,773         | 1455 | 6.0961  | 1507.468 | 1492.73 | 0.994845 |
|             | S2        | 29,682         | 1550 | 6.1652  | 1369.113 | 1529.79 | 0.993332 |
|             | S3        | 36,060         | 1595 | 6.1025  | 1568.609 | 1535.84 | 0.993563 |

Notes: S1, S2, and S3: surface water samples. OTUs: Operational taxonomic units, Shannon: the Shannon estimator, Chao1: the Chao 1 estimator, Ace: the Ace estimator, Coverage: the Good’s coverage.

A total of 42 phyla and 232 orders were detected in the water samples from Lake Nanhu. Proteobacteria (32.97–52.78%) were the most abundant bacteria, followed by Actinobacteria (7.07–33.39%) and Bacteroidetes (5.04–16.73%) (Figure 6A). In the contrast, Nitrospinae, Firmicutes and Verrucomicrobia, only represented a minor proportion (Figure 6A). Actinobacteria was significantly more abundant in March and May (mean of 32.34% and 28.66% respectively) than those in November. The abundance level of Bacteroidetes (mean of 15.93%) and Cyanobacteria (mean of 16.58%) were higher in May than those in March and November. Moreover, Chloroflexi (mean of 22.76%) and Acidobacteria (mean of 10.26%) were more abundant in November than those in March and May (Figure 6B).

Further analysis indicated that Betaproteobacteriales, Frankiales, Microtrichales, Corynebacteriales, Flavobacteriales and Rhizobiales were all abundant in Lake Nanhu (Figure 6C). The abundance levels of Betaproteobacteriales in March and May were 25.95% and 21.07%, respectively, which were higher than that in November (11.01%). The abundance levels of Frankiales, Microtrichales and Rhizobiales (9.85%, 9.82%, and 7.39%, respectively) in March were higher than those in the other two months (Figure 6D). Meanwhile, Corynebacteriales (8.44%) and Flavobacteriales (6.68%) were more abundant in May than in March and November. Moreover, the abundance of Anaerolineale (12.57%) and Steroidobacterales (5.73%) reached highest levels in November (Figure 6D).

3.4. Microzooplankton Grazing on Size Specific Phytoplankton

Size-specific phytoplankton growth rates (k), as determined by dilution experiments, were usually lower in non-bloom period (−1.00 d⁻¹ to 0.25 d⁻¹) than in bloom period (0.23 d⁻¹–1.29 d⁻¹) (Figure 7A). Meanwhile, the size-specific microzooplankton grazing rates (g) were also higher in bloom period (0.61 d⁻¹–1.05 d⁻¹) than in non-bloom period (−1.15 d⁻¹ to −0.17 d⁻¹) (Figure 7B), which were positively correlated with phytoplankton growth rates significantly (p = 0.03, r = 0.81). Phytoplankton growth rates and microzooplankton grazing rates for phytoplankton < 5 µm groups were both significantly lower than other groups in January and November (p = 0.03), and did not differ significantly among the three size fractions in other months (p > 0.05). In May, microzooplankton grazing rates for <5 µm groups were higher than other groups (p = 0.04), and phytoplankton growth rates were lower than other groups (p = 0.04).
Figure 6. Community composition and relative abundance of bacteria. Classification structure and relative abundance in each water sample from Lake Nanhu (A) at the phylum level and (C) at the order level. At the specific level, “Others” means those that account for less than 1% of the total OTUs in each sample. In the overall distribution of bacteria at the phylum level (B) and at the order level (D) in each water sample, the bar graph represents the proportion of each bacterial phylum’s or order’s abundance in the samples. The difference in bacterial abundance was significant with a p-value of <0.05. * p < 0.05; ** p < 0.01; *** p < 0.001. S1, S2, and S3: surface water samples. (A–C) represent March, May, and November respectively.

The grazing pressure of microzooplankton on standing stocks (P<sub>i</sub>) for phytoplankton in different size fraction ranged from 2.73–74.01% (Figure 7C). P<sub>i</sub> for phytoplankton in 20–200 µm groups and 5–20 µm groups both had the highest level in July (bloom period, 74.01%, 67.56%) and the lowest level in November (non-bloom period, 2.73%, 9.18%). By comparison, P<sub>i</sub> for phytoplankton with particle size <5 µm had the highest (68.05%) and lowest (4.49%) level both in non-bloom period. The grazing pressure of microzooplankton on primary production (P<sub>p</sub>) for different particle size phytoplankton was 41.13–350.07% (Figure 7D). P<sub>p</sub> for phytoplankton with particle size 20–200 µm and 5–20 µm both had the highest level in January (non-bloom period, 350.07%, 325.61%) and the lowest level in May (bloom period, 48.24%, 41.13%). In addition, P<sub>i</sub> for phytoplankton with particle size <5 µm had the highest level in non-bloom period (307.78%) and lowest level in bloom period (74.95%).
3.5. Microzooplankton Grazing on Pigment Specific Phytoplankton

Pigment-specific phytoplankton growth rates were ranged from 0.37 d\(^{-1}\) to 2.54 d\(^{-1}\) (Figure 8A). Among them, the highest growth rate was peridinin (represented for Pyrrophyta) in September (bloom period, 2.54 d\(^{-1}\)) and the lowest growth rate was lutein (represented for Chlorophyta) in March (non-bloom period, 0.37 d\(^{-1}\)). According to Figure 8B, the grazing rates of microzooplankton on phytoplankton were 0.37 d\(^{-1}\)–2.75 d\(^{-1}\). Microzooplankton showed highest grazing rate on fucoxanthin (represented for Bacillariophyta, 2.75 d\(^{-1}\)) and lowest grazing rate on alloxanthin (represented for Cryptophyta, 0.37 d\(^{-1}\)) both in non-bloom period.

The grazing pressure of microzooplankton on standing stocks (\(P_i\)) and primary production (\(P_p\)) were ranged from 30.71 to 92.48% and from 40.20 to 253.88%, respectively (Figure 8C,D). For most pigments, \(P_p\) were around 90–120%, and the maximum values of \(P_p\) were recorded in lutein (253.88%) in non-bloom period. The maximum values of \(P_i\) for zeaxanthin (represented for Cyanophyta, 88.18%), alloxanthin (76.31%), violaxanthin (represented for Chlorophyta, 91.51%), and fucoxanthin (87.69%) were recorded in non-bloom period, while the maximum values of \(P_i\) for peridinin (92.48%) were recorded in bloom period.
Figure 8. Variation of microzooplankton grazing on pigment specific phytoplankton (Peridinin, Fucoxanthin, Zeaxanthin, Alloxanthin, Lutein, and Violaxanthin) during non-bloom and bloom period. The (A) growth rates, (B) grazing rates and (E) grazing preference index of phytoplankton with different pigments. The grazing pressure of microzooplankton on pigment specific phytoplankton (C) standing stocks ($P_i$) and (D) primary productivity ($P_p$).

The grazing preference index ($\alpha_i$) of microzooplankton for pigment-specific phytoplankton in two periods was shown in Figure 8E. During non-bloom period, microzooplankton showed preference to Cryptophyta ($\alpha_{Cryptophyta} = 0.46 > 0.20$) in November, and it showed preference to Pyrrophyta, Bacillariophyta, and Cryptophyta ($\alpha_{Pyrrophyta} = 0.27 > 0.20$, $\alpha_{Bacillariophyta} = 0.24 > 0.20$, $\alpha_{Cryptophyta} = 0.23 > 0.20$) in March. During bloom period, microzooplankton showed preference to Pyrrophyta and Cyanophyta ($\alpha_{Pyrrophyta} = 0.37 > 0.25$, $\alpha_{Cyanophyta} = 0.26 > 0.25$) in June, and it showed preference to Bacillariophyta ($\alpha_{Bacillariophyta} = 0.37 > 0.33$) in September.

4. Discussion

4.1. Changes in Phytoplankton Communities between Bloom and Non-Bloom Period

In Lake Nanhu, both *A. circinalis* and *M. flos-aquae* were considered to be the causative organisms in bloom period. *Anabaena* and *Microcystis* were most pervasive bloom-forming cyanobacteria in freshwater ecosystems [47,48]. Many *Anabaena* and *Microcystis* can produce algal toxins [49]. Thus, the occurrence of cyanobacteria blooms can have adverse effects on water quality, recreation, food web dynamics and human health [50–52]. Previous studies have often focused on a single type of bloom, while our study found that both *Anabaena* and *Microcystis* blooms occurred in freshwater eutrophic lakes. HPLC characteris-
tic pigments also showed that Cyanophyta was the algae that caused the bloom in Lake Nanhu (Figure 5).

As a primary producer in water, phytoplankton species composition can objectively reflect the changing law of water environment and play an important indicator role. In this study, the composition of the phytoplankton community in Lake Nanhu exhibited changes between bloom and non-bloom period. Bacillariophyta and Cyanophyta were the main dominant species in non-bloom and bloom period, respectively. The result of HPLC characteristic pigments also confirmed this conclusion. There are many factors that influence the change of phytoplankton community (such as physical indicators, nutrients, grazing pressure). In this study, when the temperature increased from April to May, the abundance of Cyanophyta increased rapidly and became the dominant species in water (Figure 4A). Previous study has shown that Cyanophyta can reduce the abundance of other phytoplankton species by allelopathic mechanism [53]. Therefore, Bacillariophyta cannot continue to be dominant species in water even though their abundance increased with the increased of TP concentration. Studies have shown that Cyanophyta abundance is positively correlated with TN concentration [54,55], which is inconsistent with the results of this study. The possible explanation is that the Microcystis blooms could reduce the nitrogen in water bodies [56]. In addition, the discharge of domestic sewage and aquaculture are also important reasons for the changes in the phytoplankton community. Compared with deep lakes, shallow-water lakes are more susceptible to external changes, and the interaction between phytoplankton and its ecosystem is more significant. Therefore, the relationship between phytoplankton and environmental factors in shallow eutrophic lakes needs more in-depth research.

4.2. Changes in Zooplankton Communities between Bloom and Non-Bloom Period

Zooplankton, as the primary consumers of aquatic ecosystems, play an important role in the food chain and affect the quality of water environment. Zooplankton is an important part of the water environment and is crucial to maintaining the stability of freshwater ecosystems [50]. In this study, rotifers were the main components of zooplankton in Lake Nanhu, followed by cladocerans. The abundance of rotifers and cladocerans in bloom period was higher than that in non-bloom period. This may be due to the higher abundance of phytoplankton and water temperature during the bloom. Within the appropriate range of temperature and food density, the population density of rotifers and cladocerans increase with the increase of temperature and food density [57–63].

*B. calyciflorus* was the most dominant zooplankton in the non-blooming period, while the most dominant species in the blooming period was *M. macrocopa*, followed by *B. calyciflorus*. There are many factors influencing the relationship between rotifers and cladocerans, which are often the result of the combined action of biological and non-biological factors, such as temperature [64,65], food [66], individual size [67,68], and grazing [69,70]. When the individual size of cladocerans is less than 1200 µm, the competitive inhibitory effect of cladocerans on rotifers is much weaker, and rotifers can coexist with them at a higher density [71]. Previous studies reported that the individual size of *M. macrocopa* is mostly around 1200 µm [72,73]. Therefore, when *M. macrocopa* competes with rotifers, environmental factors such as temperature and food may have a greater effect.

Zooplankton, especially rotifers and cladocerans, are very sensitive to temperature changes in shallow lakes [74]. Many studies have shown that the quantity, quality and type of food have a significant impact on the abundance, diversity and interspecific competition outcome of zooplankton [75–78]. Previous study has shown that the cyanobacteria bloom was observed together with the high abundance of small-sized zooplankton [79]. Studies have shown that *B. calyciflorus* could adapt to eutrophic water by changing their life history and grazing intensity [80]. Fulton and Paerl pointed out that rotifers could consume small *Microcystis* groups [81]. Small cladocerans have a more obvious competitive advantage than large cladocerans at higher temperatures, which is believed to be the reason why small cladocerans have higher population abundance in warm waters [82]. Previous studies have
also proposed that small cladocerans were more resistant to cyanobacteria and ingesting bacteria than large cladocerans, so they are more adaptable to algal blooms [83,84]. This may be the reason why *M. macrocopa* become the dominant species during bloom period of the eutrophic shallow lake Nanhu.

### 4.3. Changes in Bacteria Communities between Bloom and Non-Bloom Period

The composition of the plankton bacterial community in water is regulated by a variety of biological and non-biological factors such as phytoplankton, zooplankton, temperature [85], and nutrients [86]. Temperature can indirectly affect the community composition of bacteria by affecting the community structure of phytoplankton and zooplankton [87]. The organic matter produced by phytoplankton and zooplankton provides energy for the growth of bacteria [88-90]. In addition, zooplankton can also directly graze bacteria, which affects the number and distribution of bacteria [91]. In lake ecosystems with different nutrient levels, nutrients have different effects on bacteria. In oligotrophic lakes, nutrients can become a limiting factor for the growth of bacteria [92,93]. In mesotrophic lakes, nutrients have different limiting effects on bacteria in different seasons [94]. In eutrophic lakes, the effect of nutrients on bacteria is less than that of phytoplankton [95]. Studies have also found that during the lake bloom period, the number and species of bacteria have changed significantly, and this change could be used to predict the algal bloom [96]. Therefore, planktonic bacteria in water are regarded as important environmental indicators of water ecology.

In this study, bacteria communities were mainly dominated by Proteobacteria and Actinobacteria, which contributed more than 50% to relative abundance of bacteria in both bloom and non-bloom periods (Figure 6A). Moreover, the mean proportion of Bacteroidetes in May (bloom period) was significantly higher than that in March and November (non-bloom period) (Figure 6B). The result also supported the increase of cyanobacteria could promote the growth of Proteobacteria, Actinobacteria and Bacteroidetes [97]. Previous studies have found that the organic secretions of cyanobacteria could adsorb Bacteroidetes, and the Bacteroidetes could lyse cyanobacteria cells [98,99]. Pinhassi et al. also found that the Bacteroidetes may play a vital role in the processing of organic matter during the algal bloom [100]. Thus, it is also an effective method to remove cyanobacteria by bacterial adhesion to surface [101,102]. All in all, bacteria were believed to participate in certain important activities during algae blooms, and need to be study further.

### 4.4. Microzooplankton Grazing on Different Phytoplankton Groups between Bloom and Non-Bloom Period

Microzooplankton grazing rates varied similarly with the growth rates of phytoplankton in different size groups during bloom period (Figure 7A,B). The result showed that microzooplankton grazing can respond quickly to the increase in phytoplankton abundance [103]. During non-bloom period, grazing pressure of microzooplankton on the standing stocks (P<sub>i</sub>) for phytoplankton <5 µm groups were relatively higher than that for the other two size groups. In comparison, P<sub>i</sub> were not significantly different among those three size groups of phytoplankton during bloom period (p > 0.05), even though all the P<sub>i</sub> values increased. This indicates that small size groups of phytoplankton could be effectively controlled by microzooplankton during non-bloom period.

Grazing pressure of zooplankton can be related to the type of food, body size, feeding mode, selectivity and tolerance to prey [104]. Copepods and cladocerans display selectivity on size of food particles and type of food, while rotifers display selectivity in regard to condition of algal cells as well as type of food [105]. In this study, microzooplankton showed preference to Pyrrophyta and Bacillariophyta both in bloom and non-bloom periods, while they showed preference to Cryptophyta and Cyanophyta during non-bloom period and bloom period, respectively. During bloom period, phytoplankton community was dominated by Cyanophyta and microzooplankton community was dominated by rotifers. Although phytoplankton such as *Anabaena* and *Microcystis* were poor quality prey to rotifers, microzooplankton still showed preference on Cyanophyta. This indicates
that grazing preference of microplankton during the bloom period was relatively mainly affected by the community composition of phytoplankton.

4.5. Ecological Restoration of Eutrophic Lakes

In the present study, microzooplankton grazing could quickly respond to phytoplankton growth. However, the improved growth conditions at the onset of a bloom allow phytoplankton to escape microzooplankton grazing pressure [106]. Therefore, the regulation of cyanobacteria blooms in Lake Nanhu requires more other methods. In the past 30 years, the main pollution sources of Lake Nanhu were the discharge of domestic sewage and the release of aquaculture feed. At present, under the management of relevant departments and policies, the sewage outlets of Lake Nanhu have been basically blocked. To further improve the water environment, more approaches need to be undertaken. Biomanipulation is an important theory first proposed by Shapiro et al. to control algae in eutrophic lakes [107]. Since then, traditional and non-traditional biomanipulation has been widely used in the prevention and control of eutrophication of water bodies in Europe, North America, and China [107–109]. Due to the lack or slow proliferation of original microzooplankton in the natural environment, the process of improving water quality by biomanipulation is relatively slow or inefficient [109]. In this study, it was also found that microzooplankton played little role during algal blooms occurred in freshwater eutrophic lakes. Nevertheless, this study still provides some basic information for improving water environment quality according to biomanipulation.

5. Conclusions

The community of phytoplankton, zooplankton, and bacteria changed between bloom and non-bloom periods: Firstly, Cyanophyta, especially *A. circinalis* and *M. flos-aquae*, were mainly dominant species during bloom period; secondly, *B. calyciflorus* and *M. macrocopa* were the most dominant zooplankton in the non-bloom and bloom period, respectively; thirdly, *Bacteroidetes* showed significantly higher mean proportion during bloom period than that in non-bloom period. Moreover, microzooplankton grazing could respond quickly to the increase in phytoplankton abundance. However, microzooplankton grazing has little effect during the outbreak period in the natural environment. This study will help us to understand the role of microbial webs during algal blooms in freshwater eutrophication lakes and provide basic data for the application of biomanipulation in the future.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13091296/s1, Figure S1. Rarefaction curves base on high-throughput sequencing. A: the period of pre-bloom, B: the period during algal bloom, C: the period of post-bloom., Table S1: The composition of phytoplankton species in Lake Nanhu (+++: Predominant species; ++: second dominant species; +: present)., Table S2: The composition of zooplankton species in Lake Nanhu (+++: Predominant species; ++: second dominant species; +: present).

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