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D. magna in Combination with *M. aquaticum* Inhibited the Bacterioplankton in Eutrophic Water

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Abstract: *D. magna* can affect the composition of planktonic bacteria, and can also significantly inhibit the growth of *Cyanobacteria* at high density. In this study, the inhibitory effects of low-density *D. magna* groups were stronger than high-density groups due to increases in *Acidobacteria* abundance in water. Meanwhile, *D. magna* can inhibit the growth of *Planktothrix* and *Microcystis*, but especially the growth of the latter. Alternatively, *M. aquaticum* and *C. demersum* can change the community structure of planktonic bacteria. Among them, the inhibitory effect of *M. aquaticum* on *Microcystis* and *Planktothrix* is strong, but it also increases the relative abundance of *Chlamydia* in water and the risk of pathogenic bacteria. In contrast, the inhibitory effect of *C. demersum* was more significant on *Microcystis* than on *Planktothrix*. Therefore, the combination of "submerged plants-*Daphnia*", especially the combination of low density *D. magna* with *M. aquaticum*, had a significant inhibitory effect on *Planktothrix* and *Microcystis*.

Keywords: submerged macrophytes; *D. magna*; plankton; eutrophic water; purification effect; microbial

1. Introduction

Human activities such as agricultural production and rural life have accelerated the discharge of nitrogen, phosphorus, and other nutrients into the water environment [1], resulting in a rapid increase in algae and plankton and a significant decline in transparency and dissolved oxygen in the water column [2]. Some algae metabolites rapidly deteriorate water quality, causing serious damage to the ecological balance of the water environment [3]. In particular, closed water bodies such as ponds and lakes have weak self-purification ability, which aggravates eutrophication [4]. Eutrophication of ponds and lakes will lead to overgrowth of algae and inhibit the growth and reproductive ability of other aquatic organisms, and eventually lead to their high mortality [5].

Although several methods have been investigated to mitigate eutrophication [6,7], biomanipulation is considered one of the most effective ecological methods to control algal growth, mainly through reducing algae abundance by plankton-feeding fish [8,9]. An increase in plankton can promote the growth of highly efficient filter-feeding phytophagous macrozooplankton, and consequently, the efficiency of other feeders such as phytoplankton or zooplankton [10–12]. This implies that, ultimately, phytoplankton biomass can be reduced. As a common macrozooplankton in lakes and reservoirs, *Daphnia magna* has long been a dominant species, with a fast growth and reproduction rate as well as strong feeding potential [13]. At present, there have been many successful cases of purifying

eutrophic water by controlling algae growth using cladoceran zooplankton, both in these regions and abroad [14,15]. For example, Gremberghe et al. [16] designed two co-culture experiments: (1) a *D. magna* culture medium filtrate, and (2) a commercially available *Daphnia* infochemical consisting of the addition of sodium octyl sulfate to study the effects on strain growth and microcystin production when they were co-cultured with eight *Microcystis* strains. The results show that both the *D. magna* culture filtrate and sodium octyl sulfate had a negative effect on the growth of half of the strains. Chislock et al. [17] conducted an enclosure test using *Daphnia pulicaria* and found that it could tolerate microcystins and significantly reduce *Cyanobacteria* biomass in *Cyanobacteria*-dominated waters. Additionally, other studies showed that filter-feeders could not withstand microcystins, giving rise to poor adaptability and a eutrophic environment with *Cyanobacteria*-dominated waters [18].

Concurrently, the remediation of hot-spot areas through macrophytes, algae bloom inhibition, and the recovery of the aquatic system has been witnessed in recent years [19]. Basically, macrophytes (i) absorb nutrients such as nitrogen and phosphorus that occur in the water during its growth [20]; (ii) boost huge root systems that can provide a living environment and attachment sites for the growth and reproduction of microorganisms, which would entail an increase in the diversity of microorganisms in water [21]; and (iii) are susceptible to some higher plants such as *Vallisneria natans*, *Hydrilla verticillata*, *Solidago canadensi*, *Ceratophyllum demersum*, or *Myriophyllum verticillatum* that secrete organic acids, organic esters, ketones, and other allelochemicals during their growth that inhibit algae growth by destroying their cell membrane, and also affecting cell division and photosynthesis [22].

Over recent years, there have been some achievements made in combined treatments for the remediation of eutrophic waters. However, to our knowledge, research on the use of biological treatments for the remediation of eutrophic water bodies by combining *D. magna* and macrophytes is limited, and its effectiveness has not yet been evaluated. Therefore, we assessed the potential remediation effect of a single organism and its combination with eutrophication water to provide new insights for better remediation of eutrophic waters.

2. Materials and Methods

2.1. Source of Original Materials for Testing

In this study, raw water was taken from a pond nearby farmland in Ninghe County, Tianjin (N 39°33′, E 117°82′). Water characteristics were determined as follows: pH 8.77, total nitrogen (TN) 7.03 mg L^{-1}, ammonia-nitrogen (NH_4^+-N) 0.21 mg L^{-1}, nitrate nitrogen (NO_3^--N) 6.59 mg L^{-1}, total phosphorus (TP) 0.28 mg L^{-1}, dissolved oxygen (DO) 9.28 mg L^{-1}, water transparency (WT) 17.20 cm, and chlorophyll a (chla) content was 272 μg L^{-1}.

The original macrozooplankton of the sampled water were filtered (no. 13 zooplankton net), diluted with a sterilized BG-11 (4%) culture solution in a 1:1 ratio, and an appropriate living space for phytoplankton and bacterial communities was left. This water sample was used to investigate the effect of the test setup on the native phytoplankton and bacterial community in the eutrophic pond.

Given the economic value of aquatic plants, *Myriophyllum aquaticum* and *Ceratophyllum demersum* were selected as the study test plants. These were purchased from a flower market in Tianjin and cultured in 4% BG-11 culture solution for 15 days to adapt to the test environment. Well-grown plants of similar size were then selected for the test.

D. magna was purchased from the Institute of Applied Ecology, Chinese Academy of Sciences in Shenyang and its cultivation enlarged in the laboratory before the test. The flask containing *D. magna* was put into an artificial climate box for more than 60 days before the test at a temperature of 25 °C, light intensity of 1200 lux, and light-to-dark ratio of 12:12 h. *Scenedesmus obliquus* (20 mL) was fed to the flask daily. Before the test, the filial generation made up of individuals alive, healthy, and of similar size was selected through a filter screen with a 2 mm pore size. Then, they were cleaned in pure water for 24 h so that their intestines were emptied as much as possible for the test.

2.2. Experiment Design

Test barrels were constructed of polyvinylchloride (PVC) with a 30 cm diameter and a 30 cm height. Before the experiment, the barrels were sterilized with ethanol (75%) and repeatedly rinsed with pure water. Fluorescent lamps were used to simulate the sunlight in spring and summer. The light intensity was 1200 ± 100 lux and the light-to-dark ratio was 12:12 h. The test water was mixed thoroughly and then packed into each test barrel. The initial water content was 20 L. The remaining water was used for measurement, which was used at the initial value of 0 d. The heating rod in each test barrel was set to a constant temperature of 25 °C. Thermometers were set to monitor water temperature to ensure constant water temperature during the test. The test time lasted for 20 d. The test setups were all based on the results of the pre-test, and there was a total of nine groups with three replicates (Table 1) (i.e., Time 0: original water samples; K2, K10, and K20 refer to the 2-, 10-, and 20-day water samples in the control treatment; A2, A10, and A20 refer to the 2-, 10-, and 20-day water samples in low-density of the *Daphnia magna* treatment; B2, B10, and B20 refer to the 2-, 10-, and 20-day water samples in high-density *Daphnia magna* treatment; C2, C10, and C20 refer to the 2-, 10-, and 20-day water samples in the *Ceratophyllum demersum* treatment; D2, D10, and D20 refer to the 2-, 10-, and 20-day water samples in the *Myriophyllum spicatum* treatment; E2, E10, and E20 refer to the 2-, 10-, and 20-day water samples in the low-density combination of *Daphnia magna* and *Ceratophyllum demersum* treatment; F2, F10, and F20 refer to the 2-, 10-, and 20-day water samples in the high-density combination of *Daphnia magna* and *Ceratophyllum demersum* treatment; G2, G10, and G20 refer to the 2-, 10-, and 20-day water samples in the low-density combination of the *Daphnia magna* and *Myriophyllum spicatum* treatment; and H2, H10, and H20 refer to the 2-, 10-, and 20-day water samples in the high-density combination of the *Daphnia magna* and *Myriophyllum spicatum* treatment.

Table 1. Experimental design of different treatments.

Treatments		*Daphnia magna*	*Ceratophyllum demersum*	*Myriophyllum spicatum*
K (control)		/	/	/
Daphnia magna	A	25 ind L^{-1}	/	/
	B	100 ind L^{-1}	/	/
Submerged macrophytes	C	/	3 g L^{-1}	/
	D	/	/	3 g L^{-1}
Submerged macrophyte + *Daphnia magna*	E	25 ind L^{-1}	3 g L^{-1}	/
	F	100 ind L^{-1}	3 g L^{-1}	/
	G	25 ind L^{-1}	/	3 g L^{-1}
	H	100 ind L^{-1}	/	3 g L^{-1}

2.3. Collection and Determination of Samples

2.3.1. Collection of Samples

This study was conducted in June 2020, when the temperature varied from around 20 to 28 °C, which is why we put heating rods (20 cm height) in the barrels to keep the water at a constant temperature. Sampling was conducted between 8:00–9:00 am, and before sampling, we slightly stirred the water in each barrel to avoid uneven heating. The water level was recorded after each sampling to reduce errors. Distilled water was used to make up the evaporation before the next sampling. The diversity of planktonic bacteria was determined on days 2, 10, and 20 of the test.

2.3.2. Community Structure of Planktonic Bacteria

On day 0 of the test, a fully mixed 300 mL of the test water sample was taken. On days 2, 10, and 20 of the test, a 100 mL water sample was taken from barrels in each test group and mixed well. The 300 mL mixed water sample was then filtered with a sterile filter membrane with a pore size of

0.22 μm and a diameter of 4.5 cm. DNA of the sample on the filter membrane was extracted by a Powerwater DNA Isolation kit (MO BIO, Jefferson City, MO, USA) and purified by a MObio power clean®DNA clean-up kit (MO BIO, Jefferson City, MO, USA). The DNA concentration was detected by Qubit 2.0. Its integrity was detected by 1% of agarose gel.

PCR in the V3–V4 region of the 16S rRNA gene was amplified by the specific primers (338F:5′-ACTCCTACGGGAGGCAGCAG-3′, 806R:5′-GGACTACHVGGGTWTCTAAT-3′). The PCR 20 μL reaction system included a 4 μL 1 × FastPfu buffer, 2.5 mM dNTPs, 5 μM forward and backward primers, 1 unit FastPfu enzyme (2.5 unit μL^{-1}), and a 10 ng DNA template. Initially, it was pre-denatured at 95 °C for three minutes, then went through 27 cycles. Each cycle included denaturation at 95 °C for 30 s and annealing at 55 °C for 30 s, and extended at 72 °C for 45 s, then to 72 °C for 10 min. The reaction product was detected with 2% of agarose gel, and the target band was cut off and then recycled by an AxyPrepGel Kit (Axygen, CA, USA). The products were subjected to double-end sequencing on the Illumina MiSeq platform (Illumina Miseq, Illumina, CA, USA, Shanghai Major Biomedical Technology Co. Ltd., Shanghai, China), according to standard methods.

2.4. Data Analysis and Processing

2.4.1. Chlorophyll a (Chla) and Microcystin (MC) Measurement

Chla was determined through the fluorometric technique using a Turner Designs Model 10–005 fluorometer. The chl-a was extracted at 20 °C in darkness over 24 h with acetone. Microcystin concentrations were measured using enzyme-linked immunosorbent assay (ELISA) following the manufacturer's protocol (Abraxis®, Warminster, PA, USA).

2.4.2. Operational Taxonomy Units (OTUs) Taxonomic and Microbial Diversity Analysis

The measured data were screened by QIIME (Version 1.17) and matched after it had its low-quality sequence removed. After removing chimeras using UCHIME, at a similarity level of 97%, operational taxonomy units (OTUs) were generated using UCLUST to cluster. Comparative analysis and species of OTU representative sequences were performed and annotated by SILVA libraries. The high-quality sequences were compared with the SILVA (128) database [23], and reference sequences with a confidence level of more than 70% were identified [24]. OTU groups were identified according to the reference sequences, and the information was annotated to phylum, class, order, family, and genus. In addition, OUT numbers, Chao, and Shannon and Simpson indices were used to indicate the microbial richness and diversity. Calculation methods for these indices can be found at http://www.mothur.org/wiki.

2.4.3. Statistical Analysis

Reshape2, ggplot2, and other R language packages (Version 3.6.3, http://www.r-project.org/)) were used to draw the histogram of relative abundance at the phylum level, and the pheatmap package was used to draw the heat map of relative abundance at the genus level.

According to the plankton bacteria relative abundance data, when entering the formation and stable phases of the microflora structure (days 10 and 20), hypothesis testing was performed on the species groups within the bacterial communities in different groups. The significance level of the abundance difference of the species groups was evaluated using one-way analysis of variance (ANOVA), a Tukey's honestly significant difference (HSD) test, and redundancy analysis (RDA), with a significance level of 0.05 to obtain the groups with significant differences among them. The RDA analyses between planktonic bacteria in the formation and stable phases and water environmental factors were carried out using Canoco (Version 5.0) software.

3. Results

3.1. Influence of "Submerged Macrophytes—D. magna" Combination on Planktonic Bacteria Diversity

The 415.0 ± 2.9 reads representing mean length amplicons were retained for further analysis (Table S1). Alpha diversity index of each sample was evaluated, and the results are shown in Table 2. Each sample coverage index was above 98%, indicating that the sequencing results in the test water best reflect the composition of planktonic bacterial communities. Values of the richness index (Chao1 index) and diversity indices (Shannon and Simpson index) of each sample are shown in Table 2. The Shannon index of the high-density *D. magna* treatments (F, H) decreased over the first ten days and increased at the end of the experiment, but the overall trend was downward. In contrast, the Shannon index of the low-density *D. magna* treatments (E, G) showed the opposite trend. In general, our experimental treatments failed to improve water bacterial diversity.

Table 2. Microbial richness and community diversity indexes of "submerged macrophytes—*Daphnia magna*" combination in different treatment group.

Treatment	Coverage	Shannon	Simpson	Chao	Treatment	Coverage	Shannon	Simpson	Chao	Treatment	Coverage	Shannon	Simpson	Chao
K2	0.988	5.28	0.0145	1246	K10	0.989	4.40	0.0595	1053	K20	0.987	4.36	0.0659	1175
A2	0.988	5.32	0.0140	1249	A10	0.989	4.27	0.0718	1037	A20	0.991	5.25	0.0101	973
B2	0.989	5.09	0.0224	1174	B10	0.990	5.35	0.0110	1097	B20	0.991	4.87	0.0169	914
C2	0.989	5.04	0.0280	1184	C10	0.987	5.37	0.0107	1255	C20	0.991	5.57	0.0080	1082
D2	0.988	5.42	0.0123	1320	D10	0.990	4.40	0.0558	944	D20	0.990	4.78	0.0322	1042
E2	0.988	5.20	0.0196	1240	E10	0.990	5.49	0.0111	1171	E20	0.989	5.06	0.0158	1108
F2	0.988	4.99	0.0262	1219	F10	0.988	4.82	0.0542	1165	F20	0.990	5.39	0.0130	1140
G2	0.987	5.32	0.0166	1343	G10	0.988	5.34	0.0128	1258	G20	0.989	4.79	0.0231	1070
H2	0.989	4.81	0.0422	1151	H10	0.987	4.14	0.0860	1156	H20	0.988	5.25	0.0114	1135

Compositional similarity between two or more sample bacterial colonies can be characterized using beta diversity. Two commonly-used methods to calculate beta diversity are cluster analysis (CA) and non-metric-multi-dimensional scaling (NMDS) (Figure 1). In the dendrogram, samples of high-density *D. magna* treatments (B, F, H), low-density *D. magna* treatments (A, E, G), and the two aquatic plant treatments (C, D) were clustered at similar coordinates at 2 d during the experiment, indicating that there was no difference in planktonic bacterial community species composition among treatment groups (Figure 1). On the 10th and 20th days of the experiment, the treatment groups A and B, E and F, G and H samples were gathered into one branch, and the samples of the control group K were gathered into another branch, far away from the samples of these groups. This indicates that, over time, both *D. magna* and submerged plants have an impact on planktonic bacteria community structure (Figure 1).

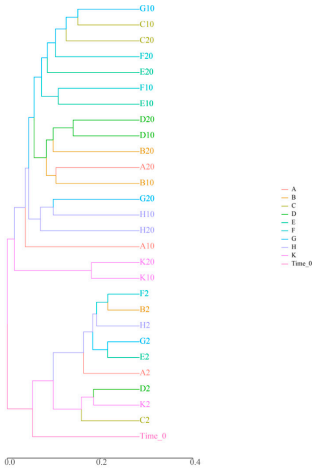

Figure 1. Analysis results of the beta diversity of microbiota. Note: The axis represents the evolutionary distance among different microbials.

3.2. Influence of "Submerged Macrophytes—D. magna" Combination on Planktonic Bacterial Community Structure

Bar graphs of the relative abundance of each sample at the phylum level during the experiment are shown in Figure 2. Overall, *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Actinobacteria*, *Planctomycetes*, and *Firmicutes* were the predominant bacteriophyta in the samples, and their cumulative relative abundance accounted for more than 80% of each sample. *Proteobacteria* contained the most bacterial species, with ten genera among the top 20 in relative abundance. The bar graphs show that different samples had the same dominant species at the phylum level, but the relative abundance was different in each sample.

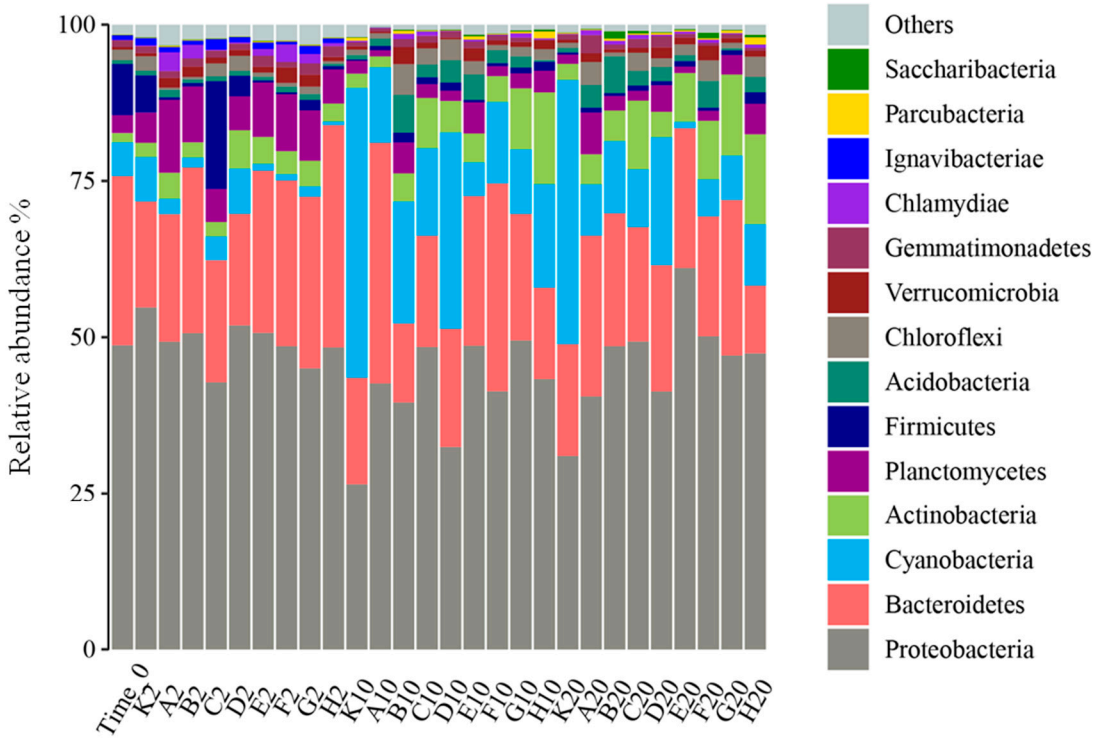

Figure 2. Bacterial community composition at the phylum level.

During the entire experiment, *Flavobacterium* relative abundance was continuously higher in each sample, while by the second day of the experiment, the dominant species in each group were *Proteobacteria*, with relative abundance of 42.83–54.81%. Among *Proteobacteria*, the proportions of *Unclassified-f-Rhodobacteraceae*, *Hydrogenophaga*, and *Woodsholea* were relatively higher in each group, and the composition at the genus level was similar in each group, which is consistent with the sample clustering results. At day 10 of the experiment, *Planctomycetes* appeared in all treatment groups, so the living space for other microorganism phyla was greatly compressed. Compared to the control (K10, K20), all the treatments better inhibited the growth of *Microcystis*, which is a *Cyanobacteria* (Figure 3). It is notable that *Mycobacterium* grew markedly from 10 to 20 days of the experiment in all treatments except K, A, and B. To further analyze the impacts of different treatments on planktonic bacterial community composition, differences in relative abundance among groups were analyzed for the top 20 species (Figure 3). At the phylum level, *Cyanobacteria*, *Actinobacteria*, and *Proteobacteria* significantly differed among all groups ($p < 0.05$), while at the genus level, *Mycobacterium*, *Microcystis*, and *Planktothrix* were the significantly dominant species ($p < 0.05$).

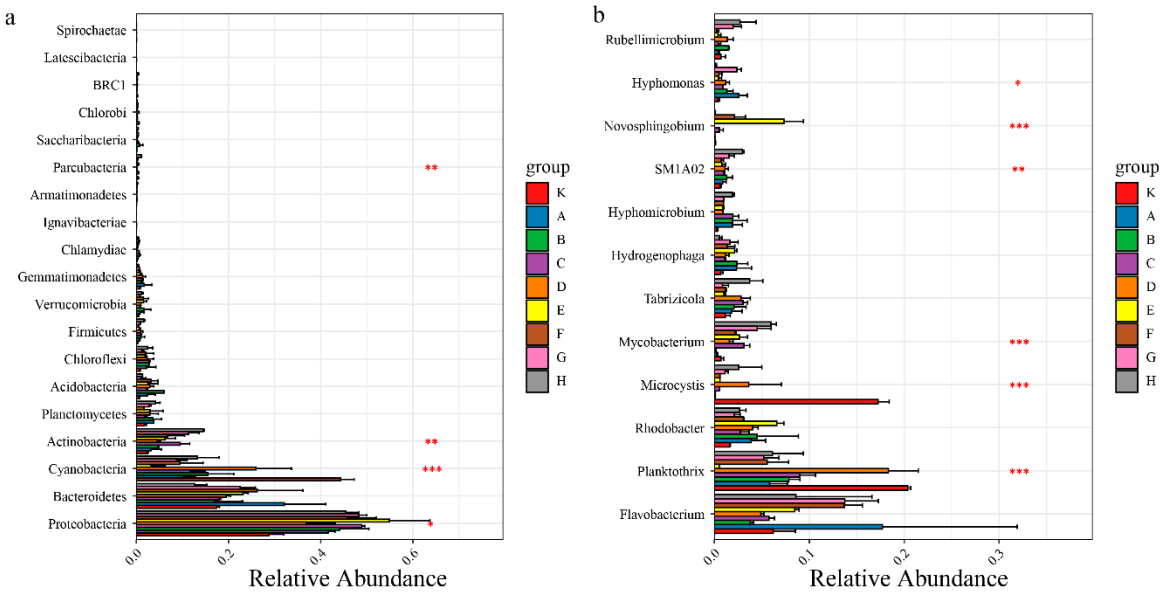

Figure 3. Univariate ANOVA of intergroup differences in Microphylum (**a**) and genus (**b**) levels. Note: Asterisks indicates the significance among different microbials; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.3. Relationships between Water Environmental Factors and the Planktonic Bacterial Community during "Submerged Macrophytes—D. magna" Combined Remediation

To further investigate interactions between water environmental factors and bacterial community composition during *D. magna* remediation, redundancy analysis (RDA) was performed. Water environmental factors including water TN, TP, and Chla concentrations, microcystins (MCs), and water transparency (WT) were used in a ranking analysis of relative abundance among groups of the top ten species at the genus level in all treatments (Figure 4). Chla concentration and water transparency were the key environmental factors that affected the bacterial community structure of the eutrophic water, and most bacterial species were positively correlated with WT.

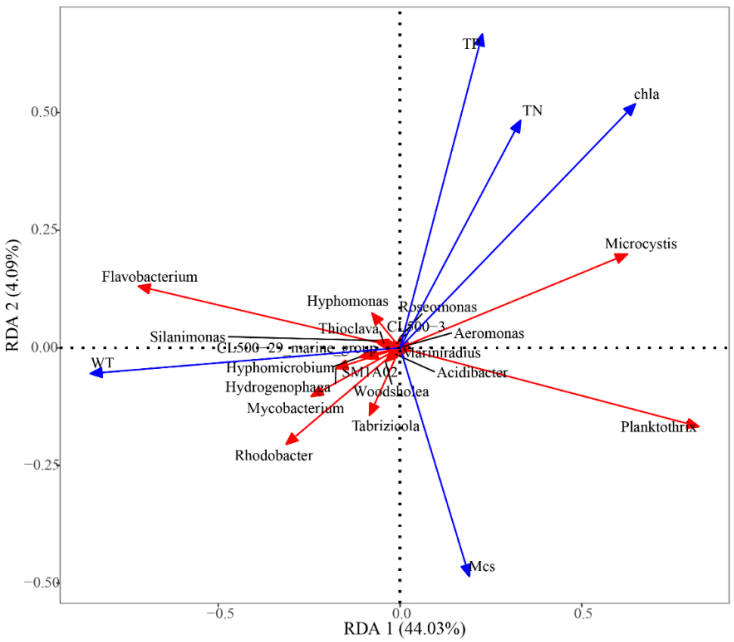

Figure 4. Redundancy analysis of microbial community gate levels. The scale on the x- and y-axes represent the values generated by regression analysis with environmental factors for each sample or species.

4. Discussion

4.1. Effects of D. magna on Bacterioplankton in Eutrophic Water

D. magna exhibited different inhibitory effects due to *Cyanobacteria* of varying toxicity [25]. For *Microcystis*, the inhibitory effect of *D. magna* was visible; however, *Planktothrix*, a type of filamentous *Cyanobacteria*, can interfere with zooplankton abdominal movement in a winding manner, blocking their filter-feeding organs and causing them damage [26]. Therefore, *D. magna* has a weaker inhibitory effect on *Planktothrix*, and their feeding effects even increased the relative abundance of *Cyanobacteria* (Figure 2). It has been speculated that strong *D. magna* excretion accelerates nutrient recycling, facilitating *Cyanobacteria* growth [27]. Notably, high-density *Daphnia* has a stronger respiration that releases more CO_2 into the water, due to the lower pH in B10 and B20 samplings, so the relative abundance of *Acidobacteria* (grows in acidic environment) was higher in the two samples.

4.2. Effects of Submerged Macrophytes on Bacterioplankton in Eutrophic Water

Our research results suggested that *Myriophyllum spicatum* and *C. demersum* have less influence on bacterial diversity. Liu et al. [28] found that bacterioplankton diversity responds slowly to environmental changes. Additionally, although our research period was short (20 d), the growth of submerged plants takes a certain amount of time and releasing allelochemicals into the water is a slow process; therefore, there were no significant changes in bacterial diversity. During the experiment, *Bacteroidetes* abundance was lower when *Cyanobacteria* abundance was high in the combined groups, indicating that these two bacteria have a competitive relationship. It has been confirmed that both *C. demersum* and *M. spicatum* have allelopathic inhibitory effects on *Microcystis* [29,30], but have large differences. In our study, this explains why *M. spicatum* had an obvious inhibitory effect on *Microcystis* and *Planktothrix* while *C. demersum* exhibited a strong inhibitory effect on *Microcystis*, but no significant inhibitory effect on *Planktothrix*.

4.3. Combined Effects of Submerged Macrophytes and D. magna on Bacterioplankton in Eutrophic Water

Notably, *Mycobacterium* grew better in the E, F, G, H treatments from 10 to 20 days, and this confirmed that the "*phytoplankton + D. magna*" treatment groups lowered eutrophication since *Actinobacteria* grow well in less eutrophic water [31]. While the inhibitory effect on *Cyanobacteria* in the combined group was better, the abundance of other taxa increased slightly, implying that the combination of "phytoplankton + *D. magna*" protected the stability of bacterioplankton composition due to submerged plant communities providing major habitats and refuges for *D. magna*, forming a benign water ecosystem [32]. The reduction of water transparency caused by algae proliferation is one of the biggest bottlenecks in the ecological restoration of eutrophic waters. The use of large fleas to quickly filter and eat algae and particles in eutrophic waters can quickly improve water transparency in the short-term. This makes it possible to transplant submerged plants, laying a foundation for further application of submerged vegetation for water purification [33]. This result is consistent with Kim et al. [34], who used an artificial food web composed of phytoplankton and *Daphnia magna* to remove nutrients from sewage.

4.4. Relationships between Environmental Factors and Bacterioplankton

TN concentration was negatively correlated with *Mycobacterium*, and it has been revealed that *Mycobacterium* possesses a denitrification function [35]. We also found that WT was negatively correlated with *Planktothrix* and *Microcystis*, indicating that their excessive growth may lower WT and inhibit the growth of other bacterioplankton. *M. spicatum* and *C. demersum* improved WT to some extent; however, since submerged plants need a certain time to grow, their effect on WT was not significant, implying that single submerged macrophytes only slowly improve water quality slightly [36]. It has been shown that *Rhodobacter* and *Novosphingobium* were negatively correlated with TN and TP, which is because *Rhodobacter* is an aerobic denitrifier that can degrade TN in water [37], while *Novosphingobium*

can degrade TP in water and possesses an algicidal effect [38]. This indicates that the combination of submerged macrophytes and *D. magna* promotes the growth of functional bacteria and the removal of nitrogen and phosphorus.

4.5. Application Prospects

Some previous studies have tried to use biomanipulation in eutrophic water. In the case of typical South Asian eutrophication water bodies in Nanjing Yueya Lake, the application of ecological restoration combination techniques of submerged macrophytes and *D. magna* was conducted. The results showed that *D. magna* feeds on algae and organic suspended matter particles, and it achieved transformation of eutrophication material [39]. In addition to nitrification/denitrification, the oxidation sedimentation of phosphorus can also be achieved in submerged macrophytes. Nutrient salt changes caused by macrophytes and *D. magna* indirectly promoted bacterioplankton change. Eutrophication water quality could be improved, and therefore, it has practical significance and application prospects.

5. Conclusions

Compared with the control group, the low-density *Daphnia magna* and *Myriophyllum verticillatum* L. treatment had a greater effect on lowering phytoplankton density, *Cyanophyta* density, chla concentration, and microcystin toxin content by 91.58%, 92.05%, 92.35%, and 87.56%, respectively, in water. Additionally, the inhibitory effect of *Ceratophyllum demersum* L. on microcystin toxins and *Planktothrix* could be significantly improved by the grass-*Daphnia* combination treatment. Furthermore, the grass-*Daphnia* combination could also protect the stability of bacterial community structure, and significantly inhibit *Cyanobacteria* growth among each group. With the same density of *Daphnia* and plant species, the grass-*Daphnia* treatment better inhibited the explosive growth of *Cyanobacteria* than the *Daphnia magna* or submerged plant single treatments. Moreover, the grass-*Daphnia* combination had a significant inhibitory effect on *Planktothrix* and *Microcystis* growth, with the strongest inhibitory effect induced by the low-density *Daphnia magna* and *Myriophyllum verticillatum* L. combination. Furthermore, the grass-*Daphnia* combination could increase the relative abundance of the denitrification bacteria *Mycobacterium* in the water. Moreover, RDA analysis showed that the correlation coefficients of environmental factors with planktonic bacterial community ranked in decreasing order were WT, chla, TN, MCs, and TP. The relative abundance of *Rhodobacter* and *Novosphingobium* in the low-density *Daphnia magna* and *Myriophyllum verticillatum* L. combination was significantly higher than other groups.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/12/22/9548/s1. (Table S1 Next generation sequencing read statistics).

Author Contributions: B.Y., C.Z. and X.Z. designed the investigation. X.W. and H.W. conducted the experiment. B.Y., J.L. and B.L. interpreted the data. All authors were involved in writing the paper and approved the final manuscript.

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

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