Original Articles

Fatty acid and alcohol compositions in lacustrine sediments as indicators of environment and ecosystem of lakes in Eastern China

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\textbf{A B S T R A C T}

During the past hundred years, the majority of lakes along the middle and lower reaches of the Yangtze River (MLRYZ) have been greatly impacted by human intervention, resulting in a significant spatial heterogeneity in water and ecosystem quality. We quantified the fatty acid (FA), n-alkanol, sterol and long-chain alkyl diol content of surface sediments of MLRYZ lakes in order to determine how ecological differences (algal versus macrophyte-dominated state) and trophic status influence the composition, origin and abundance of lipid biomarkers in the sediments. Based on the results, variation in FAs appears to be mainly controlled by the type of organisms within the lakes. Short-chain n-alkanols, monounsaturated FAs (MUFA)s and polyunsaturated FAs (PUFAs) were generally more abundant in sediments of algal lakes (lacking macrophyte growth) than in lakes with macrophyte growth. The opposite trend was apparent for long-chain n-alkanols. Ratios of (C26 + C28)/(C22 + C24) n-alkanoic acids differed significantly between macrophyte and algal lakes. In the alcohol fraction, lipid biomarker abundance appeared to be controlled by trophic status of the lake, with the exception of cholesterol and long-chain n-alkanols whose abundances were closely related to the present organisms and exhibiting greater abundances in algal lakes. Enhanced levels of total phosphorus (TP) and total nitrogen (TN) in a lake create greater concentrations of C32 1,15 alkyl diol and brassicasterol respectively, while the abundance of dinosterol and the ratio of dinosterol/brassicasterol decline in line with increase in TP and TN, respectively. Ratios of (C26 + C28)/(C22 + C24) n-alkanol were also greatly influenced by the type of organisms present, exhibiting higher values in algal lakes. The sensitivity of these sediment lipid biomarker responses points to a potentially useful role as proxies for biological community types and trophic status in paleolimnology studies.

1. Introduction

A lipid biomarker can be defined as a molecule whose carbon skeleton can be unambiguously linked to that of a known biological precursor compound or as a complex organic compound exhibiting little to no change in its chemical structure from the precursor molecule that once existed in a living organism (Killops and Killops, 2005; Peters et al., 2005; Derrien et al., 2017). Lipids comprise a large and diverse group of naturally occurring organic compounds that are soluble in non-polar organic solvents. In lake sediments, the lipid biomarkers include original, biologically synthesized lipid materials originating from organisms within the lake; terrestrial input from the surrounding watershed introduced via stream/river inflows; and secondary lipid compounds derived from microbial activity in the water and sediment (Meyers and Ishiwatari, 1993; Lu and Meyers, 2009). Fatty acids (FAs) and alcohols (e.g., n-alkanols, sterol and stanol) are among the most abundant lipids in lakes (Dunn et al., 2008; Lu and Meyers, 2009; Derrien et al., 2017) and have been widely used as source indicators for organic matter (OM) in sediments (Muri et al., 2004; Hu et al., 2008, 2009; Ortiz et al., 2016). Short-chain n-alkanoic acids and n-alkanols can reflect input from algae and photosynthetic bacteria, while long-chain n-alkanoic acids and n-alkanols are associated with OM from terrestrial higher plants (Eglinton and Hamilton, 1967; Cranwell, 1976; Meyers, 1997). Sterols in sediments can indicate input from more specific phytoplankton taxa (Volkman et al., 1998; Ortiz et al., 2016). Dinosterol, for example, is a highly specific biomarker for dinoflagellates (Boon et al., 1979; Schubert et al., 1998; Zimmerman and Canuel, 2002), while brassicasterol reflects OM input mainly from

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diatoms (Volkman et al., 1998; Hu et al., 2008). The biological sources of cholesterol on the other hand, are relatively complex, including zooplankton, diatoms and cyanobacteria (Volkman, 1986). Overall, the source, composition and distribution of lipid biomarkers in sediments have been found to be closely dependent on environmental and ecological factors in the overlying water column (Hostettler et al., 1999; Tolosa et al., 2008; Bragée et al., 2013; Zhang et al., 2017). For example, rising growth of phytoplankton in eutrophic lakes leads to a greater accumulation of phytoplankton biomarkers such as C16:1 and C18:1 FAs, short-chain n-alkanols and long-chain alkyl diols (Routh et al., 2004, 2009; Zhang et al., 2015, 2017). The ratio of dinosterol/brassicasterol also changes with the nutrient status of lakes, with lower values in eutrophic water (Zimmerman and Canuel, 2002; Zhang et al., 2018). Sediments covered by dense macrophyte growth contain abundant sitosterol, whereas cholesterol levels increase in the sediments of algae-dominated areas (Zhang et al., 2017).

The middle and lower reaches of China’s longest watercourse, the Yangtze River, run through one of the most industrialized and urbanized regions in Southeast Asia before discharging into the western Pacific Ocean (Wu et al., 2012; Zhao et al., 2016). Historical processes along the river have given rise to numerous lakes that benefit local human populations, providing opportunities for aquaculture, tourism and recreation, transport, as well as a supply of drinking water. In recent years, however, the water in many lakes of this region has declined in quality, becoming mesotrophic or eutrophic through inputs from agricultural, industrial and urbanization development (Xue et al. 2010). For example, Lake Chaohu and northern region of Lake Taihu have undergone serious deterioration, becoming infamous for summer cyanobacterial blooms (Dong et al., 2014; Xu et al., 2017). In contrast, lakes such as Lakes Liangzi, Xiliang and Honghu have been not much influenced by pollution and remain dominated by clear water habit with abundant macrophytes (Wu et al., 2012; Dong et al., 2012). The variability in water quality and ecosystem structure could lead to divergence in sedimentary records of lipid biomarkers (Tolosa et al., 2008; Xu et al., 2014; Zhang et al., 2017), but as yet there has been no attempt to map the distribution, composition and origin of FAs and alcohols across the region. The present study addresses this knowledge gap by analyzing the abundances and composition of FAs, n-alkanols, sterols and long-chain alkyl diols in sediments of lakes along the middle and lower reaches of the Yangtze River (MLRYZ) and comparing the lipids from lakes with varying conditions in the overlying water column. The results carry significant implications for the future use of lipid biomarkers in paleolimnology studies.

2. Methods and materials

2.1. Study area and sampling

The Yangtze River is 6300 km long, with a catchment of 1.8 million km², equivalent to ~20% of the total land area of China. The river can be divided into upper (source to Yichang City), middle (Yichang to Hukou City) and lower (Hukou to Shanghai City) reaches by topographic and hydrologic characteristics. The MLRYZ areas cross central and eastern China and lie within the most important economic area of
Sampling took place in 2016 and for each lake, six samples of surface sediments (0–1 cm) were collected within a 200 × 200 m area in the central zone using a gravity corer with a 90-mm-diameter coring tube. Samples from each site were homogenized and packed immediately in brown glass bottles that had been prewashed with distilled water and n-hexane. Upon arrival in the laboratory, the sediments were frozen at −20 °C until processing for geochemical analysis. In each 200 × 200 m central area, the water was sampled in each of the four seasons of 2016.

2.2. Sediment and water analyses

For the water samples, total phosphorus (TP) was determined by the ammonium molybdate method after potassium persulphate digestion. Total nitrogen (TN) was determined by the alkaline potassium persulphate digestion-UV spectrophotometric method. Chlorophyll a levels (Chla) were measured spectrophotometrically after extraction in 90% acetone. Sub-samples of sediments for analysis of total organic carbon (TOC) were leached with dilute HCl to dissolve carbonate and rinsed copiously with distilled water to remove remaining chlorides, and TOC concentrations were determined using a CHNS Vario EL III elemental analyzer. The relative standard deviation for this analysis was less than 2%.

Sediment samples for biomarker analyses were Soxhlet extracted for 72 h with dichloromethane/methanol (9:1 v/v) after adding known amounts of internal standards (C13 n-alkan, 5α-androstan-3β-ol and C19 n-alkanoic 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 FA fractions were isolated with hexane after acidification to pH 1 with 3 N HCl. The neutral fraction was fractionated using silica gel column chromatography to acquire alcohols. The alcohol fractions were silylated (70 °C, 60 min) with N,O-bis(trimethylsilyl)trifluoroacetamide and FA fractions were methylated with 14% BF₃/MeOH (60 °C, 120 min) before being analyzed by gas chromatography–mass spectrometry (GC–MS).

FAs and alcohols were analyzed by GC–MS using an Agilent 5975 mass spectrometer coupled to an Agilent 7890A gas chromatograph with DB–5MS column (30 m × 0.25 mm × 0.25 μm). To determine the FAs, the GC oven temperature program started at 80 °C (2 min), followed by an increase to 290 °C at 4 °C/min, held at this temperature for 15 min. For the alcohol fraction, the oven temperature program started at 80 °C (2 min), followed by an increase from 80 °C to 220 °C at 6 °C/min. The heating rate was subsequently reduced to 3 °C/min up to 250 °C and then to 2 °C/min up to 310 °C. The final temperature was maintained for 15 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The ion source was operated at 250 °C and 70 eV, in electron impact mode. Compounds were identified by comparison with previously reported mass spectra, and by interpretation of fragmentation patterns and chromatographic retention behavior. Quantification data was determined by comparing peak areas of the internal standard with compounds of interest in reconstructed total ion current (TIC) chromatograms. Biomarker levels were normalized to TOC content in order to minimize the effect of varying sedimentation rate and degradation on biomarker content (mg g⁻¹ TOC). A precision test performed by analyzing five replicates of each sample revealed a deviation of less than 20% in the determination of each biomarker compound.

3. Results

3.1. TN, TP and Chla

TN exhibited significant spatial variation in the lakes along the MLRYZ from 0.72 to 4.91 mg/L (Fig. 2a). The lakes of the Taihu group were richest in TN, averaging 2.46 mg/L (range 1.31–4.91 mg/L), with much lower levels (ANOVA test, P < 0.05) recorded in the Poyang and Dongting groups, averaging 1.28 mg/L (range 0.72–2.11 mg/L) and...
1.47 mg/L (range 0.88–2.18 mg/L), respectively. TP varied from 0.021 to 0.234 mg/L across the studied lakes (Fig. 2b), with slightly greater concentrations in the Taihu and Poyang groups, averaging 0.081 mg/L (range 0.021–0.125 mg/L) and 0.085 mg/L (range 0.030–0.234 mg/L), respectively, compared to an average of just 0.059 mg/L (range 0.027–0.089 mg/L) in the Dongting group. Chla varied from 3.55 to 51.10 μg/L in lakes across the study (Fig. 2c), but unlike TN, the lowest values were detected in lakes of Taihu group, averaging 9.27 μg/L (range 3.55–18.18 μg/L), while the highest values occurred in the Poyang and Dongting groups, averaging 21.34 μg/L (range 6.78–51.10 μg/L) and 20.32 μg/L (range 11.13–32.10 μg/L), respectively.

3.2. FA

Surface sediments from lakes along the MLRYZ yielded n-alkanoic acids ranging from C14 to C30, characterized by a bimodal distribution (Fig. 3). In the low carbon number (< C20) fractions, the dominant peak (Cmax) denoting highest n-alkanoic acid concentrations occurred at n-C16, whereas in the high carbon number (> C21) fraction, Cmax occurred at n-C26 or n-C28 in lakes where macrophytes were found in the 200 × 200 m sampling areas in spring (Fig. 3). These water bodies were thus designated as macrophyte lakes and appear in green in the figures. By contrast, in lakes whose sampling sites lacked macrophytes in any of the four seasons, Cmax occurred at n-C22 or n-C24 (Fig. 3). These were designated as algal lakes, and appear red in the figures. The ratios of (C26 + C28)/(C22 + C24) n-alkanoic acid in macrophyte lakes varied from 1.02 to 3.07 (average 2.02) while in algal lakes the range was 0.27–1.08 (average 0.64) (Fig. 4a). Long-chain n-alkanoic acids were relatively more enriched in the sediments of macrophyte lakes (ANOVA test, P < 0.001), with TAR (terrigenous: aquatic ratio, Bourbonniere and Meyers, 1996), defined as (C24 + C26 + C28 + C30)/(C12 + C14 + C16 + C18 + C20) n-alkanoic acid, ranging from 0.43 to 1.59 and averaging 0.94 (Fig. 4b). The TAR values of algal lakes were lower, ranging from 0.09 to 0.50 (average 0.25) (ANOVA test, P < 0.001, Fig. 4b). For n-alkanoic acids with carbon numbers lower than 18, the carbon preference index (CPI), defined as CPI1 \(=\) 1/2[(C12 + C14 + C16)/(C13 + C15 + C17) + (C14 + C16 + C18)/(C13 + C15 + C17)], ranged from 2.10 to 6.00 (average 3.53) in macrophyte lake sediments, while those in algal lakes ranged from 3.35 to 12.08 (average 6.01) (Fig. 4c). The CPI values for high carbon number n-alkanoic acids (CPIH = 1/2[(C22 + C24 + C26 + C28)/(C23 + C25 + C27 + C29) + (C24 + C26 + C28 + C30)/(C23 + C25 + C27 + C29)]) varied from 4.30 to 9.26 and exhibited little difference (ANOVA test, P > 0.5) between macrophyte and algal lakes (Fig. 4d). Abundances of the n-alkanoic acid, monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) in sediments displayed a close covariance with the biological communities in the overlying water column (Fig. 5). Short-chain n-alkanoic acids (sum of C12, C14, C16, C18 and C20) were much more abundant in algal lakes (average 8.27 mg g\(^{-1}\) TOC, range 3.23–16.15 mg g\(^{-1}\) TOC) than in macrophyte lakes (average 3.19 mg g\(^{-1}\) TOC, range 2.50–6.55 mg g\(^{-1}\) TOC) (ANOVA test, P < 0.001, Fig. 5a). Long-chain n-alkanoic acids (sum of C24, C26, C28 and C30), on the other hand, were abundant in macrophyte lakes (average 3.44 mg g\(^{-1}\) TOC, range 1.81–6.74 mg g\(^{-1}\) TOC) and greatly reduced (average 1.78 mg g\(^{-1}\) TOC, range 0.84–2.85 mg g\(^{-1}\) TOC) in algal lakes (Fig. 5b). Like short-chain n-alkanoic acids, MUFAs (sum of C16:1, C18:1, C20:1 and C22:1 FAs) and PUFAs (sum of C16:4, C18:2, C18:3, C18:4, C20:4, C20:5, C20:3, C20:2, C22:5 and C22:6 FAs) were generally more abundant in algal
lakes (ANOVA test, P < 0.002), averaging 6.70 mg g\(^{-1}\) TOC (range 4.06–9.66 mg g\(^{-1}\) TOC) and 2.79 mg g\(^{-1}\) TOC (range 1.60–4.67 mg g\(^{-1}\) TOC), respectively (Fig. 5c and d). The values declined to averages of 3.71 mg g\(^{-1}\) TOC (range 2.85–5.07 mg g\(^{-1}\) TOC) and 1.61 mg g\(^{-1}\) TOC (range 1.05–2.62 \(\mu\)g g\(^{-1}\) TOC) respectively, in macrophyte lakes (Fig. 5c and d).

3.3. n-alkanols, sterols and long-chain alkyl diols

The n-alkanols detected in the surface sediments of lakes along the MLRYZ ranged from C14 to C32, with an obvious bias in abundance towards even carbon number (Fig. 3). Abundance values exhibited a bimodal distribution, which in the lower carbon number (< C20) fraction showed a \(C_{\text{max}}\) at C16 in all lakes (Fig. 3). In the higher carbon number (> C21) fraction, \(C_{\text{max}}\) occurred at C26 or C28 in algal lakes, and at C22 or C24 in macrophyte lakes, with the exception of lakes Gucheng (Y7), Shijiu (Y8), Daguan (Y14) and Wushan (Y22) where \(C_{\text{max}}\) also occurred at C26 or C28 (Fig. 3). The (C26 + C28)/(C22 + C24) n-alkanol ratios ranged from 1.07 to 2.22 (average 1.53) in algal lakes, much higher than in macrophyte lakes (average 0.95, range 0.62–1.32) (ANOVA test, P < 0.001, Fig. 6a). The n-alkanol TAR, defined as \((C24 + C26 + C28 + C30 + C32)/(C14 + C16 + C18 + C20)\) n-alkanol (Bourbonniere and Meyers, 1996), varied from 0.74 to 5.74 (average 3.15) in algal lakes, with much lower values of 0.52–3.96 (average 1.58) in macrophyte lakes (ANOVA test, P < 0.005, Fig. 6b). There was no statistically significant difference in the combined abundances of short-chain n-alkanols (sum of C14, C16, C18 and C20) in the sediments of macrophyte and algal lakes (ANOVA test, P > 0.1). Spatially, however, short-chain n-alkanol levels increased rapidly from lakes of the Taihu group (average 0.79 mg g\(^{-1}\) TOC) to the Poyang group (average 1.14 mg g\(^{-1}\) TOC), reaching highest levels (average 1.40 mg g\(^{-1}\) TOC) in the Dongting group (Fig. 6c). In contrast, combined long-chain n-alkanol (sum of C24, C26, C28, C30 and C32) abundances were low (average 1.52 mg g\(^{-1}\) TOC) in macrophyte lakes, and increased to 2.59 mg g\(^{-1}\) TOC on average in algal lakes (Fig. 6d). Beside the n-alkanols, a series of C27 to C29 4-des-methyl sterols, dinosterol and C32 1,15 alkyl diol were found in relatively high abundances in the sediments. Cholesterol levels were generally high (average 1.32 mg g\(^{-1}\) TOC, range 0.75–2.28 mg g\(^{-1}\) TOC) in algal lakes, but declined rapidly (average 0.76 mg g\(^{-1}\) TOC, range 0.56–1.24 mg g\(^{-1}\) TOC) in macrophyte lakes (Fig. 7a). Meanwhile the abundances of brassicasterol, campesterol, stigmasterol, sitosterol, dinosterol and C32 1,15 alkyl diol showed little difference between algal and macrophyte lakes (ANOVA test, P > 0.05). Spatially however, brassicasterol was relatively abundant (average 0.66 mg g\(^{-1}\) TOC) in lakes of the Taihu group, while stigmasterol and dinosterol levels were low (average 0.76 and 0.15 mg g\(^{-1}\) TOC, respectively) (Fig. 7b, 7d, 7f). Average values of sitosterol and campesterol showed some minor variation between the three lake groups (ANOVA test, P > 0.6, Fig. 7c and e). C32 1,15 alkyl diol was relatively abundant in lakes of the Poyang group, averaging 0.18 mg g\(^{-1}\) TOC (Fig. 7g). Ratios of dinosterol/brassicasterol varied from 0.08 to 0.84 and exhibited no clear trend between algal and macrophyte lakes (ANOVA test, P > 0.4, Fig. 7h). However, the values were low (average 0.28, range 0.08–0.68) in lakes of the Taihu group, intermediate (average 0.44, range 0.12–0.72) in the Poyang group and high (average 0.52, range 0.25–0.84) in the Dongting group (Fig. 7h).
4. Discussion

4.1. Origin of lipid biomarkers in the sediments

Lipid biomarkers in lake sediments derive from multiple biogenic sources, including phytoplankton, aquatic macrophytes, terrestrial plants and bacteria (Meyers, 2003). The abundances and distribution patterns of lipid biomarkers are helpful in establishing a direct and unique link with OM sources (Zhang et al., 2015). High concentrations of short-chain \( n \)-alkanoic acids with maxima at C14:0, C16:0 and C18:0, as shown in the current study (Fig. 3), are a shared feature of algal and bacterial OM (Cranwell et al., 1987; Woszczyk et al., 2011; Derrien et al., 2017). The presence of abundant C16:1 MUFA is generally attributed to input from diatoms (Volkman et al., 1998; Woszczyk et al., 2011); C18:1 MUFAs reflect contributions from algae, cyanobacteria and bacteria (Cranwell, 1978; Volkman et al., 1980; Alfaro et al., 2006); C18:3 and C18:2 PUFAs are putative indicators of cyanobacterial and green algal input (Rezanka et al., 1983; Bechtel and Schubert, 2009; Xu et al., 2015); and C20:5\( \omega_3 \) and C22:6 \( \omega_3 \) MUFAs feature most in material of diatom and dinoflagellate origin respectively (Nichols et al., 1983; Volkman et al., 1998; Lowe et al., 2014). C18:2 \( \omega_6 \) PUFA was previously reported to be abundant in aquatic plants (Xu et al., 2014). The similarity in variation between C18:2 \( \omega_6 \) and C20:5 \( \omega_3 \) PUFA observed in the current study (R\(^2\) = 0.33, n = 30) may indicate a phytoplankton origin for C18:2 \( \omega_6 \) (Yoshinaga et al., 2008). The C18:2 \( \omega_6 \) enrichment of material from algal lakes in the current study (ANOVA test, P < 0.001) provides additional evidence for the major importance of phytoplankton input. Long-chain \( n \)-alkanoic acids are major components of the waxy coatings on plant leaves, flowers, and pollen (Lu and Meyers, 2009; Derrien et al., 2017). Submerged and floating macrophytes can also produce long-chain \( n \)-alkanoic acids, especially the C22 and C24 homologues (Ficken et al., 2000). In the current study, abundances and C\(_{\text{max}}\) of long-chain \( n \)-alkanoic acids both differ significantly between macrophyte and algal lakes (Figs. 3 and 5b), arguing for a mixed source for these compounds (Holtvoeth et al., 2010). In macrophyte lakes, the sources might be aquatic macrophytes or organisms associated with macrophyte growth, such as bacteria and periphyton (Fang et al., 2014; Zhang et al., 2017). Conversely, in algal lakes the majority of long-chain \( n \)-alkanoic acids are likely to derive from terrestrial higher plant sources (Meyers, 2003).

Short-chain \( n \)-alkanols are ubiquitous in phytoplankton and their presence in sediments can represent OM input from a full range of phytoplankton taxa (Robinson et al., 1984; Pearson et al., 2007; Bechtel and Schubert, 2009), an interpretation supported by covariation in the abundances of short-chain \( n \)-alkanols and Chla (R\(^2\) = 0.37, n = 30). Much like long-chain \( n \)-alkanoic acids, long chain \( n \)-alkanols are abundant in both aquatic macrophytes and higher plant waxes (Eglinton and Hamilton, 1967; Meyers, 1997; Woszczyk et al., 2011). In particular, C22 \( n \)-alkanol is known to dominate in eustigmatophytes, a small class of phototrophic marine and freshwater microalgae (Volkman et al., 1998), and in certain epiphytes associated with grass (Jaffé et al., 2001). In most macrophyte lakes investigated in the current study, long chain \( n \)-alkanols have maxima at C22 or C24, and their distribution probably indicates an origin in aquatic macrophytes and epiphytes (Ficken et al., 2000). Long chain \( n \)-alkanols are generally present in relatively high levels in algal lakes (ANOVA test, P < 0.002), reflecting input from terrestrial higher plants (Meyers, 2003; Zhang et al., 2017). Some sterols in sediments derive from specific phytoplankton taxa in the surrounding habitat (Volkman et al., 1998; Pearson et al., 2007). For example, dinosterol is produced exclusively by dinoflagellates (Boon et al., 1979; Volkman, 1986). Meanwhile, the high abundances of brassicasterol in the sediments of...
The current study can be largely attributed to input from diatoms, typically abundant in lakes of MLRYZ (Wang and Dou, 1998), although a small contribution from other phytoplankton such as haptophytes cannot be excluded (Volkman et al., 1998). Aside from brassicasterol and dinosterol, the dominant sterols in the sediments include stigmasterol, campesterol, sitosterol and cholesterol (Fig. 7). In the current study, cholesterol was found to be more abundant in the sediment of algal lakes (ANOVA test, P < 0.001, Fig. 7a), most likely as a result of input from zooplankton, diatoms and cyanobacteria (Nishimura and Koyama, 1977; Pearson et al., 2007; Bechtel and Schubert, 2009). Stigmasterol and sitosterol levels show no correlation with brassicasterol and exhibit little difference in abundance between algal and macrophyte lakes (ANOVA test, P > 0.8, Fig. 7c and d), probably reflecting mixed contributions from terrigenous plants, algae and aquatic macrophytes (Volkman, 1986; Gómez-Gutiérrez et al., 2011; Derrien et al., 2017). The small observed correlation between campesterol and brassicasterol (R² = 0.35, n = 30) probably reflects inputs from algae, such as green algae and diatoms (Gómez-Gutiérrez et al., 2011; Zhang et al., 2015). In addition to the sterols, C32 1,15 alkyl diol is believed to be a taxon-specific biomarker found exclusively in the eustigmatophyceae (Volkman et al., 1998).

4.2. Distribution of lipid biomarkers in the sediments

The FA content of sediments is commonly ascribed to input from algae and terrestrial plants (Meyers, 2003; Fang et al., 2014). In the current study, however, the FA profiles of lake sediments are shown to be greatly influenced by the presence or absence of macrophytes in the overlying water. Average levels of short-chain n-alkanoic acids, MUFAs and PUFAs in sediments increased up to 117%, 81% and 73% from macrophyte lakes to algal lakes, whereas the average values of long-chain n-alkanoic acids decreased 48% from macrophyte to algal lakes. Our results demonstrate algae to be the major contributors of short-chain n-alkanoic acids, MUFAs and PUFAs, while macrophytes or organisms associated with macrophytes appear to be largely responsible for the presence of long-chain n-alkanoic acids (Volkman et al., 1998; Ficken et al., 2000; Meyers, 2003; Zhang et al., 2015). The variable Cmax exhibited by long-chain n-alkanoic acids was also influenced by the type of organisms present in the water column, with macrophyte lake samples exhibiting Cmax at n-C26 or n-C28 and algal lakes peaking at n-C22 or n-C24 (Fig. 3). The difference could be the result of large inputs of n-C26 and n-C28 alkanoic acids from aquatic macrophytes (Volkman et al., 1998; Ficken et al., 2000; Meyers, 2003; Zhang et al., 2015). The variable Cmax exhibited by long-chain n-alkanoic acids was also influenced by the type of organisms present in the water column, with macrophyte lake samples exhibiting Cmax at n-C26 or n-C28 and algal lakes peaking at n-C22 or n-C24 (Fig. 3). The difference could be the result of large inputs of n-C26 and n-C28 alkanoic acids from aquatic macrophytes (Ficken et al., 2000), but this interpretation seems at odds with previous findings suggesting a terrestrial plant origin for n-C26 and n-C28 homologues and an aquatic macrophyte source for n-C22 and n-C24 homologues (Ishiwatari et al., 2016; Wang and Liu, 2012). Other studies have shown that long-chain n-alkanoic acids may also arise from bacterial and algal sources (Gong and Hollander, 1997; Naraoka and Ishiwatari, 2000; Fang et al., 2014). The periphyton associated with aquatic macrophytes may produce large amounts of n-C26 and n-C28 alkanoic acids (Zhang et al., 2017). Furthermore, the high productivity of bacteria during degradation of macrophyte OM might lead to enrichment of C26 and C28 n-alkanoic acids in the sediments (Boschker et al., 1999). The average CPI values of short-chain n-alkanoic acids in our study decreased by 41% from algal to macrophyte lakes (Fig. 4c), perhaps reflecting a larger contribution of bacterial short-chain odd carbon (such as C15:0 and C17:0) n-alkanoic acids in the latter (Kawamura et al., 1987; Alfaro et al., 2006; Ortiz et al., 2016). Alternatively, the lower CPI values recorded in macrophyte lake sediments may result from greater input from emergent macrophytes, such as reeds (Holtvoeth et al., 2010). In the macrophyte lakes, PUFAs exhibited a high abundance correlating with elevated TP levels in the
Fig. 7. Variation in concentrations (mg g$^{-1}$ TOC) of sterols (a-f) and C32 alkyl diol (g) and the ratios of dinosterol/brassicasterol (h) in sediments of lakes along the MLRYZ. Symbols appear red for algal lakes and blue for macrophyte lakes.
water ($R^2 = 0.72$, $n = 15$). The correlation between MUFAs and TP was much less obvious ($R^2 = 0.30$, $n = 15$). In algal lakes, however, a weak correlation was found between PUFAs and TP ($R^2 = 0.25$, $n = 15$), indicating that PUFAs abundance in sediments is controlled by nutrient status of the lake to some extent. In contrast, the observed variation of short-chain $n$-alkanoic acids, long-chain $n$-alkanoic acids and MUFAs in sediments bore little relation to nutrient levels in the overlying water.

In the alcohol fraction, average concentrations of cholesterol and long-chain $n$-alkanols in sediments increased by 73% and 71% respectively from macrophyte lakes to algal lakes. Elevated levels of cholesterol in algal lakes reflect the origin of this sterol from algae and zooplankton (Gómez-Gutiérrez et al., 2011). Meanwhile, the high levels of long-chain $n$-alkanols in algal lakes are probably caused by loading with significant terrestrial OM, a phenomenon previously identified in algal areas of Lake Taihu (Zhang et al., 2017). Sediment OM derived from submerged macrophytes can produce long-chain $n$-alkanols with $C_{\text{max}}$ at $n$-$C_{22}$ or $n$-$C_{24}$ (Ficken et al., 2000), as observed in most macrophyte lakes (Fig. 3). However, massive loading with terrestrial OM results in a shift in $C_{\text{max}}$ to $n$-$C_{26}$ or $n$-$C_{28}$ (Fig. 3) owing to the large amounts of long-chain $n$-alkanols (> $C_{26}$) in higher plants (Meyers, 2003). Previous assessments of the applicability of sediment sterols as indicators for the past trophic status of lakes have involved empirical assumptions of associations between sterols and specific phytoplankton groups and the response of phytoplankton communities to nutrient forcing (Zhang et al., 2016). In the current study, cholesterol abundances exhibited a relatively good correlation with TN in macrophyte lakes ($R^2 = 0.61$, $n = 15$), but the correlation was much weaker in algal lakes ($R^2 = 0.28$, $n = 15$). In combined lakes of the two types, brassicasterol showed a relatively weak abundance correlation ($R^2 = 0.39$, $n = 30$) with TN (Fig. 8a), while $C_{32}$ 1,15 alkyl diol presented a moderate correlation ($R^2 = 0.49$, $n = 30$) with TP (Fig. 8b). Overall negative correlations were found between abundances of dinosterol and TP ($R^2 = 0.39$, $n = 30$) and between values of dinosterol/brassicasterol and TN ($R^2 = 0.44$, $n = 30$) (Fig. 8c and d). The results suggest a significant influence of trophic status on phytoplankton-derived sterol levels in the sediments (Zhang et al., 2017) and suggest high TN levels are responsible for enrichment with cholesterol and brassicasterol. An increase in N flux would result in a reduction of dinosterol relative to brassicasterol. Elevation of TP enhances the production of $C_{32}$ 1,15 alkyl diol by eustigmatophyceae (Volkman et al., 1998). The causal relationships presented here are supported by sediment historical records of variation in sterols and long-chain alkyl diol in response to anthropogenic N and P loading (Zhang et al., 2015, 2018). The implication that reduced dinosterol levels are driven by excessive P input agree with the analyses of sediments in Lake Taihu showing that dinosterol is much more abundant (ANOVA test, $P < 0.001$) in the oligotrophic East Bays than in the eutrophic Meiliang Bay and Zhushan Bay (Zhang et al., 2017). However, historical records obtained from sediment cores indicate an increase of dinosterol as eutrophication took place (Zhang et al., 2015, 2018). The data in the current study show a notable increase in dinosterol when TP concentrations rose from zero to $\sim 0.5$ mg/L, but a general reduction of dinosterol with increasing TP when the entire data range is considered (Fig. 8c). The records in sediment cores (Zhang et al., 2015, 2018) may therefore reflect the increase in dinosterol production forced by the initial stages of TP elevation.
4.3. Implications for paleolimnology studies

A macrophyte-dominated clear state and a turbid algal state are likely to be alternative equilibria over a range of nutrient levels in most shallow lakes (Scheffer et al., 1993), and the factors controlling the transition between the two states have attracted considerable scientific interest (Kenney et al., 2002; Sayer et al., 2010; He et al., 2015; Randalsu-Wendrup et al., 2016). Lipid biomarkers used in paleolimnology studies have generally focused on the past input of algae, aquatic macrophytes and terrestrial plants to lakes (Bourbonniere and Meyers, 1996; Ficken et al., 2000; Meyers, 2003). The method developed by Ficken et al. (2000), based on the distribution of n-alcanes, was frequently used to assess OM input from aquatic macrophytes versus terrestrial higher plants. The reliability of molecular geochemical method, however, was still poorly known in determining the transition between macrophyte and algal lakes. The results of our study of extractable FA and alcohol biomarkers in sediments have revealed a number of compositional differences between macrophyte and algal lakes. The algal state corresponds with high levels of short-chain n-alkanoic acids, MUFAs, PUFAcs, cholesterol and long-chain n-alkanols (ANOVA test, P < 0.002), while sediments from macrophyte lakes are enriched with long-chain n-alkanoic acids (ANOVA test, P < 0.001). Elevated values of n-alkanoic acid TAR and n-alkanol TAR are associated with macrophyte and algal states respectively (ANOVA test, P < 0.005). Reduced CPI of short-chain n-alkanoic acids corresponds to macrophyte lakes (ANOVA test, P < 0.02). In particular, ratios of (C26 + C28)/(C22 + C24) n-alkanoic acid and (C26 + C28)/ (C22 + C24) n-alkanol were found to be sensitive indicators of the transition between states, and showing high values in macrophyte and algal lakes respectively (ANOVA test, P < 0.001). For each ratio, the ranges had a small overlap between macrophyte and algal lakes (Figs. 4a and 5a). Ratio variation was not influenced by differences in preservation potential because the long-chain compounds exploited have similar chemical structure and thus exhibit similar variation in sediments. These two ratios thus have great potential as indicators of historical transitions between macrophyte and algal states in shallow lakes. In addition, our results offer evidence that the abundance of long-chain alkyl diols and dinosterol in sediments can reflect past variation in P, while variations in dinosterol/brassicasterol ratios and the abundance of brassicasterol are largely dependent on N.

5. Conclusion

Our study presents a first inventory of FA and alcohol biomarkers in total lipid extracts from the sediments of MLRYZ lakes. The results reveal significant spatial heterogeneity in the abundances and composition of lipid biomarker profiles. A predominance of either algae or submerged macrophytes within lake water columns was shown to exert a significant influence on the abundances of short-chain n-alkanoic acids, long-chain n-alkanoic acids, MUFAs, PUFAcs, cholesterol and long-chain n-alkanols in the sediments, and on values of short-chain n-alkanoic acid CPI, n-alkanoic acid TAR, n-alkanol TAR, (C26 + C28)/ (C22 + C24) n-alkanoic acids and (C26 + C28)/(C22 + C24) n-alkanols present. Variation in the abundances of long-chain alkyl diols, brassicasterol and dinosterol and the ratios of dinosterol/brassicasterol in sediments was shown to be linked to the trophic status of the lakes. We believe that the sediment lipid biomarkers analyzed in this study may provide powerful tools to investigate past changes in lake environment and ecology. In particular, ratios of (C26 + C28)/ (C22 + C24) n-alkanoic acid and (C26 + C28)/(C22 + C24) n-alkanol are shown to be excellent candidates for characterizing the transition between macrophyte and algal lake states, while long-chain alkyl diol abundances and dinosterol/brassicasterol ratios appear highly applicable in the reconstruction of past TP and TN profiles respectively.

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Bourbonniere, R.A., Meyers, P.A., 1996. Sedimentary geolipid records of historical transitions between the two states have attracted considerable scientific interest. The method developed by Ficken et al. (2000), based on the distribution of n-alcanes, was frequently used to assess OM input from aquatic macrophytes versus terrestrial higher plants. The reliability of molecular geochemical method, however, was still poorly known in determining the transition between macrophyte and algal lakes. The results of our study of extractable FA and alcohol biomarkers in sediments have revealed a number of compositional differences between macrophyte and algal lakes. The algal state corresponds with high levels of short-chain n-alkanoic acids, MUFAs, PUFAcs, cholesterol and long-chain n-alkanols (ANOVA test, P < 0.002), while sediments from macrophyte lakes are enriched with long-chain n-alkanoic acids (ANOVA test, P < 0.001). Elevated values of n-alkanoic acid TAR and n-alkanol TAR are associated with macrophyte and algal states respectively (ANOVA test, P < 0.005). Reduced CPI of short-chain n-alkanoic acids corresponds to macrophyte lakes (ANOVA test, P < 0.02). In particular, ratios of (C26 + C28)/(C22 + C24) n-alkanoic acid and (C26 + C28)/ (C22 + C24) n-alkanol were found to be sensitive indicators of the transition between states, and showing high values in macrophyte and algal lakes respectively (ANOVA test, P < 0.001). For each ratio, the ranges had a small overlap between macrophyte and algal lakes (Figs. 4a and 5a). Ratio variation was not influenced by differences in preservation potential because the long-chain compounds exploited have similar chemical structure and thus exhibit similar variation in sediments. These two ratios thus have great potential as indicators of historical transitions between macrophyte and algal states in shallow lakes. In addition, our results offer evidence that the abundance of long-chain alkyl diols and dinosterol in sediments can reflect past variation in P, while variations in dinosterol/brassicasterol ratios and the abundance of brassicasterol are largely dependent on N.


