

Strong influence of the littoral zone on sedimentary lipid biomarkers in a meromictic lake

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ABSTRACT

Planktonic sulfur bacteria growing in zones of photic zone euxinia (PZE) are important primary producers in stratified, sulfur-rich environments. The potential for export and burial of microbial biomass from anoxic photic zones remains relatively understudied, despite being of fundamental importance to interpreting the geologic record of bulk total organic carbon (TOC) and individual lipid biomarkers. Here we report the relative concentrations and carbon isotope ratios of lipid biomarkers from the water column and sediments of meromictic Mahoney Lake. The data show that organic matter in the central basin sediments is indistinguishable from material at the lake shoreline in both its lipid and carbon isotopic compositions. However, this material is not consistent with either the lipid profile or carbon isotope composition of biomass obtained directly from the region of PZE. Due to the strong density stratification and the intensive carbon and sulfur recycling pathways in the water column, there appears to be minimal direct export of the sulfur-oxidizing planktonic community to depth. The results instead suggest that basinal sediments are sourced via the littoral environment, a system that integrates an indigenous shoreline microbial community, the degraded remains of laterally rafted biomass from the PZE community, and detrital remains of terrigenous higher plants. Material from the lake margins appears to travel downslope, traverse the strong density gradient, and become deposited in the deep basin; its final composition may be largely heterotrophic in origin. This suggests an important role for clastic and/or authigenic minerals in aiding the burial of terrigenous and mat-derived organic matter in euxinic systems. Downslope or mineral-aided transport of anoxygenic, photoautotrophic microbial mats may have been a significant sedimentation process in early Earth history.

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INTRODUCTION

To understand the evolution of Earth's redox cycles, it is critical to understand the mechanisms that establish, maintain, and record the geologic history of marine euxinia—including its temporal and spatial scales (Meyer & Kump, 2008; Ozaki & Tajika, 2013), as well as its biomarker record to sediments (Grice *et al.*, 2005; Brocks & Schaeffer, 2008; Meyer *et al.*, 2011; French *et al.*, 2014). The carotenoid okenone and its degradation product, okenane, are biomarkers for euxinic conditions due to their taxonomically specific source from sulfide-oxidizing, phototrophic bacteria [purple sulfur bacteria (PSB)]. This compound is found definitively only in planktonic species of PSB (Brocks & Schaeffer, 2008), although there also is debate about possible microbial mat sources (e.g., Meyer *et al.*, 2011). To examine processes controlling the delivery

of organic matter to sediments in euxinic systems—including okenone and other lipids—we extracted and analyzed biomarkers from stratified Mahoney Lake, British Columbia, Canada. Modern analogues such as meromictic lakes are useful for their compact redox gradients and dense chemocline microbial communities, offering end-member scenarios for the processes operating in sulfidic systems.

Mahoney Lake is a small lake (surface area, 11.5 ha; maximum depth, 14.5 m; Overmann, 1997) with an oligotrophic mixolimnion overlying a permanently stratified monimolimnion that contains extremely high concentrations of sulfate and sulfide (Northcote & Hall, 1983). The chemocline is maintained near 7 m depth throughout the year, where it supports the densest population of PSB (*Chromatiaceae*) ever measured (bacteriochlorophyll *a* > 20 mg l⁻¹; Overmann *et al.*, 1991). The dominant

species in this layer had been believed to be the photoautotroph *Lamprocystis purpurea* (formerly *Amoebobacter purpureus*; Overmann *et al.*, 1991), but companion analyses to this work now show that the major organism is closely related to *Thiobalocapsa* spp., rather than to the genera *Lamprocystis* or *Amoebobacter* (Klepac-Ceraj *et al.*, 2012; Hamilton *et al.*, 2014).

In addition to photoautotrophy, autotrophic sulfide and sulfur oxidation and extensive sulfate reduction complete the sulfur and carbon cycles in the chemocline (Overmann, 1997; Hamilton *et al.*, 2014). Measurements of exported sulfur—which accumulates intracellularly in *Chromatiaceae*—show that <0.2% of the upwelling flux of sulfur (as H₂S and SO₄²⁻) sinks from the chemocline as S⁰ (Overmann *et al.*, 1996a). Instead, it has been proposed that this system loses its organic matter through a combination of *in situ* respiration, plus upwards-vertical and lateral rafting of organic debris (Overmann *et al.*, 1994, 1996b). Lateral rafting brings planktonic material to the shoreline, where it is deposited among a well-developed microbial mat community. The littoral sediments are a mixture of this degraded material, plus organic matter and weathering products from the surrounding landscape, including clastic mineral debris as well as authigenic mineral formations (visible salt crusts; the major lake ions are Mg²⁺, Ca²⁺, Na⁺, SO₄²⁻, and CO₃²⁻; Northcote & Hall, 1983). The shoreline mats also host their own population of PSB, including (but possibly not limited to) the cultured species *Thiorhodovibrio winogradskyi*, which has higher oxygen, salinity, and phototolerance than typical planktonic *Chromatiaceae* spp. (Overmann *et al.*, 1992).

Sedimentary evidence shows that euxinia has predominated in Mahoney Lake for at least 9 Kyr (Lowe *et al.*, 1997; Coolen & Overmann, 1998). Although direct export of the PSB community to sediments is very low, it is commonly believed that much of the organic matter buried in the deep lake sediments ultimately does originate from the biomass-rich chemocline. Evidence includes the presence in sediments of the carotenoid biomarker, okenone (Coolen & Overmann, 1998). However, it remains unclear how (or if) the planktonic PSB layer of Mahoney Lake physically reaches the lake-bottom sediments to be preserved. PSB primary production generally does not enter macrobiotic ecosystems (Fry, 1986); in addition, Mahoney Lake is eukaryote-poor, including being free of fish (Northcote & Halsey, 1969).

Because of these many unusual characteristics, Mahoney Lake has been proposed as a modern analogue for Earth systems that may have been sulfidic into the shallow photic zone (Canfield & Teske, 1996; Brocks *et al.*, 2005; Meyer & Kump, 2008; Lyons & Reinhard, 2009), including environments in which sulfidic photic zones intercept shallow, sulfide-oxidizing mats (Meyer *et al.*, 2011; Voorhies *et al.*, 2012). Here we use lipid distributions and

compound-specific δ¹³C analyses to resolve the sources and processes governing organic matter burial in Mahoney Lake.

METHODS

Samples, lipid extraction

Water samples from 7 m and 8 m depth in Mahoney Lake (49°17'N, 119°35'W), a grab core of the underlying lake sediment (15 m depth), and a sample of shoreline microbial mat were collected in July 2008 and stored on dry ice (−70 °C) as reported previously (Klepac-Ceraj *et al.*, 2012; Hamilton *et al.*, 2014). Upon return to laboratory, water samples were centrifuged in 50-ml Falcon tubes to pellet biomass; all samples subsequently were stored at −80 °C until use. Glassware was combusted (450 °C, 6 h), and all Teflon caps, stopcocks, and syringes were rinsed with methanol (MeOH), dichloromethane (DCM), and hexane. Water was Nanopure[®] grade, and solvents were Burdick & Jackson GC² or equivalent.

Total lipid extracts (TLEs) of each sample were obtained using a modified Bligh and Dyer extraction (Bligh & Dyer, 1959; Nishihara & Koga, 1987). Five grams of shoreline and lake-bottom sediment or 0.24 g of 7 m and 8 m biomass were extracted in 1:2:0.8 chloroform:MeOH:H₂O, where the H₂O contained 0.5% trichloroacetic acid. Each sample was sonicated and vortexed (3 × 5 min) with 5 min on ice (0 °C) between each round. Phase separation was achieved by changing the solvent ratio to 1:1:0.8. The organic layer was removed, and the aqueous layer was extracted again with chloroform. The combined extracts were washed against 1 M NaCl and H₂O, and the resulting TLEs were dried under N₂(g) with Na₂SO₄ added to remove residual water. TLEs (in DCM) were desulfurized in columns containing 2 g Na₂SO₄ plus 10 g HCl-activated copper pellets. Each TLE was dried just to completeness and weighed.

Total lipid extracts were separated into polarity fractions on columns containing 15 ml of silica gel (70–230 mesh). Fractions were eluted with two column volumes of the following (modified from Pearson *et al.*, 2001): hexane (A), 5% ethyl acetate (EtOAc) in hexane (B), 15% EtOAc in hexane (C), 20% EtOAc in hexane (D), 25% EtOAc in hexane (E), 75% EtOAc and 25% MeOH (F), and MeOH (G). Fractions were stored at −20 °C until they were analyzed.

Additional aliquots of 7 m PSB biomass, lake-bottom sediment, and shoreline material were extracted by sonication sequentially with DCM and acetone in the dark to yield lipid extracts suitable for pigment analysis. The samples were acidified by the dropwise addition of 12 N HCl to remove carbonate and quantitatively convert porphyrins to pheophytins to simplify analysis. Water was removed by Na₂SO₄, and the pigment extracts were redissolved in

acetone and filtered through 0.45- μm PTFE syringe filters before analysis. Extracts were stored at 4 °C and analyzed within 1 week of extraction.

Gas chromatography–mass spectrometry

For analysis of sterols and *n*-alcohols, trimethylsilyl (TMS) derivatives were prepared from fractions C, D, and E using equal parts pyridine and bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane. These derivatives were analyzed relative to injections of a quantitative external standard (stigmasterol) on an Agilent (Santa Clara, CA, USA) 6890N gas chromatograph with a 30 m \times 0.25 mm \times 0.25 μm Agilent DB-5MS column coupled to an Agilent 5973 mass spectrometer (GC-MS). The programmable temperature vaporization (PTV) inlet of the instrument was maintained at 112 °C for 0.85 min, then ramped to 320 °C (720 °C min⁻¹), held for 2.35 min, and ramped to 450 °C (720 °C min⁻¹; hold 5 min). Simultaneously, the oven was maintained at 112 °C for 2 min, ramped to 130 °C (20 °C min⁻¹), then to 280 °C (6 °C min⁻¹), then 320 °C (3 °C min⁻¹; hold 25 min). Helium carrier gas flow rate was 1.2 ml min⁻¹, the MS transfer line was held at 300 °C, and the mass spectrometer scanned *m/z* 50–750 at 70 eV.

For analysis of fatty acids derived from intact polar lipids (IPLs), 50% of fractions F and G were transesterified with known $\delta^{13}\text{C}$ MeOH containing 5% HCl (4 h, 70 °C). Fatty acid methyl esters (FAMES) were extracted using 9:1 hexane: DCM and analyzed by GC-MS. The GC-MS programme was identical to the TMS-derivative programme, but with starting temperatures of 65 °C and a carrier gas flow rate of 1.4 ml min⁻¹. External standards of nonadecanoic acid FAME (C_{19:0}-FAME) were used to prepare calibration curves for quantitation; the response factor was linear over the range of reported sample peaks.

All compounds were identified, integrated, and quantified using AMDIS (Stein, 1999). Detection limits (3 \times signal/noise) for FAMES and sterols were 0.36 ng and 2.16 ng, respectively, as calculated from external standards; *n*-alcohols were assumed to have the same response as sterols. This translates into FAME detection limits of ca. 0.1 ng mg⁻¹ TLE for the sediment and 7 m samples, 0.5 ng mg⁻¹ TLE for the 8 m sample, and 24 pg mg⁻¹ TLE for the shoreline sample.

Compound-specific carbon isotope analysis

For analysis of $\delta^{13}\text{C}$ values, FAMES were run on a Thermo Trace Ultra GC (60 m \times 0.32 mm \times 0.25 μm Agilent DB5-MS column) with a GC Isolink combustion interface coupled to a Thermo Delta V isotope ratio mass spectrometer (GC-C-IRMS). The injector was operated in splitless mode at 220 °C. The oven programme was 60 °C, then

10 °C min⁻¹ to 145 °C, 2.7 °C min⁻¹ to 230 °C, 5 °C min⁻¹ to 275 °C, and 7 °C min⁻¹ to 310 °C (5-min hold). Helium carrier gas flow rate was 1.2 ml min⁻¹, and compounds were combusted at 980 °C to CO₂. An alkane (*n*-C₃₂) of known $\delta^{13}\text{C}$ value (<http://geology.indiana.edu/schimmelmann/>) was co-injected as an internal standard, and each chromatogram was bracketed by CO₂ reference gas pulses. Isotope chromatograms were integrated in Isodat 3.0 using default settings. Values of $\delta^{13}\text{C}$ from the CO₂ reference gas were used to correct all compound-specific $\delta^{13}\text{C}$ values for instrumental drift, assuming a linear trend with time. Peaks with amplitudes <0.5 V or >10 V (*m/z* 44) were eliminated due to nonlinear *m/z* 44 responses. Remaining minor, but statistically significant, size-dependent biasing effects on $\delta^{13}\text{C}$ values were removed using a linear model in which dilution-series data from external FAME standards of C_{16:0}, C_{19:0}, and C_{24:0} run on the same day were fitted using least-squares approaches (calculated using the *lm* and *predict* functions in the *R* statistical package). Finally, isotope mass balance was used to correct for the derivative methyl carbon.

Bulk values of $\delta^{13}\text{C}$ were obtained on acidified (10% HCl, 4 h, 60 °C) aliquots of biomass or sediment prepared in tin capsules (Costech 5 \times 9 mm). Measurements were made at the MBL Stable Isotope Lab (<http://dryas.mbl.edu/silab/>) and reported relative to the VPDB standard.

Pigment analysis

Pigment extracts were analyzed on an Agilent model 1290 ultra-high-pressure liquid chromatography with model 6410 triple quadrupole mass spectrometer (UHPLC-QQQ) following the 'A' method of Airs *et al.* (2001) and using 0.5 M ammonium acetate as the ion-pairing reagent A. Solvents B, C, and D were methanol, acetonitrile, and ethyl acetate, respectively, and the gradient proceeded from 5/80/15/0% to 0/20/15/65% A/B/C/D over 95 min with an initial isocratic hold of 5 min. The flow rate was 0.5 ml min⁻¹, and three Kinetex C₁₈ phase (150 \times 4.6 mm, 2.6- μm particles) columns were used in series. Runs were monitored by ultraviolet-visible diode array detector (DAD) and by mass spectrometry with atmospheric pressure chemical ionization (APCI) in positive ion mode. Ionization parameters were chosen to reduce in-source fragmentation of okenone: vaporizer temperature 300 °C, drying gas flow 4 l min⁻¹ at 300 °C, nebulizer pressure 40 psi, fragmentation 135 V, and collision chamber 20 V. Compounds were identified by relative retention times, by their UV-visible spectra, and by their mass spectral fragmentation patterns, including MS² transitions and molecular ion masses, M⁺ (Airs *et al.*, 2001; Romero-Viana *et al.*, 2009). All peak areas were determined by integration of the molecular ion trace in Agilent Masshunter. As we did not have authentic pheophytin or

carotenoid standards, the reported abundances are semi-quantitative.

RESULTS

Fatty acids

Fatty acids from fractions F and G displayed similar patterns of abundance relative to each other within a given sample (Fig. 1A). The higher absolute concentration yield (Table S1) of the F (nominally ‘glycolipid,’ but also including sulfolipids and other less polar IPL head groups; Schubotz *et al.*, 2009; Close *et al.*, 2013) fraction relative to the G (nominally ‘phospholipid’) fraction may be due to partial loss of charged phospholipid compounds during extraction and/or separation on silica gel (Huguet *et al.*, 2010). However, sufficient yield was obtained from all F and G samples for quantitative analysis and for measure-

ment of $\delta^{13}\text{C}$ values, with the exception of the shoreline G sample (Fig. 1A,B).

The samples qualitatively fall into two groups: water column organic matter vs. benthic organic matter. The 7 m PSB sample from the chemocline primarily contains $\text{C}_{18:1}$, lesser amounts of $\text{C}_{16:0}$ and $\text{C}_{16:1}$, and minor amounts of $\text{C}_{14:0}$, $\text{C}_{18:0}$, and $\text{C}_{20:1}$. Both the relative and absolute quantities of other trace fatty acids in this sample are below those of the other three samples, with the exception of $\text{C}_{15:0}$ and $\text{C}_{17:0}$ (Fig. 1A; Table S1). The ratio of total C_{18} chain lengths ($\text{C}_{18:0} + \text{C}_{18:1}$) to C_{16} chain lengths ($\text{C}_{16:0} + \text{C}_{16:1}$) is 1.4 (calculated for fraction F) (Table 1). There are no fatty acids of chain length $>\text{C}_{20}$ that are above detection limits. The 8 m sample represents material from the dark monimolimnion sampled below the chemocline, yet its fatty acid composition is broadly similar to the chemocline: $\text{C}_{18:1}$ is the dominant compound, but with relatively more $\text{C}_{16:0}$ and $\text{C}_{18:0}$. Here, the ratio of C_{18}

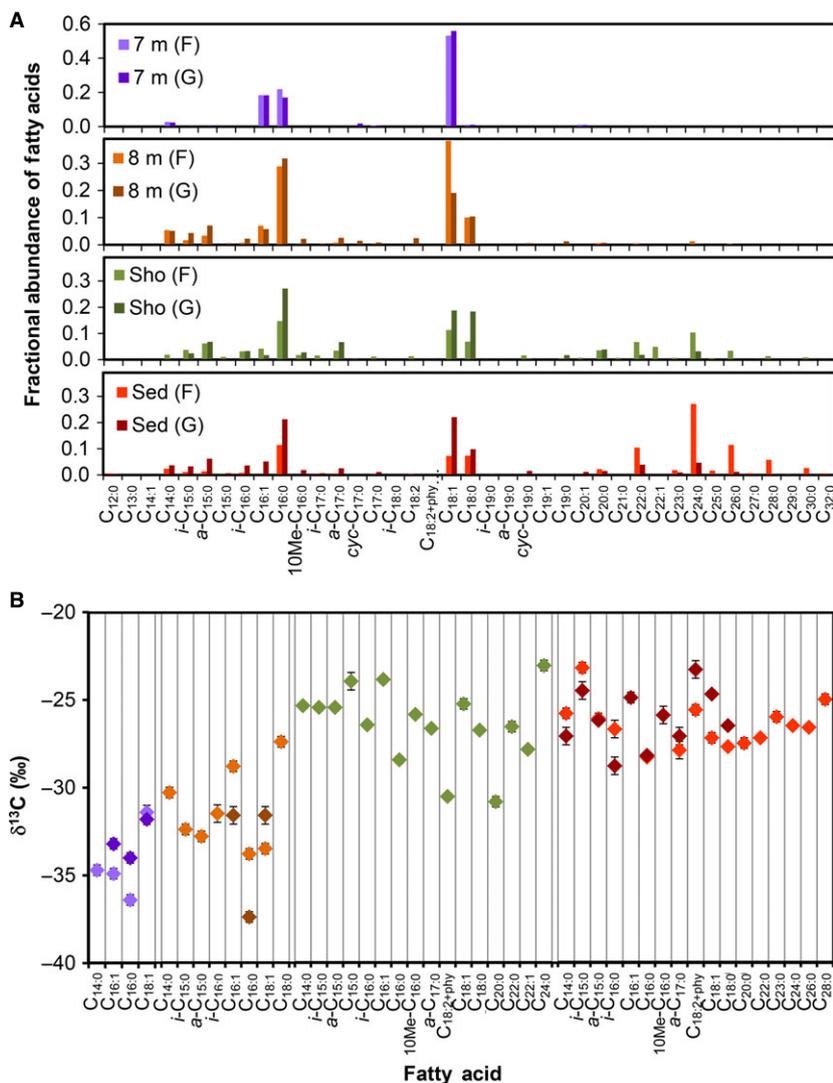


Fig. 1 (A) Relative concentrations of fatty acids in each sample. Fractions F (lighter colors) and G (darker colors) are plotted together. (B) Carbon isotope values of individual fatty acids sorted by sample from fractions F (lighter colors) and G (darker colors). Purple, 7 m; brown, 8 m; green, shoreline; red, basin sediment.

Table 1 Key diagnostic ratios of fatty acids, fraction F (*n/a*, not available)

	7 m	8 m	Sho	Sed
$\Sigma C_{18}/\Sigma C_{16}$	1.4	1.3	0.82	1.2
$\Sigma brC_{15}/C_{16:0}$	0.011	0.18	0.67	0.22
$\Sigma brC_{15}/nC_{15:0}$	0.54	8.9	9.3	8.1
$C_{24:0}/(\Sigma C_{16}+\Sigma C_{18})$	<i>n/a</i>	0.015	0.24	1.0
$\geq C_{20}, \Sigma_{even}/\Sigma_{odd}$	<i>n/a</i>	<i>n/a</i>	16	13

chain lengths ($C_{18:0} + C_{18:1} + C_{18:2}$) to C_{16} chain lengths ($C_{16:0} + C_{16:1} + iso-C_{16:0}$) is 1.3. At 8 m, there also are detectable fatty acids of chain length $>C_{20}$, including minor amounts of $C_{20:0}$, $C_{22:0}$, $C_{24:0}$, and $C_{26:0}$ (maximum at $C_{24:0}$). The ratio of $C_{24:0}$ to the sum of all C_{16} chain lengths, however, is only 0.015, indicating that both the 7 m and 8 m samples primarily consist of C_{16} and C_{18} fatty acids.

The shoreline and sediment samples quantitatively and qualitatively have a greater diversity of fatty acids (Fig. 1A; Fig. S1; Table S1), with both having almost twice as many detectable components as the 7 m and 8 m samples. The sediment sample contains a regular series of longer chain fatty acids with a strong even-over-odd predominance ($\geq C_{20}$ evens:odds = 13; Table 1) and a maximum at $C_{24:0}$. In the sediment, $C_{18:1}$, $C_{18:0}$, and $C_{16:0}$ fatty acids are approximately equally abundant, and the ratio of total C_{18} chain lengths to C_{16} chain lengths is 1.2 (Table 1). The longer chain components dominate this sample, and the ratio of $C_{24:0}$ to the sum of all C_{16} chain lengths is 1.0. The shoreline sample qualitatively resembles the basinal sediment. It contains the regular series of longer chain fatty acids, also with a maximum at $C_{24:0}$. $C_{16:0}$ is the dominant shorter chain compound, although $C_{18:1}$ and $C_{18:0}$ are also major components. The ratio of total C_{18} chain lengths to C_{16} chain lengths is 0.82, and the ratio of $C_{24:0}$ to the sum of C_{16} chain lengths is 0.24.

Specific bacterial fatty acids are widespread in all samples. The lipid 10-methyl- $C_{16:0}$ is characteristic of anoxic sulfur cycling, being a common component of sulfate reducers such as *Desulfobacter* spp. (Taylor & Parkes, 1983). It was detectable in all samples except the 7 m sample, and its relative abundance was highest in the shoreline (Fig. 1A; Table S1). All samples also contained detectable quantities of the bacterial lipids *iso*- and *anteiso*- $C_{15:0}$, and most contained *iso*- and *anteiso*- $C_{17:0}$ as well. These lipids tend to be common in heterotrophic, gram-positive bacteria such as Firmicutes (Kaneda, 1991) and are thought in some cases to be synthesized *de novo* in anoxic systems (Li *et al.*, 2009). These components were fractionally most abundant in the shoreline sample (ratio of branched C_{15} compounds to $C_{16:0}$ of 0.67; Table 1) with lesser amounts in the 8 m and sediment samples (branched C_{15} to $C_{16:0}$ ratios of 0.18 and 0.22, respectively). Polyunsaturated fatty acids were not detected in any sample.

Fatty acid isotopes

Fatty acid $\delta^{13}C$ values (Fig. 1B) also distinguish the shoreline and sediment samples from the 7 m and 8 m samples. Values for individual compounds from the water column are relatively depleted in ^{13}C , ranging from -31.4% to -36.4% at 7 m and from -27.4% to -37.4% at 8 m. These numbers are similar to other measurements of purple sulfur bacterial populations in meromictic lakes (Hartgers *et al.*, 2000). The 7 m sample yielded only four fatty acids above the analytical threshold for GC-C-IRMS analysis. In contrast, more compounds were above the detection limit at 8 m. However, when comparing all water column data, there appear to be no systematic patterns, either inter- or intrasample. For example, the odd-chain and branched lipids plot within the range of the non-branched components, and unsaturated lipids are neither systematically enriched nor depleted in ^{13}C . The mass-weighted average $\delta^{13}C$ value for all fatty acids at 7 m is -33.3% , while at 8 m, it is -32.4% (both for Fraction F; Table 2).

In comparison, fatty acid values of $\delta^{13}C$ from the shoreline and sediment samples are enriched in ^{13}C , with a mass-weighted average of -26.2% for the shoreline and -26.8% for the sediment (range -23.0% to -30.8% for shoreline, and -23.2% to -28.8% for sediment) (Fig. 1B). Among saturated compounds, there is minimal isotopic difference between samples. For example, $C_{16:0}$ measures -28.3% and -28.4% in the sediment and shoreline, respectively (fraction F). The heterogeneity is larger for unsaturated and branched compounds, but again without notable patterns.

Unusually, the absence of isotopic patterns also extends to the long-chain compounds, $C_{22:0}$ to $C_{28:0}$. There is no evidence for a sawtooth pattern of ^{13}C enrichment in even-chain lengths relative to odd-chain lengths as is sometimes observed (Drenzek *et al.*, 2007; Galy *et al.*, 2011). There also is no systematic decrease in overall ^{13}C content of the long-chain compounds relative to the short-chain compounds as has been seen in other settings (Gong & Hollander, 1997; Naraoka & Ishiwatari, 2000; Drenzek *et al.*, 2007). The mean $\delta^{13}C$ values of long-chain ($\geq C_{20}$) and short-chain ($< C_{20}$) fatty acids are identical in the sediment (-26.5%) and very similar in the shoreline (mean

Table 2 Carbon isotope values for TOC and average fatty acids (*n/a*, not available; F, fraction F; G, fraction G)

	TOC		Weighted average fatty acids	
	$\delta^{13}C$ (‰)	Weight % C	$\delta^{13}C$ (‰) (F)	$\delta^{13}C$ (‰) (G)
7 m	-27.2	<i>n/a</i>	-33.3	-32.5
8 m	-27.2	<i>n/a</i>	-32.4	-34.9
Shoreline	-23.6, -24.0	3.4%	-26.2	<i>n/a</i>
Sediment	-24.2, -25.0	20.5%	-26.8	-26.4

long-chain $\delta^{13}\text{C}$ value: -27.0‰ ; mean short-chain $\delta^{13}\text{C}$ value: -26.1‰).

Sterols and fatty alcohols

Both the shoreline and the 7 m sample are low in total sterol content, while sterols are a higher proportion of total mass of recoverable lipids in the 8 m sample and the sediments (Table S2). The sterol distribution in all samples is enriched in C_{29} sterols (50–90% of total) relative to C_{27} sterols (10–37%), with low relative amounts of C_{28} sterols ($\leq 12\%$) (Fig. 2A).

Concentrations of *n*-alcohols are low in all samples (Table S3). The primary fatty alcohol at 7 m is *n*- C_{18} with

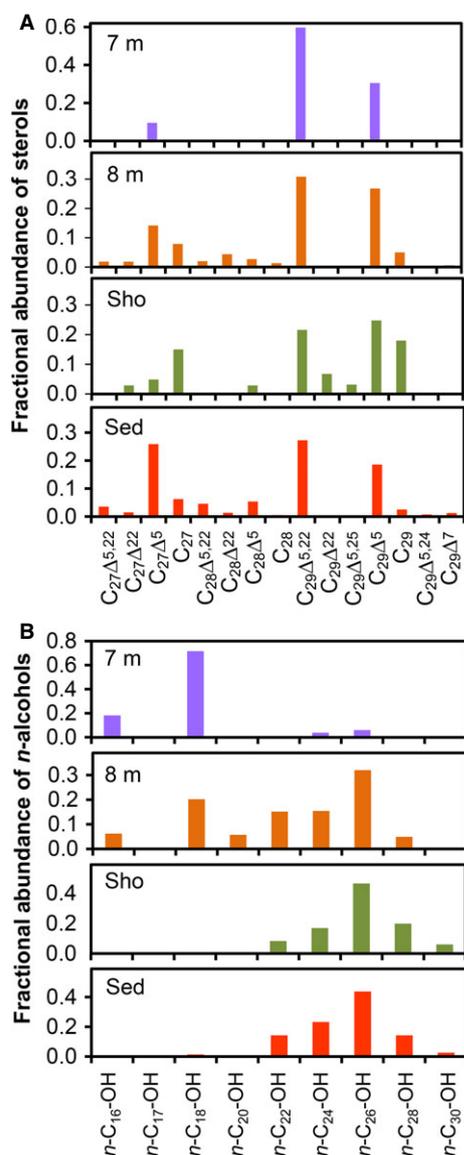


Fig. 2 (A) Relative concentrations of sterols and (B) relative concentrations of *n*-alcohols; colors are as in Fig. 1.

a lesser amount of *n*- C_{16} (Fig. 2B). Elevated levels of C_{18} and C_{16} fatty alcohols are also commonly observed in marine systems (Wakeham, 1982; Mudge & Norris, 1997). The shoreline contains a continuous series of *n*-alcohols with strong even-over-odd predominance, peaking at *n*- C_{26} . The *n*-alcohol profile from the sediment also strongly resembles the shoreline profile. Unlike the fatty acid profiles, the *n*-alcohol fraction from 8 m appears to resemble a mixture between the composition at 7 m and the composition in the shoreline.

Bulk carbon

Bulk organic carbon isotopes approximately track the fatty acid $\delta^{13}\text{C}$ values, with the samples again falling in two groups: ^{13}C -enriched sediments and ^{13}C -poor planktonic material. In the water column, total organic carbon (TOC) values (-27.2‰ for both samples; Table 2) are 6.6‰ and 4.7‰ enriched in ^{13}C relative to average fatty acids at 7 m and 8 m, respectively. The offsets for the shoreline and sediment samples are smaller: shoreline TOC is 2.5‰ enriched in ^{13}C compared to its average fatty acids, while the sediment TOC (average -24.6‰) is 1.7‰ enriched in ^{13}C compared to fatty acids (for Fraction F, Table 2).

Pigments

Bacteriopheophytin *a*, a major degradation product of the PSB pigment bacteriochlorophyll *a*, was found in all samples, so we report the concentrations of all other compounds relative to it (Fig. 3). Additionally, pheophytin *a* and pyrobacteriopheophytin *a*, degradation products of chlorophyll *a* and bacteriochlorophyll *a*, respectively, both were present in the shoreline and sediment, but pyrobacteriopheophytin *a* appeared to be absent in the 7 m PSB layer. Okenone was the most abundant carotenoid in the 7 m sample and in the sediment, but it also had significant

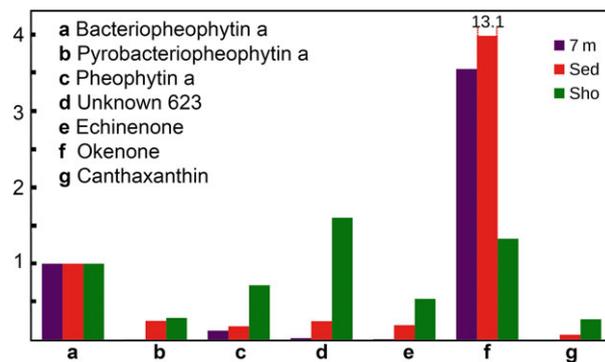


Fig. 3 Relative concentrations of the most abundant pigments for 7 m (purple), shoreline (green), and lake-bottom sediment (red) samples, normalized to bacteriopheophytin *a*.

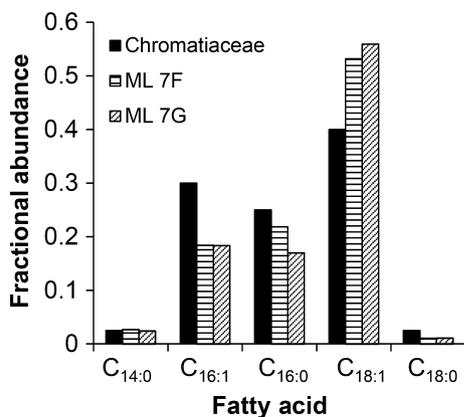


Fig. 4 Comparison between a typical fatty acid profile for *Chromatiaceae* (Imhoff & Bias-Imhoff, 1995) and the Mahoney Lake 7 m and 8 m samples.

abundance in the shoreline. The most abundant carotenoid in the shoreline is an unknown with an $[M+H]^+$ ion of 623.3, a fragment at 591.2, and UV-visible maxima of 490 and 518 nm. Echinenone and canthaxanthin also were detected in the shoreline and sediment, but not in significant amounts at 7 m.

DISCUSSION

Compound distributions

The purple sulfur bacteria, *Chromatiaceae*, typically have fatty acid patterns with ca. 25% C_{16:0}, 30% C_{16:1}, 40% C_{18:1}, and minor amounts of C_{14:0} and C_{18:0} (Imhoff & Bias-Imhoff, 1995). The profile of the 7 m sample would be consistent with almost all of the fatty acids coming from these phototrophs (Fig. 4). Indeed, biomass in the chemocline of Mahoney Lake is reported to be dominated by a single species (Overmann *et al.*, 1991), now classified as *Thiohalocapsa* sp. strain ML 1 (Tank *et al.*, 2009; Hamilton *et al.*, 2014). Cell counts by dilution (Overmann *et al.*, 1991) and metagenomic coverage data (Hamilton *et al.*, 2014) indicate that this species may account for >90% of the standing biomass in the phototrophic layer at 7 m. Based on the similarity of fatty acid profiles, the material at 8 m also may contain sinking biomass of this species, but with a small but significant fraction from other species that supply compounds such as 10-Me-C_{16:0} (*i.e.*, sulfate reducers). The sample from 8 m also contains higher relative abundances of the minor fatty acids, again pointing to additional sources with different endmember composition(s).

Clues to two potential additional sources are found by examining the alcohol fractions. The shoreline and sediment samples have abundant long-chain saturated fatty acids and alcohols, with regular distribution around C_{24:0}

fatty acid and *n*-C₂₆-OH. Such patterns are commonly explained by a strong contribution from terrestrial plants (Cranwell, 1981; Rao *et al.*, 2009). Low levels of C₂₈ sterols and a predominance of C₂₉ over C₂₇ sterols also are typical of terrestrial plant inputs (Huang & Meinschein, 1979; Volkman, 2003). The majority (60%) of the pollen preserved in recent Mahoney Lake sediment is from *Pinus* (Pine), with slightly <10% each of *Pseudotsuga* (Fir), *Alnus* (Alder), and *Poaceae* (grasses) (Cawker, 1983). The surrounding vegetation also is believed to contribute to the high concentration of dissolved organic carbon (DOC) in the lake (Hall & Northcote, 1990; Overmann, 1997).

Thus, the distribution of lipid biomarkers in all four samples would suggest that planktonic material mainly is remineralized in the water column, while the sediments derive primarily from terrestrial organic matter, possibly mixed with the remains of heterotrophic bacteria bearing a higher $\Sigma brC_{15}/C_{16:0}$ signature (Table 1). A primarily terrigenous flux would be consistent with the sulfur budget proposed by Overmann (1997), which suggests that PSB biomass does not sink. However, it fails to explain the high concentration of okenone (Overmann *et al.*, 1993; Coolen & Overmann, 1998) – a compound that must derive from PSB – and it is not completely consistent with the $\delta^{13}C$ patterns in bulk organic matter and individual lipids. As we discuss below, the pattern of ^{13}C distribution in basinal sediments is not clearly planktonic vs. terrigenous. Instead we suggest that heterotrophic reprocessing and a strong shoreline bacterial component affect the ultimate signatures preserved in the system.

Constraints from $\delta^{13}C$ values

The 7 m PSB sample is the least complex and is consistent with a nearly exclusive source from bacterial autotrophy, having average values around -33‰ and -27‰ for individual fatty acids and bulk materials, respectively. *Thiohalocapsa* sp. strain ML1 and other related PSB use Rubisco Type 1A to fix dissolved CO₂ (Badger & Bek, 2008; Hamilton *et al.*, 2014). The chemocline of Mahoney Lake contains ca. 40 mM dissolved inorganic carbon at pH 8.1, suggesting the dissolved CO₂ concentration should not be limited and that typical fractionations for bacterial Type 1A Rubisco ($\epsilon = 20\text{--}24\text{‰}$; Sirevåg *et al.*, 1977; Madigan *et al.*, 1989; Scott *et al.*, 2007) could generate the values observed. Similar or even more negative values have been seen in other contemporary stratified systems (*e.g.*, Velinsky & Fogel, 1999; Hartgers *et al.*, 2000). Our values for fatty acids at this depth are somewhat more ^{13}C -depleted than previous reports for purified okenone and bacteriochlorophyll *a* from the Mahoney Lake water column ($-27.2 \pm 1.9\text{‰}$; $-27.8 \pm 0.7\text{‰}$; Overmann *et al.*, 1996b). It is unclear whether these differences represent temporal change in the system, differences in analytical methods between laboratories, or may be partially

explained by the natural ^{13}C enrichment of ca. 0–3‰ (Schouten *et al.*, 1998; Hayes, 2001) in isoprenoid lipids over acetogenic lipids (Schouten *et al.*, 1998). Regardless, autotrophy appears to dominate the lipids from the water column, but this signature is not carried to the sediments.

Long-chain fatty acids in the sediments would most commonly be assigned a higher plant origin. Here, their $\delta^{13}\text{C}$ values (ca. –26‰) argue against any direct or first-order connection to the 7 m PSB biomass, for example via early-stage heterotrophy by the few known bacteria that produce long-chain lipids. In agreement with this argument, there is only a slight enrichment of ^{13}C observed in the 8 m fatty acids relative to the 7 m fatty acids (average value ca. –32‰ rather than –33‰). This is consistent with minimal heterotrophic reworking of the material descending from 7 m (DeNiro & Epstein, 1978; Hayes, 1993) and is evidence that the sediment fatty acid concentration pattern and $\delta^{13}\text{C}$ values are not the result of alteration of 7 m and 8 m material within the water column.

The *intrasample* pattern of compound-specific $\delta^{13}\text{C}$ values for the sediment also is not fully consistent with overprinting by a dominant, allochthonous C_3 plant contribution. Although the *n*-alcohol and sterol abundance profiles from the sediments are typical of higher plants, we unfortunately could not measure values of $\delta^{13}\text{C}$ for these two compound classes, precluding definitive knowledge of the isotopic composition of the terrigenous endmember. Values of $\delta^{13}\text{C}$ for *Pinus* should represent the majority of this endmember, however, and pine needles and needle litter in the area have values of –27.2‰ and 24.5‰, respectively (Overmann *et al.*, 1996b). The latter value is a possible explanation for the ^{13}C -enriched bulk values observed in shoreline and sediment samples. The total system may be fed by detrital plant matter having a ^{13}C -enriched endmember value typical for moderately arid but still C_3 -dominated environments (Brooks *et al.*, 1998; Diefendorf *et al.*, 2010). However, fatty acids would be derived from the primary pine biomass and should retain the biosynthetic signature, that is, $\delta^{13}\text{C}$ values ca. 3–5‰ lower than the non-degraded needles (Hayes, 1993). This would predict terrigenous fatty acid values ca. –31‰, which is inconsistent with the values measured for long-chain fatty acids in the shoreline and basinal sediment samples.

Instead, there is no statistical difference between the observed $\delta^{13}\text{C}$ values for long-chain and shorter chain fatty acids in the shoreline or sediment samples (< C_{20} chain lengths vs. $\geq \text{C}_{20}$ chain lengths; two-tailed *t*-test). By contrast, in sediments of the Mackenzie Shelf, values of $\delta^{13}\text{C}$ for fatty acids show a stepwise decrease from ca. –26‰ (chain lengths < C_{20}) to ca. –31‰ (chain lengths $\geq \text{C}_{20}$) (Drenzek *et al.*, 2007). A similar pattern also is observed for fatty acids of the nearby Washington Margin (Feng *et al.*, 2013). Such a bimodal distribution, with ^{13}C -

depleted values for long-chain compounds, is interpreted to reflect the influence of C_3 -dominated plant detritus only at longer-chain lengths. This pattern also would be expected for Mahoney Lake sediments if the fatty acids reflected mixed sources. Alternatively, if the lake sediments contained solely terrigenous lipids, then all compounds might be expected to have values ca. –31‰, not near –26‰. The homogeneous values we observe near –26‰ argue that all of the fatty acids have the same source – either from a single type of organism, or from a mixture of species that are all dependent on the same food source – but that this material is not directly from C_3 plants.

As an alternative, bacteria occasionally have been proposed as a source of long-chain fatty acids (Volkman *et al.*, 1988; Gong & Hollander, 1997). The most striking example, both for its similarity to the profiles observed here, as well as for its definitive microbial origin, is the profile of fatty acids extracted from modern ooids (Summons *et al.*, 2013). Like our samples, the ooids have long-chain fatty acids with maxima at $\text{C}_{24:0}$, similar proportions of C_{14} – C_{18} fatty acids, and abundant branched-chain and 10-Me- $\text{C}_{16:0}$ structures. Some bacteria produce long-chain lipids in other contexts (*e.g.*, heterocyst glycolipids of Cyanobacteria; Gambacorta *et al.*, 1998), but only recently has it been suggested that long-chain polyketide synthesis pathways may be widespread among environmental bacteria (Shulze & Allen, 2011). Direct synthesis by species endogenous to microbial mat and/or littoral bacterial communities potentially could explain both the Mahoney Lake and the ooid observations.

The abundant short-chain fatty acids in the shoreline, especially those that are methylated or have cyclopropyl moieties, also indicate a significant *de novo* bacterial contribution to this environment and are a fingerprint for its contribution to sediments (Perry *et al.*, 1979). Specifically, the abundance of bacterial *i*- $\text{C}_{15:0}$ and *a*- $\text{C}_{15:0}$ (Kaneda, 1991) relative to *n*- $\text{C}_{15:0}$ fatty acids (ratios of 9.3 and 8.1 in the shoreline and basinal sediment samples, respectively; Table 1) is even higher than typically reported for bacterially dominated sediments (4–4.5; Parkes & Taylor, 1983). This suggests that despite its proximity to surrounding vegetation, the lipid material in the Mahoney Lake shoreline is dominated by products of the microbial mat community (Overmann *et al.*, 1992). This community may be sustained primarily by rafting PSB biomass, with a secondary contribution from degraded plant biomass. This mixture is then recycled and resynthesized by a complex heterotrophic and autotrophic microbial mat community, yielding *de novo* fatty acids (including long-chain acids) enriched in ^{13}C . Such an argument is consistent with the ooid hypothesis recently offered by Summons *et al.* (2013) and points to a broad importance for bacteria in reprocessing sedimentary organic matter. Similarly, a shoreline-basinal link also is consistent with the detection of

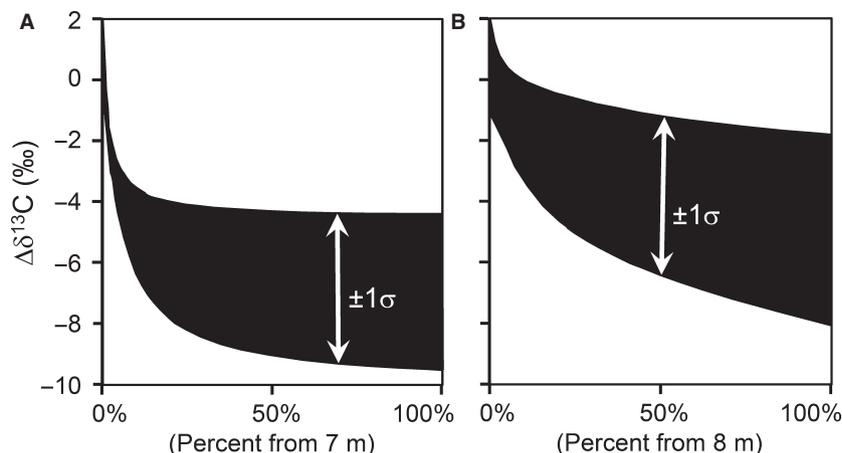


Fig. 5 Results for an isotope mass-balance model that mixes fatty acids from the shoreline with fatty acids from either the 7 meter (A) or the 8 meter (B) samples. A $\Delta\delta^{13}\text{C}$ value of 0‰ indicates best fit of the model to the values of $\delta^{13}\text{C}$ measured for fatty acids from the basal sediments. Both results show the sediment cannot contain more than a few percent of material derived directly from the water column.

pyrobacteriopheophytin *a*, echinenone, canthaxanthin, and the carotenoid Unknown-623 dominantly or exclusively in these two environments, but not in the 7 m sample. Pyrobacteriopheophytin *a* is a degradation product of bacteriochlorophyll *a*, while Unknown-623 is suspected to be a degradation product of okenone.

Mixing model of sources to lake-bottom sediment

The above hypothesis, however, does not resolve the mechanism or quantitative importance of organic export to Mahoney Lake sediments. We constructed an isotope mass-balance mixing model to determine the quantity of unaltered, autochthonous organic material (from 7 m or 8 m) that may be present in the basal sediments while still being concealed by the primarily allochthonous (shoreline-derived) inputs. Our mixing model is modified from Collister *et al.* (1994) and uses fatty acid concentrations and $\delta^{13}\text{C}$ values to determine the fractional contributions of two endmembers to a total mixture. For each individual compound, we computed the predicted value of $\delta^{13}\text{C}$ for the sediment over the full range ($x = 0-1$) of mixing an autochthonous component (x) with shoreline-derived organic matter, and then determined the difference ($\Delta\delta_i$) between this modeled value and the true observation ($\delta_{sed,i}$)

$$\Delta\delta_i = \frac{xM_{auto,i}\delta_{auto,i} + (1-x)M_{shore,i}\delta_{shore,i}}{xM_{auto,i} + (1-x)M_{shore,i}} - \delta_{sed,i} \quad (1)$$

Here i is an individual compound, δ_i is the $\delta^{13}\text{C}$ value of i in a given endmember, and M_i is its relative concentration. The best fit between model and data occurs at $\Delta\delta_i = 0\text{‰}$. The mean of these differences ($\Delta\delta \pm 1\sigma$; averaged over all $\Delta\delta_i$) for the scenarios of mixing the 7 m PSB layer with shoreline material (Fig. 5A) and the 8 m sample with shoreline material (Fig. 5B) show that the basal sediment cannot contain much of either planktonic endmember.

The maximum amount of 7 m material that could be added is 1%, while the maximum amount of 8 m material is 46% (both to satisfy $\Delta\delta^{13}\text{C} = 0 \pm 1\text{‰}$). It is likely that the 8 m endmember already contains a shoreline contribution, however, making the latter number effectively smaller. More importantly, the lack of an unaltered signal of 7 m biomass in the deep sediments suggests that for okenone to ultimately reach the deep basin, it must first be rafted to the shoreline and be heavily reprocessed; the residue is then carried downslope.

The oxic photic zone of Mahoney Lake is oligotrophic and has minimal primary production (small numbers of Cyanobacteria and Chrysophyta; Northcote & Hall, 1983)—thus, we did not have sufficient shallow-water biomass to analyze surface-derived lipids, despite having previously analyzed DNA from 5 m (Klepac-Ceraj *et al.*, 2012). We therefore hypothesized that the 8 m (‘sulfidic zone’) sample qualifies as an integrated, autochthonous lipid endmember (Fig. 5B), based on the assumption that it contains contributions from all of the lake’s autotrophic sources: settling material from the oxic surface, the 7 m PSB layer, and aeolian delivery of terrigenous plant inputs. In support of this assumption, high-throughput metagenomic data show the 8 m sample contains numerous eukaryotic sequences (T. Hamilton, personal communication). As few eukaryotes are tolerant of such high sulfide levels, this is consistent with a contribution of organic matter from sinking algal biomass and/or other plants. In contrast, these signals in the 7 m layer largely are swamped by the dense PSB microbial community.

Our conclusion that the majority of the sediment flux comes from shoreline-derived lateral transport is unexpected, but it is not necessarily incompatible with the literature. Coolen & Overmann (1998) found okenone in the deep basin sediments, but the abundance of preserved *Thiohalocapsa* sp. strain ML 1 DNA was low and was not correlated with okenone concentrations. This supports the rafting and reprocessing hypothesis. But given our data, it

is also possible – although far from demonstrated – that the sediment okenone derives from a PSB community that lives in the shoreline and is different from the 7 m community. This would support a possible mat-derived source for okenone (Meyer *et al.*, 2011) and is contrary to the prevailing hypothesis that okenone is necessarily planktonic (Brocks & Schaeffer, 2008). It is notable that the -27.2% value reported for okenone from basal sediments (Overmann *et al.*, 1996b) is more similar to our shoreline and sediment fatty acids than to fatty acids from the water column. The typical ^{13}C enrichment in isoprenoid relative to acetogenic lipids (ca. $0\text{--}3\%$; Schouten *et al.*, 1998; Hayes, 2001) appears too small to allow the -27% value for okenone to have the same source as the water column fatty acids (ca. -33%); the okenone either has been affected by diagenetic fractionation or originates *de novo* in the shoreline. Unfortunately, our okenone concentration measurements cannot distinguish between these hypotheses definitively. In support of the diagenetic explanation, the only PSB taxon characterized to date from the shoreline does not contain okenone (Overmann *et al.*, 1992), suggesting the okenone in basal sediments should have its ultimate source from the water column. However, in support of the *de novo* okenone hypothesis, a second source of okenone with a higher ^{13}C content would more easily explain the observed 6% offset. A possible means to reconcile all of the observations is to invoke rafting of PSB debris to the shoreline where it is subsequently mixed with terrigenous plant detritus. Intensive bacterial heterotrophy within the microbial mat system would resynthesize all *n*-alkyl lipids (Logan *et al.*, 1995; Close *et al.*, 2011). The 6% shift during the respiration of okenone would still need to be reconciled, as it is too large to be within the normal $1\text{--}3\%$ bounds of heterotrophic processing (DeNiro & Epstein, 1978; Coffin *et al.*, 1990). However, there is still relatively little known about the extent or diversity of ^{13}C fractionation expressed at the molecular scale during heterotrophic processing (Hayes, 1993).

Processes controlling delivery of organic matter to basal sediments

The failure of the PSB layer to sink to the sediments has been noted previously. Buoyant rafting is associated with seasonal die-off, and estimates suggest $>85\%$ of the PSB is lost by this route (Overmann *et al.*, 1994). There also is a relatively small flux of elemental sulfur (S^0) and biomass to sediment traps below the chemocline (Overmann *et al.*, 1996a,b). Nearly all of the remainder of the PSB and associated community are presumed to be degraded *in situ*. The strong density gradient of the chemocline helps retain this microbial organic matter in neutrally buoyant layers where it may be remineralized in the water column, a feature also common in marine systems (MacIntyre *et al.*,

1995; Sorokin, 2002). Temporal and physical oscillations also can occur at redox interfaces, significantly increasing the remineralization of organic matter (Aller, 1998). These cycles occur in Mahoney Lake both diurnally and in association with a small seasonal migration of the chemocline (Hall & Northcote, 1990).

In Mahoney Lake, shoreline-derived organic matter may bypass these intensive remineralization processes due to its association with denser particles. In many marine systems, lateral and downslope transport is a major contributor of organic matter to sediments (Inthorn *et al.*, 2006; Mollenhauer & Eglinton, 2007). Turbidity currents also have been known to contribute to sedimentary deposition in other meromictic lakes, such as Fayetteville Green Lake (Ludlam, 1974), where they are responsible for ca. 50% of deposition. Mahoney Lake is a wider and shallower lake than Fayetteville Green Lake, so initiation and maintenance of such turbidity currents may be more difficult. Other physical transport mechanisms—such as ice rafting of shoreline sediments (e.g., Kempema *et al.*, 2001) and/or aeolian transport in the relatively arid climate of Mahoney Lake – may also play a primary role in transporting dense minerals to the center of the basin. Regardless of the specific physical transport mechanism, association with inorganic phases may help transport buoyant organic matter across salinity gradients and into subchemocline basins.

Downslope transport of anoxygenic photoautotrophic mats may have been a significant sedimentation process earlier in Earth history. Mats of non-photosynthetic sulfide oxidizers such as *Thioploca* cover thousands of square kilometers of the present-day ocean floor where oxygen minimum zones intersect sediments (Otte *et al.*, 1999). In low-oxygen Proterozoic oceans, these sulfide-oxidizing microbial mats may have migrated to shallower depths, intersecting the photic zone and supporting PSB. Such sulfide-oxidizing, phototrophic mats today exist under suboxic conditions of Lake Superior sinkholes (Voorhies *et al.*, 2012) and on the margins of Fayetteville Green Lake (Meyer *et al.*, 2011) where sulfide is produced from pore-water sulfate but is not present in the overlying water column. In similar scenarios in restricted marine basins, the water column may be suboxic, dysoxic, or even oxic, and the traditional biomarkers (okenone, chlorobactene) for photic zone euxinia (PZE) would be derived from benthic mats (e.g., Meyer *et al.*, 2011). Such an interpretation has been proposed for several geologic occurrences of PZE biomarkers (Coolen & Overmann, 2007; French *et al.*, 2014). Much as in Mahoney Lake, these biomarkers might then be laterally transported downslope into areas not overlain by PZE, thereby complicating interpretations of water column sulfide concentrations. This is a significant issue of mat vs. planktonic biomarker geochemistry that remains to be resolved by future studies. Isotope labeling

experiments or metatranscriptomic analysis of carotenoid synthesis genes in PSB mats may provide an answer.

Several episodes of Earth history may have such conflicting records that could be explained by the dominant source of PZE biomarkers being shallow anoxygenic mats overlain by non-euxinic water columns. Rock records of the Permo–Triassic boundary contain isorenieratene and aryl isoprenoids (Grice *et al.*, 2005; Hays *et al.*, 2007), but atmospheric oxygen levels may not have been notably lower than today (Knoll *et al.*, 2007). Similarly, Neoproterozoic marine rocks contain okenane, chlorobactene, and other diagenetic products of anoxygenic photoautotrophs (Brocks *et al.*, 2005), but iron speciation data suggest the ocean as a whole may not have been strongly euxinic (Johnston *et al.*, 2010; Planavsky *et al.*, 2011), pointing toward the importance of local processes. Our results suggest that biomarker interpretations of photic zone euxinia in Earth history should be evaluated in the context of local stratigraphy, lateral transport processes, and the potential for PZE to intersect with mat-bearing sediments.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1 The *m/z* 74 ion chromatograms of GC-MS runs of fractions F from each sample.

Table S1 Concentrations and carbon isotope values of all measured FAMES presented in Fig. 1A,B respectively.

Table S2 Concentrations of sterols presented in Fig. 2A.

Table S3 Concentrations of fatty alcohols presented in Fig. 2B.