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ARTICLE

Can Application of Commercial Microbial Products Improve Fish Growth and Water Quality in Freshwater Polyculture?

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Abstract

A 31-d experiment was conducted to examine the effects of three commercial microbial products (Novozymes pond plus, Zhongshui BIO-AQUA, and Effective Microorganisms) on production performance and water quality in freshwater tanks stocked with Grass Carp *Ctenopharyngodon idellus*, Gibel Carp *Carassius gibelio* and Silver Carp *Hypophthalmichthys molitrix*. Four treatments were used: blank control (BL), adding Novozymes pond plus (NO), adding BIO-AQUA (PB), or adding Effective Microorganisms (EM). The fish were fed daily with a formulated feed, and each of the microbial products was added to the tanks every 10 d. No significant differences were found in survival, weight gain, and feed conversion ratio of the fishes, Secchi depth, chemical water quality, and phytoplankton between the blank control and any other treatments (NO, PB and EM). This study indicates that the addition of these three microbial products every 10 d has limited function to improve production performance and water quality in freshwater polyculture of Grass Carp, Gibel Carp, and Silver Carp within the first 31 d of application.

Human activities in pursuing ecosystem services have perturbed the balance of ecosystems. In aquaculture practice, intensive stocking and nutrient overloading have caused a series of ecological consequences, such as overexploitation of natural productivity, environmental destruction, and disease dispersal (Lin and Yi 2003; Balcázar et al. 2006; Wang et al. 2008). To establish sustainable aquaculture, probiotics have been used in an attempt to improve survival, growth, and feed utilization of the stocked animals, as well as water quality (Gomez-Gil et al. 2000; Verschuere et al. 2000; Balcázar et al. 2006; Farzanfar 2006; Kesarcodi-Watson et al. 2008; Wang et al. 2008; Ninawe and Selvin 2009; Qi et al. 2009; Prado et al. 2010). The microbial products for improving water quality in aquaculture systems are referred to bioremediation products (Devaraja et al. 2002). The function of microbial products for improving water quality in aquaculture practices is controversial. In some studies, the addition of microbial products showed improvements in water quality and production performance of the stocked species, such as whiteleg shrimp *Penaeus vannamei* (Wang et al. 2005), Asian tiger shrimp *P. monodon* (Janeo et al. 2009; Shariff et al. 2001) and Olive Flounder *Paralichthys olivaceus* (Taoka et al. 2006). In contrast, other studies reported that the addition of microbial products did not show any evidence in improving water quality or growth of the stocked animals, such as Channel Catfish *Ictalurus punctatus* (Boyd et al. 1984; Tucker et al. 2009) and whiteleg shrimp (Zhou et al. 2009). The discrepancy between previous studies may be attributed to the great variation of environmental conditions between different aquaculture systems. In addition,

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a common feature in previous testing of microbial products was that the aquaculture systems were only stocked with a single principal species of either fish or crustacean (monoculture) and the environmental background of the systems was not clearly defined. There is a need to test the effects of microbial products on water quality and production performance in an aquaculture system with a well-defined environmental background.

Polyculture is a practice of stocking various fish species with different food habits in an aquaculture system for the effective use of available food resources. In China, polyculture of freshwater fishes, including Grass Carp *Ctenopharyngodon idella*, Black Carp *Mylopharyngodon piceus*, Gibel Carp *Carassius gibelio*, Common Carp *Cyprinus carpio*, Silver Carp *Hypophthalmichthys molitrix,* and Bighead Carp *Hypophthalmichthys nobilis*, is widely used in commercial pond culture (Lin 1982; Wang 2004; Tang et al. 2015). Compared with monoculture, polyculture is beneficial in reducing the accumulation of wasted nutrients because wastes produced by one stocked species can be utilized as food resources by other species (Tang et al. 2015). However, the increase of stocking density and excessive use of formulated fish feed have led to deterioration of water quality in freshwater polyculture (Lin and Yi 2003; Yuan et al. 2010). To our best knowledge, the role of supplementary microbial products in regulating production performance and water quality in a freshwater polyculture system has been rarely evaluated. The addition of microbial products might not substantially impact water quality in a fish polyculture system because fishes with different feeding habits may strengthen the system stability to counteract bacterial colonization. We examined the effects of three commercial microbial products on production performance and water quality in a polyculture system of Grass Carp, Gibel Carp, and Silver Carp, with an emphasis on evaluating the accumulation of nutrient wastes and an intent to provide useful information associated with probiotics application in aquaculture practices.

METHODS

Microbial products and fish polyculture system.—We tested three commercial microbial products—Novozymes pond plus (Novozymes Biological, Boston), Zhongshui BIO-AQUA (Zhongshui Fish Medicine, Wuxi, China) and Effective Microorganisms (Jiangsu Suwei Microbiology Research, Wuxi, China) at Fengqiao farm (Zhuji, China) from July 16 to August 15, 2010. These dry-powder microbial products were purchased from the manufacturers and stored at 4°C before use. They are widely used in freshwater aquaculture in China. Bacterial composition of the microbial products was determined with denaturing gradient gel electrophoresis and 16S rDNA sequence analysis (the V6-V8 variable region was used for polymerase chain reaction; Zhou et al. 2013). The Novozymes pond plus is composed of *Acinetobacter* sp., *Bacillus* sp., *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus licheniformis*, *Cedecea davisae,* and uncultured *Pseudomonas* sp. The BIO-AQUA is composed of *Aerococcus urinaeequi*, *Clostridium sticklandii*, *Desulfomicrobium norvegicum*, *Exiguobacterium marinum*, *Exiguobacterium* sp., *Frigovirgula patagoniensis*, *Microbacterium oxydans*, *Nitrobacter* sp., *Pseudomonas* sp., and uncultured *Frigovirgula* sp. The Effective Microorganisms is comprised of *Acinetobacter* sp., *Actinopolyspora* sp., *Bacillus* sp., *Carnobacterium* sp., *Corynebacterium tuberculostearicum*, *Jeotgalicoccus psychrophilus*, *Planococcus citreus*, *Rhodococcus* sp., *Saccharopolyspora taberi* and *Shigella flexneri.* The activated bacteria in Novozymes pond plus and BIO-AQUA are both 10^{10} CFU/g while for Effective Microorganisms is 10^{11} CFU/g. The polyculture systems consisting of Grass Carp, Gibel Carp, and Silver Carp were stocked in 2,000-L polyethylene tanks (136 cm in diameter and 138 cm in height). The tanks were located near an earthen pond and under a shelter made of a wood frame (3 m in height) and a black polyvinyl chloride net to prevent extremely high water temperatures (over 35°C) in the tanks due to direct sunlight. The fishes were obtained from a freshwater fish farm at Deqing (Huzhou, China) in May 2010 and were maintained in net pens suspended in an earthen pond.

Experimental design and rearing experiment.—Four treatments were tested: a blank control (BL), adding Novozymes pond plus (NO), adding Zhongshui BIO-AQUA (PB) and adding Effective Microorganisms (EM). Each treatment was replicated three times.

At the start of the experiment, the tanks were sterilized with potassium permanganate, solarized, and filled with 1,800 L of water pumped from an earthen pond. Twelve groups, each consisting of 25 Grass Carp (4.6–5.4 g), 5 Gibel Carp (8.0–9.0 g), and 3 Silver Carp (1.2–1.9 g), were captured from the net pens, bulk-weighed, and randomly distributed in the tanks. Fish were acclimated in the tanks for 2 weeks, during which any dead fish were replaced with fish of the same species and similar size.

The experiment lasted 31 d. The fishes were fed a commercial formulated feed containing 4.9% nitrogen and 2.9% phosphorus (Kesheng Feed Stock, Shaoxing, China) at 0800 and 1700 hours daily. The feeding rate was about 3–4% of initial body weight in initial stages, and adjusted daily according to the amount of uneaten feed on the feeding trays in each tank. The amounts of formulated feed used in treatments were 643 g (SD, 91) for BL, 596 g (SD, 135) for NO, 670 g (SD, 141) for PB, and 614 g (SD, 98) for EM. Water in the tanks was neither aerated nor exchanged. Illumination was measured with a ZDS-10 luxmeter (Jiading Xuelian Meter Plant, Shanghai, China) every 3 d and averaged 9,040 lx ($n = 10$) under the shelter or 33,945 lx ($n = 10$) outside of the shelter in the morning (0800–1000 hours). The Novozymes pond plus and BIO-AQUA was added at 0.3 g/tank on days 1, 11, and 21. The Effective Microorganisms was added at 1.0 g/tank on day 1 and then at 0.5 g/tank on days 11 and 21. The activated bacterial count of microbial products added in the tanks was 10^3 to 10^4 CFU/mL. To add the microbial products, the dry powder was weighed, mixed with 50 mL water collected from the tanks, and then poured into the tanks. At the end of

the experiment, the fish were captured from each tank and bulk weighed.

Sampling, chemical and biological analysis.—During the experiment, water temperature and dissolved oxygen in the tanks were measured daily with a model 85 dissolved oxygen (DO) meter (YSI Scientific, Yellow Springs, Ohio) at 0600–0800 hours and 1600–1800 hours, while pH was measured with a model 63 pH meter (YSI Scientific). Water clarity in each tank was measured using a Secchi disk. Water samples were collected every 3 d (at 0800–1000 hours) from each tank at about 30–50 cm below the surface via a 5-L sampling vessel, and the water samples were stored in two sterile glass bottles (volume = 1 L). One bottle was used for chemical analysis, and the other bottle contained phytoplankton specimens preserved with 10% Lugol's solution. The following water quality variables were measured: alkalinity (CO_3^{2-} and HCO_3^-), hardness (Ca^{2+} and Mg^{2+}), ammonia (NH_3-N and NH_4^+-N), nitrite (NO_2-N), nitrate (NO_3-N), reactive phosphate (PO_4-P), total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand oxidized with manganese (COD_{Mn}). Biological parameters including chlorophyll *a* (Chl *a*), and species and biomass of phytoplankton were measured. Ammonia, NO_2-N, NO_3-N, PO_4-P, COD_{Mn}, Chl *a*, and phytoplankton species and biomass were monitored every 3 d. Alkalinity, hardness, TN and TP were only measured on days 1, 16 and 31. Alkalinity, hardness, ammonia, NO_2-N, PO_4-P, COD_{Mn} were analyzed with the methods described in APHA (2005). Nitrate was analyzed with the method described in Parsons and Takahashi (1973). Total nitrogen and TP were analyzed using the method described in Ebina et al. (1983). Chlorophyll *a* was measured using a 10-005 R fluorometer (Turner Designs, Sunnyvale, California). Phytoplankton biomass was estimated on an E100 microscope (Nikon, Tokyo). To determine the activated bacterial count of the microbial products, 1 g of dry powder was evenly mixed with 1,000 mL sterile water in a sterile flask and incubated for 2 h. A subsample of 1 mL was taken from the flask and filtered with a 0.2-μm black polycarbonate membrane (25-mm diameter, Whatman 110656 Nuclepore Track-Etched Membranes, Whatman, Maidstone, United Kingdom) fixed in a glass filter (Merck Millipore, Billerica, Massachusetts) as described in Du et al. (2007). The bacteria on the membrane were enumerated under an Eclipse 80i fluorescence microscope (Nikon).

Calculation and statistical analysis.—Survival, weight gain, and feed conversion ratio of fish, waste accumulation and Shannon–Weaver's diversity index (Shannon and Weaver 1949) of phytoplankton were calculated:

$$\text{Survival}(\%) = 100 \times N_t/N_0$$

$$\text{Weight gain (g/fish)} = (W_t/N_t - W_0/N_0)$$

$$\text{Feed conversion ratio} = I/(W_t - W_0)$$

$$\text{Waste accumulation(mg/L)} = C_t - C_0$$

$$\text{Shannon–Weaver's diversity index} = -\sum_{i=1}^{S} P_i \log_2 P_i$$

where, N_t is the number of fish at the end of the experiment and N_0 at the start; W_t is the total final body weight (g) of fish and W_0 is the total initial body weight (g); I is dry feed consumption (g); C_t is the concentration (mg/L) of TN, TP, or COD_{Mn} at the end of the experiment and C_0 at the start; P_i is the ratio in biomass between species i and total phytoplankton; S is the number of species of phytoplankton.

Data were expressed as the mean and SD of three replicate tanks for each treatment. The effects of different microbial products on survival, weight gain, feed conversion ratio, Secchi depth, chemical water quality (DO, pH, alkalinity, hardness, ammonia, NO_2-N, NO_3-N, PO_4-P, TN, TP, COD_{Mn}), biological variables (Chl *a*, biomass of phytoplankton and Shannon–Weaver's diversity index), and accumulation of wasted nutrients were examined with one-way analysis of variance (ANOVA). The differences in the above variables among treatments were examined with Fisher's least-significant-difference test (LSD) if a significant difference was detected in ANOVA. A redundancy analysis (RDA) was performed to evaluate the relationship between weight gain of fishes and variables including Chl *a*, Shannon–Weaver's diversity index of phytoplankton, DO, ammonia, NO_2-N, TN, TP, and COD_{Mn}. The significant level was set at $P = 0.05$. The ANOVA were performed with IBM SPSS Statistics V 20.0 (IBM, Armonk, New York). The RDA was performed with Canoco for Windows 4.5 (Microcomputer Power, Ithaca, New York).

RESULTS

No significant differences were found in the survival, weight gain, and feed conversion ratio of fish among the treatments (ANOVA: $P > 0.05$; Table 1). During the experiment, water temperature fluctuated from 26.2°C to 35.4°C (mean = 30.1°C, SD = 2.4). Mean water quality values ($n = 3$, including control) ranged as follows: Secchi depth = 42–50 cm, DO = 4.03–4.57 mg/L, pH = 7.73–7.83, alkalinity = 94.46–95.73 mg $CaCO_3$/L, and hardness = 98.65–99.24 mg $CaCO_3$/L. No significant differences were found in the Secchi depth, DO, pH, alkalinity, hardness, ammonia, NO_2-N, NO_3-N, PO_4-P, TN, TP, and COD_{Mn} between the treatments (ANOVA: $P > 0.05$; Table 2). The concentrations of ammonia, PO_4-P, TN, TP, and COD_{Mn} in all the tanks increased with the progression of the experiment. There were no significant differences in the accumulation of TN, TP, and COD_{Mn} among the treatments (ANOVA: $P > 0.05$; Figure 1).

During the experiment, a total of 51 genera of phytoplankton (*Merismopedia* sp., *Chroococcus* sp., *Aphanocapsa* sp., *Microcystis* sp., *Anabaena* sp., *Coelosphaerium* sp., *Dactylococcopsis* sp., *Nostoc* sp., *Spirulina* sp., *Oscillatoria* sp., *Hammatoidea* sp., *Chamydomonas* sp., *Volvox* sp., *Eudorina* sp., *Pan-*

TABLE 1. Survival, initial body weight, weight gain and feed conversion ratio (mean ± SD, $n = 3$) of fishes in experimental polyculture tanks in which supplementary products, Novozymes pond plus (NO), BIO-AQUA (PB), and Effective Microorganisms (EM), were tested against a blank control (BL); FCR is feed conversion ratio.

	Initial body weight (g/fish)			Survival (%)			Weight gain (g/fish)			
Treatment	Grass Carp	Gibel Carp	Silver Carp	Grass Carp	Gibel Carp	Silver Carp	Grass Carp	Gibel Carp	Silver Carp	FCR
NO	4.9 ± 1.1	8.1 ± 1.0	1.2 ± 0.4	92 ± 0	87 ± 23	78 ± 19	20.3 ± 3.6	17.5 ± 3.7	50.6 ± 10.2	1.46 ± 0.23
PB	5.3 ± 0.3	9.0 ± 1.1	1.9 ± 1.3	76 ± 25	93 ± 12	67 ± 0	14.2 ± 2.8	16.3 ± 1.9	77.9 ± 16.3	1.94 ± 0.27
EM	4.6 ± 0.4	8.7 ± 1.0	1.7 ± 1.2	83 ± 6	100 ± 0	67 ± 0	14.9 ± 6.8	16.1 ± 1.3	52.0 ± 19.8	1.64 ± 0.30
BL	5.4 ± 1.1	8.0 ± 0.9	1.2 ± 0.4	92 ± 0	100 ± 0	56 ± 19	16.0 ± 3.3	16.8 ± 1.4	43.6 ± 28.5	1.47 ± 0.19

dorina sp., *Schroederia* sp., *Tetraedron* sp., *Selenastrum* sp., *Kirchneriella* sp., *Nephrocytium* sp., *Actinastrum* sp., *Pediastrum* sp., *Ankistrodesmus* sp., *Micractinium* sp., *Crucigenia* sp., *Scenedesmus* sp., *Westella* sp., *Oocystis* sp., *Mougeotia* sp., *Spirogyra* sp., *Cosmarium* sp., *Staurastrum* sp., *Arthrodesmus* sp., *Euglena* sp., *Trachelomonas* sp., *Lepocinclis* sp., *Phacus* sp., *Cryptomonas* sp., *Heterotrichales* sp., *Synura* sp., *Stephanodiscus* sp., *Coscinodiscus* sp., *Cyclotella* sp., *Melosira* sp., *Navicula* sp., *Frustulia* sp., *Cocconeis* sp., *Surirella* sp., *Synedra* sp., *Gymnodinium* sp., *Glenodinium* sp.) were identified. Phytoplankton communities were initially dominated by the blue-green algae *Merismopedia* sp. and *Nostoc* sp. in most tanks, and gradually altered to the blue-green algae *Coelosphaerium* sp. later. Blue-green algae and green algae accounted for 86.6% and 12.2% of the biomass of phytoplankton, respectively. The Shannon–Weaver's diversity index of phytoplankton increased from day 1 to day 14 and then decreased from day 14 to day 31. No significant differences were found in the Shannon–Weaver's diversity index among the treatments (ANOVA: $P > 0.05$; Figure 2).

The Chl *a* concentration decreased from day 1 to day 17, and then increased with progression of the experiment in the following days (Figure 3A). A similar trend was observed in the biomass of phytoplankton in treatment NO. However, the biomass of phytoplankton in treatments PB, EM, and BL decreased until day 17, then began increasing until day 27, followed by another decreased again (Figure 3B). No significant differences were found in the Chl *a* and biomass of phytoplankton between the treatments (ANOVA: $P > 0.05$).

The Chl *a*, Shannon–Weaver's diversity index of phytoplankton, DO, ammonia, NO_2-N, TN, TP, and COD_{Mn} explained 97.6% of the variability in fish weight gain (RDA), and the first and second ordination axis had the highest eigenvalue (0.69 and 0.24, respectively; Figure 4). The forward selection procedure indicated that TN was the dominant factor influencing fish weight gain ($F = 3.51$, $P = 0.046$).

DISCUSSION

Many factors in aquaculture practices can affect the function of microbial products in regulating production performance and water quality, including the bacterial composition and supplementation regime of microbial products (frequency and dosage), the aquaculture system (ponds or tanks), and the aquaculture mode (species composition, density and methods of husbandry management). Therefore, evaluation of microbial products function should be conducted in an aquaculture system with a well-described aquaculture mode and environmental background. In our study, we quantified species and stocking density of fish, bacterial composition and supplementation method of microbial products, husbandry management (feed composition and feeding regime, water exchange and aeration), and environmental background (water quality before microbial products addition) in experimental tanks. Therefore, the results reliably reveal the effect of three commercial microbial products on production performance and water quality in a freshwater polyculture system with a low-nutrient loading (TN < 3 mg/L, TP < 1 mg/L, COD_{Mn} < 17 mg/L).

Previous studies have indicated that addition of microbial products can increase the abundance of natural food and improve feed utilization efficiency of farmed animals (Verschuere et al. 2000; Balcázar et al. 2006). For instance, adding microbial

TABLE 2. Concentrations of nitrogen, phosphorus and chemical oxygen demand (mean ± SD, $n = 3$) in experimental polyculture tanks in which supplementary products, Novozymes pond plus (NO), BIO-AQUA (PB), and Effective Microorganisms (EM), were tested against a blank control (BL); NO_2-N is nitrite, NO_3-N is nitrate, PO_4-P is reactive phosphate, TN is total nitrogen, TP is total phosphorus, COD_{Mn} is chemical oxygen demand.

Treatment	Ammonia (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	PO_4-P (mg/L)	TN (mg/L)	TP (mg/L)	COD_{Mn} (mg/L)
NO	0.396 ± 0.290	0.011 ± 0.016	0.023 ± 0.025	0.457 ± 0.391	2.752 ± 1.306	0.804 ± 0.342	16.17 ± 0.89
PB	0.481 ± 0.246	0.016 ± 0.021	0.022 ± 0.022	0.567 ± 0.496	2.825 ± 1.311	0.940 ± 0.492	16.32 ± 0.13
EM	0.403 ± 0.197	0.013 ± 0.016	0.026 ± 0.032	0.544 ± 0.459	2.956 ± 1.467	0.981 ± 0.470	16.70 ± 0.06
BL	0.421 ± 0.232	0.009 ± 0.018	0.025 ± 0.028	0.492 ± 0.395	2.183 ± 1.413	0.881 ± 0.374	16.23 ± 0.60

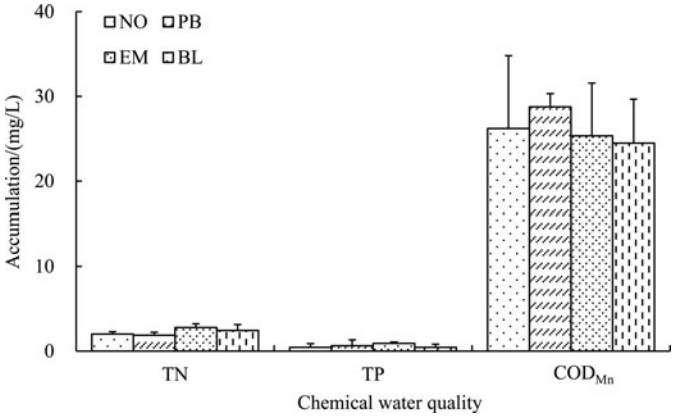

FIGURE 1. Accumulations of total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD_{Mn}) in polyculture tanks in which supplementary products, Novozymes pond plus (NO), BIO-AQUA (PB), and Effective Microorganisms (EM), were tested against a blank control (BL).

products can enhance growth performance and feed utilization efficiency of white shrimp *Litopenaeus setiferus* (Wang et al. 2005; Zhou et al. 2009) and ornamental fish (Ghosh et al. 2008). However, we found no significant differences in the weight gain and feed conversion ratio of fishes between treatments with or without microbial products addition. Our results suggest that the three commercial microbial products we used have limited function in improving short-term production performance of polyculture of Grass Carp, Gibel Carp, and Silver Carp.

The beneficial roles of adding microbial products to improve water quality in an aquaculture system include degrading organic wastes, converting toxic inorganic nitrogen (e.g., ammonia and NO_2-N), and inhibiting pathogens and harmful algae (Chiayvareesajja and Boyd 1993; Shariff et al. 2001; Wang et al. 2005; Janeo et al. 2009). Some bacteria, such as *Bacillus* sp., *Nitrosomonas* sp. and *Nitrobacter* sp., can degrade organic wastes and convert toxic inorganic nitrogen (Verschuere et al. 2000).

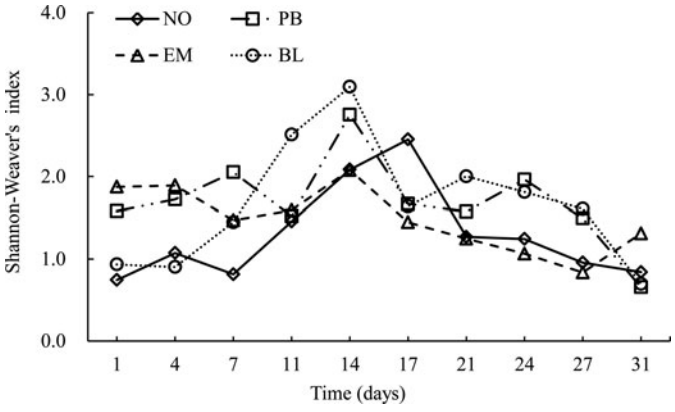

FIGURE 2. Variations of Shannon–Weaver's diversity index of phytoplankton in polyculture tanks in which supplementary products, Novozymes pond plus (NO), BIO-AQUA (PB), and Effective Microorganisms (EM), were tested against a blank control (BL).

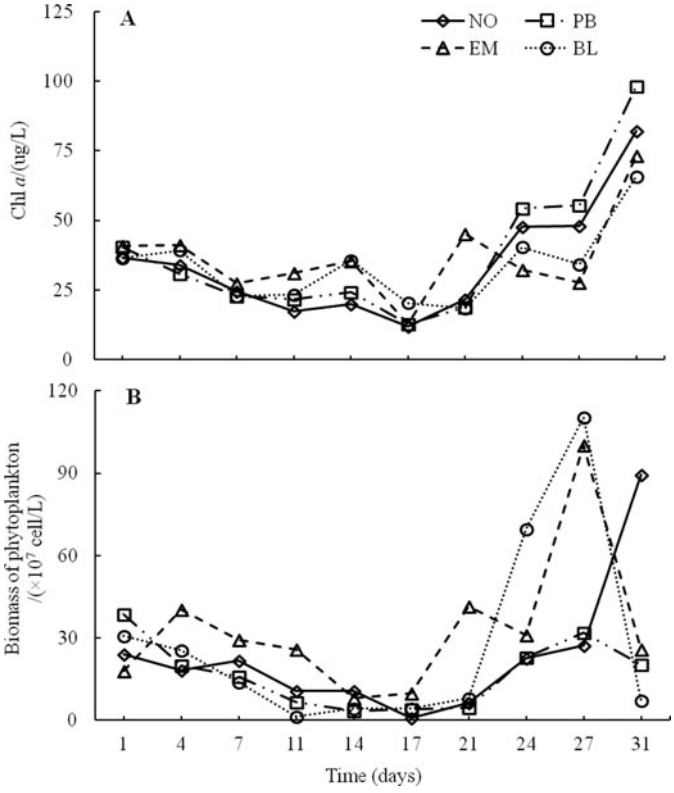

FIGURE 3. Variations of **(A)** chlorophyll *a* (Chl *a*) and **(B)** biomass of phytoplankton in polyculture tanks in which supplementary products, Novozymes pond plus (NO), BIO-AQUA (PB), and Effective Microorganisms (EM), were tested against a blank control (BL).

Ghosh et al. (2008) reported that adding *Bacillus subtilis* can reduce the concentration of ammonia and dissolved organic matter in waters where ornamental fish are stocked. Microbial products can accelerate the processes of organic matter degradation and nitrification in Asian tiger shrimp ponds (Janeo et al. 2009) and reduce the concentrations of dissolved inorganic nitrogen (ammonia, NO_2-N and NO_3-N) and COD_{Mn} in whiteleg shrimp ponds (Wang et al. 2005; Nimrat et al. 2012). However, in other studies, microbial products addition did not significantly reduce the concentration of ammonia and NO_2-N in waters stocked with white shrimp (Zhou et al. 2009) or Channel Catfish (Boyd et al. 1984; Tucker et al. 2009). Our study suggests that application of the three commercial microbial products cannot reduce concentration of ammonia and NO_2-N or the accumulation of TN, TP, and COD_{Mn} in a polyculture system of Grass Carp, Gibel Carp, and Silver Carp.

We found no significant differences in the DO and pH between the treatments with or without microbial products addition. This result is consistent with the conclusion that microbial products cannot significantly affect DO and pH in waters stocked with white shrimp (Wang et al. 2005) or ornamental fish (Ghosh et al. 2008). The limited function of microbial products in increasing DO may minimize its role to promote nitrification. In addition, our results suggests that TN

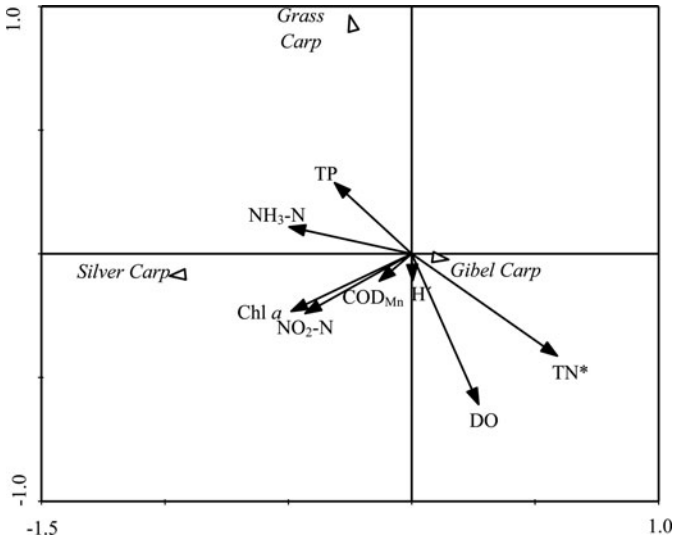

FIGURE 4. Redundancy analysis of the growth of fishes (Grass Carp, Gibel Carp and Silver Carp) in polyculture tanks as related to variables: Chl *a* = chlorophyll *a*; H′ = Shannon–Weaver's diversity index of phytoplankton; DO = dissolved oxygen; NH_3-N = ammonia; NO_2-N = nitrite; TN = total nitrogen; TP = total phosphorus; COD_{Mn} = chemical oxygen demand. Asterisks represent statistical significance ($P < 0.05$).

accumulation might be a key factor regulating weight gain of the fish. Generally, within an aquaculture production period, TN concentration gradually increases and DO decreases. Hence, the relationship between DO and TN accumulation warrants more studies in the future.

Phytoplankton is critical in regulating water quality in aquaculture ponds through photosynthesis and absorbing inorganic nutrients, e.g., ammonia. Beneficial bacteria can inhibit the growth of some harmful algae through nutrient competition or excretion of algaecide (Verschuere et al. 2000). Janeo et al. (2009) reported that adding microbial products could inhibit the dominance of cyanobacteria and increase the biomass of the edible phytoplankton species in shrimp ponds. However, adding microbial products did not reduce biomass of harmful algae in Channel Catfish ponds (Boyd et al. 1984; Tucker et al. 2009) or in water recirculation systems of farmed white shrimp (de Paiva-Maia et al. 2013). We found that adding three commercial microbial products did not result in a significant change in species, biomass, or diversity of phytoplankton, suggesting negligible impact of microbial products addition on the phytoplankton community in a polyculture system with low nutrient loading.

In conclusion, our 31-d experiment conducted in a polyculture system containing Grass Carp, Gibel Carp, and Silver Carp with low nutrient loading reveals that additions of three commercial microbial products did not significantly improve production performance or chemical water quality. This result warrants further long-term studies to test the function of microbial products in freshwater polyculture systems with different nutrient loadings.

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