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Investigation of microbial community structure of a shallow lake after one season copper sulfate algaecide treatment

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ABSTRACT

In present work we described, for the first time, the phylogenic structure of the microbial community in a shallow freshwater lake (Hawk Island Lake, located in the Lower Peninsula of the State of Michigan, U.S.A.) after one season (four times during May to August 2007) of $CuSO_4$ treatment for algae growth control. The microbial community structure was characterized by terminal restriction fragment length polymorphism (TRFLP), clone library and 454 pyrosequencing. The similar structure of water chemistry measured across three sampling sites suggested that the lake was well mixed. The concentration of chlorophyll a (chl-a) and turbidity was low, 3.35 ± 1.62 μg/L and 2.5 ± 1.9 NTU, respectively, implying that photosynthesis was suppressed. TRFLP profiles showed that the lake was dominated by 16 terminal fragments (TFs), accounting for 85.5–92.6% abundance. Analysis of similarity (ANOSIM) showed that the difference in microbial community structure between upper and lower depths of the water column was not significant ($P = 0.101$). These results suggested that the microbial community structure within the lake was similar. Clone library and 454 pyrosequencing indicated that the lake was dominated by freshwater phyla, *Proteobacteria*, *Bacteroides*, and *Actinobacteria*. Moreover, the large number of unclassified bacteria (27.4% of total 2090 454 sequences) suggested a complex microbial community structure in the lake.

Introduction

Copper sulfate ($CuSO_4$) has been widely used for algal growth control (Moore and Kellerman, 1905) and it is the activity of ionic Cu that determines toxicity (McKnight et al., 1983). Single additions of $CuSO_4$ do not effect a permanent reduction in algal abundance and continual additions should be applied to keep populations under control (Garcia-Villada et al., 2004). When additions occur over a limited time, algal communities can recover after the additions are suspended, and the recovery times have been observed to be on the order of days to months (Hanson and Stefan, 1984; Hullebusch et al., 2002).

Though $CuSO_4$ additions are effective in reducing algal populations (Prescott, 1948), studies are showing that such additions cause substantial changes on biotic and abiotic structure of the water ecosystem (Hanson and Stefan, 1984; Girvan et al., 2005; Bressan et al., 2008). For example, Hanson and Stefan (1984) reported that long term (58 years) $CuSO_4$ addition changed phosphorous recycling and shifted the distribution of algal species.

Copper sulfate can impact the microbial community structure of a body of water that receives copper (Shade et al., 2012; Atlas et al., 1991), either by the direct toxicity of copper sulfate (Effler et al., 1980) or by indirect effects such as changes in the phototrophic community structure by copper sulfate treatment (Watson and Bollen, 1952; Barranguet et al., 2003). However, the impact of copper sulfate effects on microbial community structure and function is poorly understood. Limited previous research suggested that copper sulfate addition could dramatically reduce the bacterial content of the water column immediately following addition (Effler et al., 1980), alter the metabolic profiles of the bacteria community (Tubbing et al., 1995), and change the microbial biofilm community (Massieux et al., 2004).

To the best of our knowledge, the freshwater microbial community structure after copper sulfate addition based on sequencing techniques (e.g., clone library or pyrosequencing) has not been reported. Therefore, in this study we report the microbial community phylogenetic structure in a shallow Lake, Hawk Island Lake, located in the Lower Peninsula of the State of Michigan, U.S.A.,

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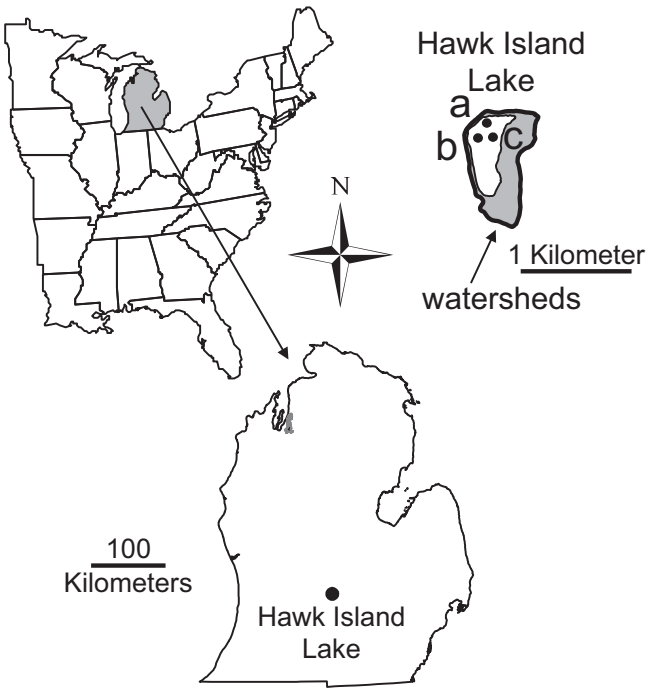

Fig. 1. Location of study lake. U.S. base map from Corel Draw™, Michigan base map Song et al. (2011), Hawk Island Lake and watershed modified from Ingham.County (2009).

which was treated with periodic additions of $CuSO_4$ to control algae. In 2007, the lake was first time treated with four times with 2 mg/L $CuSO_4$ during May to August. The water samples were obtained at 29 August, 20 days after the last time $CuSO_4$ addition. Water chemistry was characterized by 14 physiochemical parameters, including cupric ion. Microbial community pattern at different sites within the lake was evaluated by TRFLP. Microbial community structure was determined by 16 S rRNA clone library and bar-coded pyrosequencing approaches targeting the V4 variable region of the 16 S rRNA gene.

Materials and methods

Lake description

Hawk Island Lake (Lat: 42.41778, Lon: 84.314531) (Fig. 1) is an artificial lake located in the Lower Peninsula of the State of Michigan, U.S.A. It was originally a gravel pit mined for material for cement in the early 1940s (Ingham.County, 2009) and restored in 1998 with the aim of establishing a park. Water in the lake is groundwater derived. Fish and other lake species were established early in the history of the lake by occasional flooding from a nearby creek. A few islands were developed from the calcium carbonate by-products of mining activities. The lake area is 0.12 km^2 with mean depth of 4.1 m and a maximum depth of 8.2 m. The watershed area is approximately 0.075 km^2 resulting in a lake area to watershed area ratio of 1.6. The watershed is characterized as a public park with a combination of wooded areas and open grass with some trees. The lake is used for swimming, boating, and fishing, and is considered eutrophic. Copper sulfate has been used to control algae growth in Hawk Island Lake since 2007. The application data was documented at Water Bureau, Michigan Department of Environmental Quality. Briefly, 2 mg/L copper sulfate was added into lake four times during May to August, 2007.

Sampling

Sampling was carried out in 18th Sep 2007. To eliminate the human activity impact on sampling, the west area of this lake was selected as the sampling area because it was rarely disturbed. Three sampling sites (marked as A, B, C) were picked, A and B are near the shore, and C is on the center of the area (Fig. 1, geographic location was listed at Table 1).

Before sampling, a pre-calibrated Horiba U-10 Water Quality Checker™ was used to measure water temperature, pH, DO, conductivity and turbidity at 0.5 m depth increments. Temperature and DO profiles were made to decide sampling depth. The temperature and DO profile (Fig. 2a and c) showed that lake had not been stratified well and sampling depths were selected at 2.0 and 4.0 m and marked upper depth as "U" and lower depth as "L".

Water samples were collected by a 70% ethanol sterilized 4.2 L Vertical Beta Water Sampler (Wildlife Supply Company, USA) and then distributed for chemical and microbial community samples individually through ultra clean Tygon Tubing driven by a Watson-Marlow 302 S vacuum pump.

Necessary filtration was carried on the field for each sample and all sampling containers were ultra cleaned. One liter sample was filtered through 0.45 μm thermopor membrane (AquaPrep, Pall Corporation, USA) into 1 L ultra cleaned Nalgene HDPE sample bottles (Fisher Scientific, USA) for DOC and aromaticity % determination. Chlorophyll a (Chl-a) was filtered through 0.6 μm glass/fiber filters with 47 mm diameter (Sterili Tech, USA) and the filter with retained material was stored in a clean 250 mL dark HDPE bottle (Fisher Scientific, USA). Two hundred and fifty ml water was filtered through 0.45 μm ultra clean driven filter units (Millipore Corporation) using a connected ultra-clean syringe into a 60 mL ultra-cleaned Nalgene HDPE sample bottles for anion analysis. The rest water was kept for total phosphorus (TP) and total nitrogen (TN) analysis. One hundred ml water was filtered through a sterilized 0.22 μm filter (Millipore, USA) for microbial community analysis.

The used bottles and syringes were ultra-clean washed inside an EPA class 100 clean room and the washing protocol documented elsewhere (Song et al., 2011). The filtered samples were stored on ice for shipping. At the lab, the samples for microbial community analysis and Chl-a determination were stored at −20 °C and the samples for water chemistry analysis were stored at 4 °C.

Chemicals analysis

DOC was determined by a high temperature catalytic oxidation on an O.I 1100 TOC analyzer (O.I Analytical, USA) followed by 5310 B method (APHA, 1998). TN and TP were determined by the persulfate digestion methods 4500-N C and 4500-P E, respectively (APHA, 1998). Aromaticity % was evaluated by a Shimadzu UV-160 Spectrophotometer at 280 nm and calculated as: Aromaticity% = $0.05\varepsilon + 6.74$ (ε is the molar absorptivity and its unit is (mole of OC^{-1}) cm^{-1}) (Chin et al., 1994). Chl-a was estimated by absorbance at 664, 647 and 630 nm using 90% acetone extraction following the 10,200 H method (APHA, 1998). Anions (chloride, nitrate, and sulfate) and cations (calcium, magnesium, sodium, and potassium) were determined by ion chromatography (Dionex 2000 i/sp) and atomic absorption spectrophotometer (Perkin-Elmer 1100), respectively. The copper analysis was performed using a Thermo Platform inductively coupled plasma mass spectrometer (Thermo Fisher Scientific, Inc.) with hexapole collision cell technology using the EPA 200.8 method. Indium and bismuth were used as the internal standards and certified NIST 1643e was used as check standard.