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Responses of periphyton morphology, structure, and function to extreme nutrient loading

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ABSTRACT

Periphyton have been widely applied in aquaculture systems, however, little information is available on how periphyton respond to such high nutrient levels in water. Thus, changes in the morphological characteristics, community structure, and metabolic function of periphyton under high eutrophic waters were evaluated. The results indicated that the morphology of periphyton was affected by increasing the nutrient concentration of water, which shifted the micromorphology of periphyton from spheriform to filamentous. The periphyton under higher water nutrient levels were able to utilize more carbon source types. Additionally, higher water nutrient levels increased the bacterial and protozoal proportions in periphyton. This study fills the gap in knowledge about the responses of periphytic communities to extremely eutrophic waters. It provides valuable information on the full understanding of the periphyton-nutrient relationship in aquaculture systems, which is beneficial for regulating the microbial species or communities in periphyton by manipulating the nutrient levels in water.

1. Introduction

The hyper-eutrophication phenomena frequently occurs in cage aquaculture systems and its surrounding waters, which often causes many negative effects on the flora and fauna and the well-being of humans, such as the deterioration of the water quality and aquatic ecosystem balance and toxic generation (Guo and Li, 2003; Jegatheesan et al., 2011; Paerl et al., 2011). The most important causes of hyper-eutrophic waters are the excessive loads of nutrients (nitrogen and phosphorus) to the water, which are the result of increased loads of agricultural soil containing chemical fertilizers, the excess agricultural wastewater from the intensive farming of fish, livestock and poultry, and the large discharge of municipal wastewater, the biodegradable organics of which have been removed but most of the nutrients in the effluent have remained (Woodward et al., 2012).

Periphyton (biofilm) are a complex microbial assemblage of algae, fungi, bacteria, protozoa, metazoan, epiphytes and detritus (Azim et al., 2005; Characklis and Marshall, 1990). It is ubiquitously distributed in approximately every aquatic ecosystem, especially in shallow waters such as lakes, rivers, and streams. Previous studies reported that periphyton were capable of improving the water quality in eutrophic waters such as aquaculture ponds (Crab et al., 2007; Khatoon et al., 2007; Sanz-Lázaro et al., 2011). As a result, periphyton-based cage cultures have been extensively developed to reduce the use of artificial feeds in aquaculture (Azim et al., 2005; Huchette and Beveridge, 2003; Richard et al., 2010). As an environmentally friendly and cost-effective measure, periphyton have also been widely applied to remove nutrients from water and/or wastewaters (Boelee et al., 2011; Guzzon et al., 2008).

Previously, much attention was given to study the periphytic response to water nutrients (DeNicola et al., 2006; McCormick et al., 2001; Ozkan et al., 2010; Stelzer and Lamberti, 2001). It is

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well documented that nutrient types and concentration play a significant role in the periphytic composition, structure and productivity (Cashman et al., 2013; Havens et al., 1999; McCormick et al., 1996; Sanz-Lázaro et al., 2015; Schiller et al., 2007). Generally, enhanced nutrient availability will lead to shifts in the taxonomic composition and increases in the periphytic biomass, density and thickness (Fermino et al., 2011; Ferragut and de Campos Bicudo, 2010). For example, it was reported that periphyton in lakes with increased nutrient loading would have a greater relative abundance of filamentous chlorophyte, which are the most abundant species associated with high nutrient concentrations in lakes (DeNicola et al., 2006). Although these studies provide a great deal of valuable information on the response of periphyton to nutrients in waters, the results may not be very helpful or practical when applied to hyper-eutrophic waters whose levels of nutrients, in particular, N and P, are extremely high. This is because most of the research has been based on the normal eutrophic level of waters (<10 mg L^{-1}), which are relatively lower than hyper-eutrophic wastewater (>100 mg L^{-1}), such as turtle aquaculture wastewater (Chen et al., 2013).

Therefore, in this study, an extremely high concentration of water nutrients was simulated. The responses of periphyton to such high nutrient levels were investigated using some widely accepted and established technologies such as Biolog, phospholipid fatty acids (PLFAs) and environmental scanning electron microscope (ESEM) methods. AWCD is an important index to indicate the overall ability of microorganisms using different carbon sources, which is closely related to the growth and activity of microorganisms that can use a single carbon source (Choi and Dobbs, 1999). Phospholipid fatty acids (PLFAs) are an essential structural component of all microbial cellular membranes. PLFAs are widely used in microbial ecology as chemotaxonomic markers of bacteria and other organisms (Helfrich et al., 2015). According to the principle of PLFAs, microorganisms can be differentiated into bacteria (Gram-positive bacteria/Gram-negative bacteria), fungi, and protozoa.

The primary objective of this study is to investigate (i) the change in the microbial function (carbon metabolic capability) of periphyton, (ii) the change in the community structure of periphyton, and (iii) the change in the morphology of periphyton in waters with a high degree of eutrophication. The results of this study will provide insight into the responses of periphytic structures and functions to extremely eutrophic waters. It will also offer some valuable information towards a full understanding of the periphyton-nutrient relationship.

2. Experimental materials and methods

2.1. The culture of periphyton

Commercial substrates (stereo elastic fillings, Yixing environmental filling Co., Ltd., Jiangsu province, China) were used for the substrate of periphyton concentrated with a BG-11 medium (Rippka et al., 1979). The culture process was conducted outside and the air temperature ranged from 20 to 36 °C. When the color of the periphytic assemblages started to become olive-drab (at approximately 30 days), the periphyton with their substrates were then collected for the following experiments.

2.2. The experimental process

The experimental water for preparing the experimental medium was collected from Xuanwu Lake, Nanjing city, eastern China, where the water quality suffers from eutrophication and harmful algal blooms. The main chemical properties were as follows: pH = 7.8, total nitrogen = 1.9 mg L^{-1}, $NO_3^- + NO_2^-$ = 0.73 mg L^{-1}, NH_4^+ = 0.53 mg L^{-1}, total phosphorus = 0.1 mg/L, and dissolved phosphorus = 0.035 mg/L. The collected periphyton with their substrates were placed in a glass container holding a 5.0-L experimental medium. To produce high N- and P-levels similar to those in cage aquaculture waters, $NaNO_3$ and K_2HPO_4 were added to the experimental medium and then the pH was adjusted to 7.1 ± 0.2. In the experiments, one control level (T1: TN = 10 mg L^{-1}, TP = 10 mg L^{-1}) and two degrees of hyper-eutrophication (T2: TN = 350 mg L^{-1}, TP = 350 mg L^{-1} and T3: TN = 550 mg L^{-1}, TP = 550 mg L^{-1}) were established by adding chemicals to the water. Each trophic state was triplicated.

The experiment was conducted on the roof of an experimental building, at the Institute of Soil Science. During the experiment, the periphyton grew as the water temperature increased from 20 °C to 36 °C. The experiment was then moved to a greenhouse when the ambient air temperature was less than 20 °C. To avoid any impacts from insects, the glass containers were covered with a sheer nylon net (hole dia. < 0.2 mm).

2.3. Sampling and analysis methods

The periphyton with substrates (each length = 25 cm) were sampled using sterile scissors and were then filtrated with a 20-mesh nylon griddle (0.85 mm) until the water ceased to drip. Then, the periphyton with substrates were heated at 40 °C for 1 h for detearing superfluous water. At that time, the periphyton were considered to be saturated (moisture 100%). Then, the periphyton with substrates were weighed. After the experiments, the biomasses of the periphyton in different hyper-eutrophic treatments were determined and calculated as the difference between the weight of the periphyton and the substrate and only the substrate. To maintain a high degree of eutrophication of the medium in which the periphyton lived, the solution was replaced every month.

To keep the biofilm cells for the morphology study intact, the periphyton samples were collected with their substrates on the 57th day. The morphology of the periphyton was observed using an environmental scanning electron microscope (ESEM, QUANTA 200, FEI Company of America). To capture representative pictures of the periphyton, periphyton in each treatment were sampled at the same position as the substrates and at the same depth in the beakers for further observation with the ESEM. To conduct Biolog (Balser and Wixon, 2009) and PLFA analyses (Chang et al., 2001), the periphyton were then gently removed by hand from their substrates to avoid destroying the biofilm cells. The sampled periphyton were immediately refrigerated (4 °C) and analyzed within one day.

Periphytic samples for Biolog analyses were collected at days 30, 58 and 86. Biolog™ ECO microplates (Hayward, CA, USA) were used for determining the functional diversity of microbial communities of periphyton. The Biolog system is based on interpreting patterns of sole-carbon substrate utilization indicated by color development in a 96-well microtiter plate. All of the wells of the plate contained a redox-sensitive tetrazolium dye, which would turn purple when respiratory electrons were transported in metabolically active cells (Balser and Wixon, 2009). Briefly, for all treatments, 2 g periphyton (moisture 100%) was used to prepare the microbial solution (50 mL) in a shake incubator (200 rpm, 30 min, 25 °C); then, a 1-mL microbial solution was diluted to 20-mL aliquots, and 150-μL aliquots were added into each well and analyzed according to Guckert et al. (1996). The plates were cultured at a constant temperature of 25 °C and the color development (590 nm) was scored every 24 h for one week using a Biolog microplate reader.

Periphytic samples for the PLFA analyses were collected at day 29 and day 85. Fatty acids were expressed as nanomoles per gram