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Experimental and geochemical evidence for Δ7/Δ5 sterol ratios as indicators of trophic status in Lake Fuxian, a great lake in Yunnan Province, SW China

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ABSTRACT

The reliability of Δ7/Δ5 sterol ratios as an indicator of trophic status in Lake Fuxian was tested with in situ nutrient enrichment incubation experiment and a comparison of Δ7/Δ5 values in the strata of a sediment core with the known chronology of nutrient enrichment in the lake. The results indicated that the Δ7/Δ5 sterol ratios (including sum of Δ7/sum of Δ5 sterol ratios and C27-C29 Δ7/C27-C29 Δ5 sterol ratios) in the incubation water increased consistently with total phosphorus (TP) and total nitrogen (TN) concentration across the full experimental range from 0.012 to 0.130 mg/L and from 0.068 to 1.351 mg/L, respectively. The covariation between Δ7/Δ5 sterol ratios and TP and TN concentration could be explained by enhanced productivity of Δ7 sterol-producing green algae (e.g., *Scenedesmus* sp.) under the prevailing nutrient conditions. A sediment core from Lake Fuxian showed a two-stage increase in C27-C29 Δ7/C27-C29 Δ5 sterol ratios over time, including a small rise from 1951 to 1986, and a much greater increase since 1986. These results coincide with the two-stage eutrophication previously documented in Lake Fuxian using geochemical evidence and water quality monitoring data. This study therefore demonstrates Δ7/Δ5 sterol ratio as a credible descriptor of trophic status in Lake Fuxian.

1. Introduction

Eutrophication has been the pre-eminent environmental problem affecting the world's lakes over the past four decades (Schindler, 2006), resulting in major changes to lentic primary production such as the promotion of cyanobacterial blooms and subsequent reductions in water transparency, deteriorating oxygen conditions, changes in the composition and structure of aquatic food webs, and toxicity effects (Paerl, 1988; Muri et al., 2013). Assessing the past trophic status of lakes may provide valuable baseline information with which to inform and improve management protocols and strategies for restoration (Smol, 2008). However, attempts at long-term direct monitoring of nutrient levels in the water column (e.g. total phosphorus, TP) have been sporadic and fairly inconsistent, with detailed data only available for limited periods of time at sparsely-distributed locations around the world. Fortunately, there are alternatives. For example, the evolution of trophic status in lakes is accompanied by variations in phytoplankton productivity and community structure forced by nutrient abundance (Naeher et al., 2012; Parsons et al., 2006). These variations are recorded in sediments laid down over time, which may provide a convenient multiproxy archive for evaluating long-term (e.g., 100–200 years) ecosystem changes (Yuan et al., 2014; O'Beirne et al., 2015).

The biogenic specificity and relative simple diagenetic transformation of certain lipid compounds found in lake sediments render them useful as biomarkers, which have been used to infer historic phytoplankton productivity values and reconstruct past community structures (Pinturier-Geiss et al., 2002; Lu and Meyers, 2009; Xu and Jaffé, 2009). For example it is widely accepted that long-chain 1,15-alkyl diols and 24-methylcholesta-5,24(28)-dien-3β-ol in sediment cores are indicative of the past productivity of Eustigmatophyceae and diatoms respectively in the overlying water column (Volkman et al., 1992; Schubert et al., 1998; Zhang et al., 2011). The applicability of sediment biomarkers to reflect past

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trophic status of lakes is an empirical assumption based on the association between biogenic compounds and specific phytoplankton groups and the response of phytoplankton communities to nutrient forcing. In the sediments of Lake Fuxian, C_{30} 1,15-alkyl diols have exhibited enhanced concentrations since the 1980s, reflecting the increasing trophic status of this lake, in agreement with water column monitoring records (Zhang et al., 2015a). However the technique may not be universally applicable. For example, the variation in trophic status of Lake Lugu inferred from sediment C_{30} 1,15-alkyl diol runs counter to the results of water column monitoring (Zhang et al., 2015b). Thus in each case, the reliability of any putative biomarker as an indicator of trophic status should be confirmed by direct evidence. As biomarkers in sediment are archives of former conditions in the water column, the response of biomarker proxies to experimental nutrient forcing in modern lake water provides a suitable test of their suitability as indicators of the past trophic status of lakes. Previous short-term (about 1 week) nutrient manipulation studies have confirmed the response of phytoplankton productivity and community structure to nutrient forcing (Xu et al., 2010). Likewise, the response of phytoplankton biomarkers to nutrient forcing could be directly established under similar experimental condition.

Lake Fuxian has undergone significant environmental change over recent decades (Zhang et al., 2015a). While significant anthropogenic impact is known to date back at least to 1950, routine monitoring of lake condition was only begun in 1980. The early monitoring data are limited (for example, the spatial and temporal variability of TP is not examined), and contains artificial error due to inconsistencies in the sampling and measuring protocols carried out by different institutions and individuals. In contrast, sediment lipid biomarkers represent a continuous archive of temporally and spatially integrated "snapshots" of phytoplankton productivity and community structure dating back 100–200 years (Zhang et al., 2015a). The record of long-term trophic evolution in Lake Fuxian previously established using lipid biomarkers in sediment (Zhang et al., 2015a) is in partial agreement with the limited water quality data, but the response to nutrient forcing of the biomarkers used was not explicitly examined in that study (Zhang et al., 2015a). Thus the current investigation seeks to establish the response of common lipid biomarkers to nutrient manipulation in an incubation experiment in the lake. In particular, the reliability of $\Delta 7/\Delta 5$ sterol ratio as an indicator of trophic status in Lake Fuxian was assessed by this experiment and also by the stratigraphic variances of this ratio in sediment cores of the lake.

2. Methods

2.1. Study site

Lake Fuxian (24°17′–24°37′N, 102°49′–102°57′E) is a large, deep, oligotrophic lake, situated at 1721 m a.s.l. in central Yunnan Province, SW China (Fig. 1). It has a surface area of 212 km^2, a maximum depth of 155 m, and a water residence time of 167 years (Zeng and Wu, 2009; Liu et al., 2009). The lake currently exhibits TN concentrations of 0.126 mg/L, TP levels of 0.021 mg/L, chlorophyll a (Chla) concentrations of 4.5 μg/L and a Secchi depth (SD) of 6 m. Lake Fuxian is surrounded on four sides by mountains, and receives water from more than 20 rivers (including the Jianshan River, Dongda River, and Xida River), of which seven flow through cultivated fields. Agriculture around the lake began developing rapidly in the 1950s, whereas most industrial development and urbanization (e.g., food processing, phosphorus fertilizer and cement manufacturing) has occurred since the 1980s. This history is reflected in geochemical records, which indicate a two-stage increase in nutrient abundance in the lake, comprising a minor rise beginning in 1951 and a much greater increase since 1986 (Zhang et al., 2015a).

2.2. Nutrient manipulation incubation experiments

Nutrient supplementation experiments were performed in situ during July 2014 to address the effects of nutrient inputs on phytoplankton growth and the corresponding variation in lipid biomarkers. Pooled water samples containing natural phytoplankton assemblages were collected from the center of Lake Fuxian, 0–5 m below the surface (the most phytoplankton-rich layer) in precleaned 20-L polyethylene carboys. Each water sample was screened through a 200-μm mesh to remove large zooplankton grazers and redistributed into two or four precleaned, chemically inert, unbreakable and transparent 5 L containers. The water samples were numbered from F-1 to F-8. Half of the lake water in containers of the F-1 sample was poured away, and replaced with the same volume of deionized water (Table 1). No further treatment was given to sample F-2. Because both TN and TP exhibited an increase in the water column of Lake Fuxian since 1980 (Zhang et al., 2015a), samples F-3 to F-8 were supplemented with gradually increasing amounts of N and P (as KNO_3 and $K_2HPO_4{\cdot}3H_2O$, respectively) to simulate the trophic evolution in this lake (Table 1). After the addition of nutrients, all containers were incubated for 6 d in the lake, supported on a steel frame just below the water surface (Fig. 1). This in situ treatment replicated natural light and temperature conditions. All treatments were repeated in triplicate.

2.3. Phytoplankton biomass, TN, TP and Chla

At the end of the incubation experiment, phytoplankton species were classified and counted and biomass was measured for all water samples. Sub samples were collected in 1 L plastic bottles and preserved in the field by the addition of 2%, Lugol·s acid iodine fixative solution after which they were allowed to settle for at least 48 h. Following decanting and an additional period of settling in a 100 mL glass measuring cylinder, about 70 mL of supernatant was siphoned off. A 0.1 mL aliquot of the remaining supernatant was placed in a counting chamber and examined under a Nikon inverted microscope. Chemical analyses of water samples including TP and TN were performed by combined persulfate digestion, followed by spectrophotometric analysis. Chla was determined spectrophotometrically after extraction in 90% acetone.

2.4. Lipid biomarkers in incubation water

The remaining water (about 9–19 L) from each incubation treatment was filtered through pre-combusted glass fiber filters (GFF filter) to collect particulate organic matter (POM). Filters were folded carefully, wrapped with pre-combusted aluminium foil and frozen at −20 °C until extraction. The POM filters were Soxhlet extracted for 72 h with dichloromethane/methanol (9:1 v/v) after adding known amounts of internal standards (*n*-tetracosane-d50, tridecanol, 5α-androstan-3β-ol and nonadecanoic acid). Sulfur was removed by addition of activated copper. The extracts were saponified with 5% KOH-methanol solution. The neutral fraction was isolated with hexane by extraction. The fatty acid (FA) fractions were isolated with hexane after acidification to pH 1 with 3 N HCl. The neutral fraction was fractionated by silica gel column chromatography. Aliphatic hydrocarbon was eluted with hexane, and *n*-alkanol/sterol fraction was eluted with dichloromethane/methanol (9:1). The *n*-alkanol/sterol fraction was silylated (70 °C, 60 min) with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and FA fractions were methylated with 14% BF_3/CH_3OH (60 °C, 120 min) before being analyzed by gas chromatography-mass spectrometry (GC–MS).