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Tracking aquatic change using chlorin-specific carbon and nitrogen isotopes: The last glacial-interglacial transition at Lake Suigetsu, Japan

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[1] Joint carbon and nitrogen isotope measurements were made from chlorins (chlorophyll *a*, phaeophytin *a* and pyrophaeophytin *a*) extracted from the last glacial-interglacial transition sediments of Lake Suigetsu, central Japan. These data highlight both the potential and difficulty of using chlorin-specific isotopes to track aquatic change from lake sediments. $\delta^{13}C$ and $\delta^{15}N$ of the three chlorins show coherent patterns with time, supporting the theory that phaeophytin *a* and pyrophaeophytin *a* are early diagenetic products of chlorophyll *a* and that despite this transition, their isotopic signatures remain intact. However, our data suggest that the isotopic composition of phaeophytin *a* and pyrophaeophytin *a* can be imprecise proxies for the isotope composition of chlorophyll *a*, possibly owing to the complex array of factors which affect the synthesis, transformation and sedimentation of these phaeopigments in nature. The total accumulation of organic matter in Lake Suigetsu appears to be controlled by the balance of allocthonous and authocthonous material as reflected by the C/N ratio. However, both bulk organic and chlorin-specific $\delta^{13}C$ show similar changes, suggesting that the first order variability in bulk organic $\delta^{13}C$ reflects aquatic change. By contrast, there is no similarity between chlorin and bulk $\delta^{15}N$, suggesting that interpretation of bulk $\delta^{15}N$ in this setting is compromised by diagenetic alteration. The isotopic composition of chlorins are interpreted to reflect the response of aquatic primary productivity to post-glacial environmental change. However, further research into the synthesis and transformation of chlorins in the modern environment is required in order to facilitate a more rigorous approach to interpreting isotope ratios in chlorins extracted from sediments.

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1. Introduction

[2] The carbon and nitrogen isotopic compositions of photoautotrophs have long been targets in contemporary and paleo-ecology and biogeochemistry [*Wada and Hattori*, 1978; *Wada*, 1980; *Hayes et al.*, 1987; *Altabet et al.*, 1991; *Altabet and Francois*, 1994]. Sedimentary organic matter $\delta^{13}C$ and $\delta^{15}N$ are valuable biogeochemical tracers, providing information concerning organic matter source, synthesis and taphonomy [*Hayes*, 1993; *Meyers and Lallier-Verges*, 1999; *Ohkouchi et al.*, 2005], they can be used to infer past aquatic primary productivity [*Laws et al.*, 1995] and in some instances, the pCO_2 of past waters/atmospheres [*Freeman and Hayes*, 1992; *Pagani et al.*, 2005]. In contemporary studies, the $\delta^{13}C$ and $\delta^{15}N$ of primary photoautotrophs are used for determination of trophic level within aquatic food webs [*Wada*, 1980; *Fry*, 2006] and $\delta^{15}N$ in particular can be used to trace the passage of pollutants through aquatic communities [*Wolfe et al.*, 2001; *Enders et al.*, 2008]. However, determination of the isotopic composition of photoautotrophs from either contemporary waters or sediments is not trivial. Traditionally, such measurements have been made from bulk suspended or sedimentary organic matter, which is limited by the inherent heterogeneity of organic matter – derived from a mixture of higher plant, algal, bacterial and animal sources – and altered by the variable effects of diagenesis during sedimentation [*Wada*, 1980; *Macko and Estep*, 1984; *Altabet and Francois*, 1994; *Sachs and Repeta*, 1999; *Freudenthal et al.*, 2001; *Lehmann et al.*, 2002]. Compound-specific isotope analysis offers a solution to such issues if those particular compounds can be assigned to specific aquatic organisms or functional groups [e.g., *Hayes et al.*, 1987; *Hayes*, 1990].

[3] A wide range of algal biomarkers are known [*Peters et al.*, 2005], however where photoautotrophic processes are concerned, chlorins - here, broadly defined as chlorophylls and their phaeopigment derivatives [*Sachs and Repeta*, 2000] - are logical target compounds [*Bidigare et al.*, 1991; *Kennicutt et al.*, 1992; *Laws et al.*, 1995; *Qian et al.*, 1996; *Sachs and Repeta*, 1999, 2000; *Chikaraishi et al.*, 2007; *York et al.*, 2007; *Higgins et al.*, 2009, 2010]. Chlorins (Appendix A) are central to photosynthesis and inherently linked to primary production and primary producers [*Sanger*, 1988; *Falkowski and Raven*, 1997; *Nakajima et al.*, 2003]. Furthermore, chlorins degrade rapidly in light and under oxic conditions, which means that chlorins extracted from water or sediments are often believed to derive from synthesis at or near the site of collection, reducing the influence of transport and re-working. It is commonly believed that breakdown of chlorins during senescence and transport heavily biases lake sediments toward aquatic sources, although chlorins are also synthesized by land plants and it is possible that terrestrially derived chlorins also contribute to lake sediments [*Sanger*, 1988; *Leavitt and Hodgson*, 2001]. Finally, chlorins contain four nitrogen atoms to each molecule, offering a rare opportunity for compound-specific nitrogen isotope analysis. To date, the isotopic composition of chlorins, including intact chlorophyll *a* (Chl *a*), has been determined from contemporary waters and cultured algae [*Laws et al.*, 1995; *Qian et al.*, 1996; *Sachs and Repeta*, 1999; *Sachs et al.*, 1999; *Ohkouchi et al.*, 2005; *York et al.*, 2007]. In addition, chlorin isotope composition has been analyzed from late Quaternary marine and lake sediments, however these studies have relied upon phaeopigments [*Sachs and Repeta*, 1999, 2000] or on the amalgamation of several chlorin fractions [*Enders et al.*, 2008; *Higgins et al.*, 2010]. *Chikaraishi et al.* [2007] determined the $\delta^{13}C$ of Chl *a* derived phytol extracted from the sediments of Lake Haruna, Japan, but only from the uppermost 50 cm. Predictably, where ancient sediments are concerned, analyses of diagenetic products of chlorins are more common, such as metalloalkylporphyrins [*Hayes et al.*, 1987; *Boreham et al.*, 1989, 1990; *Chicarelli et al.*, 1993; *Ohkouchi et al.*, 2006; *Kashiyama et al.*, 2007] and maleimides [*Grice et al.*, 1996; *Pancost et al.*, 2002]. To date, there have been no reported measurements of $\delta^{13}C$ and $\delta^{15}N$ from intact Chl *a* extracted from sediments older than ~200 years.

[4] Recent developments in HPLC purification of chlorins [*Sachs and Repeta*, 2000; *Chikaraishi et al.*, 2007] and establishment of sensitive elemental analyzer isotope ratio mass spectrometry

(EA-IRMS) [*Ogawa et al.*, 2010] now make it possible to determine both carbon and nitrogen isotopic compositions from individual chlorin compounds extracted from sediments. Here, we explore this potential by analyzing $\delta^{13}C$ and $\delta^{15}N$ in Chl *a*, phaeophytin *a* (Phe *a*) and pyrophaeophytin *a* (Ppe *a*) extracted from late Quaternary sediments of Lake Suigetsu, central Japan. These sediments span a period of marked climate and environmental change, characterized by a warming of 8–10°C as reconstructed from pollen assemblages preserved in Lake Suigetsu [*Nakagawa et al.*, 2003, 2006]. The pollen data demonstrate a shift in the terrestrial vegetation from a predominantly cool-temperate or boreal coniferous woodland (*Tsuga*, *Picea*, *Pinus*) to a greater proportion of temperate evergreens and conifers (*Cryptomeria*, *Quercus*). Lake ecosystems are sensitive to climate and environmental change [*Battarbee*, 2000] and thus significant changes within the aquatic system would be expected in response to the glacial-interglacial transition at Lake Suigetsu. Therefore, the last glacial-interglacial sediments of Lake Suigetsu present an ideal opportunity to evaluate the potential of using compound-specific analysis of chlorins to track changes in primary productivity across a period of marked climatic change.

2. Materials and Methods

2.1. Site

[5] Lake Suigetsu (35°35′08″ N, 135°52′57″ E, 0 AMSL) is a 34 m deep lake formed in a subduction fault in Fukui prefecture, Japan (Figure 1). Currently, the lake is characterized by eutrophic and saline conditions, owing to intense agriculture in the catchment and seasonal marine incursions due to a drainage canal built during the 17th century A.D [*Matsuyama and Saijo*, 1971; *Matsuyama*, 1973]. The lake is meromictic with an anoxic hypolimnion for much of the year characterized by accumulation of inorganic phosphate, ammonia and hydrogen sulphide [*Matsuyama and Saijo*, 1971; *Matsuyama*, 1973; *Kondo et al.*, 2000, 2006; *Kondo and Butani*, 2007; *Kondo et al.*, 2009]. Based on observations of chlorophyll *a* and abundant bacteriochlorophylls (probably *a*, *c* and *e*), *Takahashi and Ichimura* [1968] concluded that organic matter was produced mainly by purple (*Chromatium* spp.) and green (*Chlorobium* spp.) sulphur bacteria in the halocline [*Takahashi and Ichimura*, 1968; *Okada et al.*, 2007]. Unfortunately, due to the marked human impact on Lake Suigetsu over the past 400 years, it is currently unclear whether such photosynthetic bacteria accounted for the majority of primary productivity prior to human intervention, and this is the subject of ongoing research. However, well preserved annually laminated sediments (varves) do suggest that persistent hypolimnetic and sediment anoxia existed during the past ~70 ka ($\times 10^3$ calendar years before present (1950)) [*Kitagawa and van der Plicht*, 1998a, 1998b, 2000; *Yasuda et al.*, 2004], preventing bioturbation by burrowing animals and leading to exceptional preservation of organic compounds.

2.2. Sediment Coring and Dating

[6] Sediments were retrieved from four boreholes taken at 34 m water depth during a coring expedition between July 3rd and August 11th 2006. A continuous, 73.19 m overlapping sediment sequence was taken which spans the past ~140 ka, the most recent ~70 ka of which consists of varves [*Kitagawa and van der Plicht*, 1998a, 1998b, 2000; *Yasuda et al.*, 2004; *Staff et al.*, 2010].

[7] Radiocarbon analyses of plant macrofossil remains extracted from the Lake Suigetsu sediments are ongoing and are one of the principal objectives of the 'Suigetsu Varves 2006' project (www.suigetsu.org). For the present paper, a preliminary chronology was derived using 10 accelerator mass spectrometer (AMS) radiocarbon dates from the section of core under investigation, as calibrated against the international consensus radiocarbon calibration curve IntCal 09 [*Reimer et al.*, 2009] (Figure 2). Of these 10 measurements, five were performed at the Oxford Radiocarbon Accelerator Unit (ORAU) and five at the NERC Radiocarbon Facility-Environment (NRCF-E), East Kilbride. The preliminary radiocarbon age model suggests linear sediment accumulation throughout the section, with an increase in sediment accumulation rate at ~13 ka (Figure 2). The reasons behind changes in sediment accumulation rate are subject to continued investigation by microsedimentology, inorganic geochemistry and additional radiocarbon measurements.

2.3. Extraction and Analysis of Chlorins

[8] From core SG06, 5–10 cm subsamples (20–40 cm^3) were taken from 20 discrete levels between 7.9 and 20.6 m (composite sediment depth) for chlorin extraction. These 12.7 m represent a time interval of approximately 15,000 years between 21 ka and 6 ka, chosen to include sediments deposited during glacial conditions (~21–17 ka),

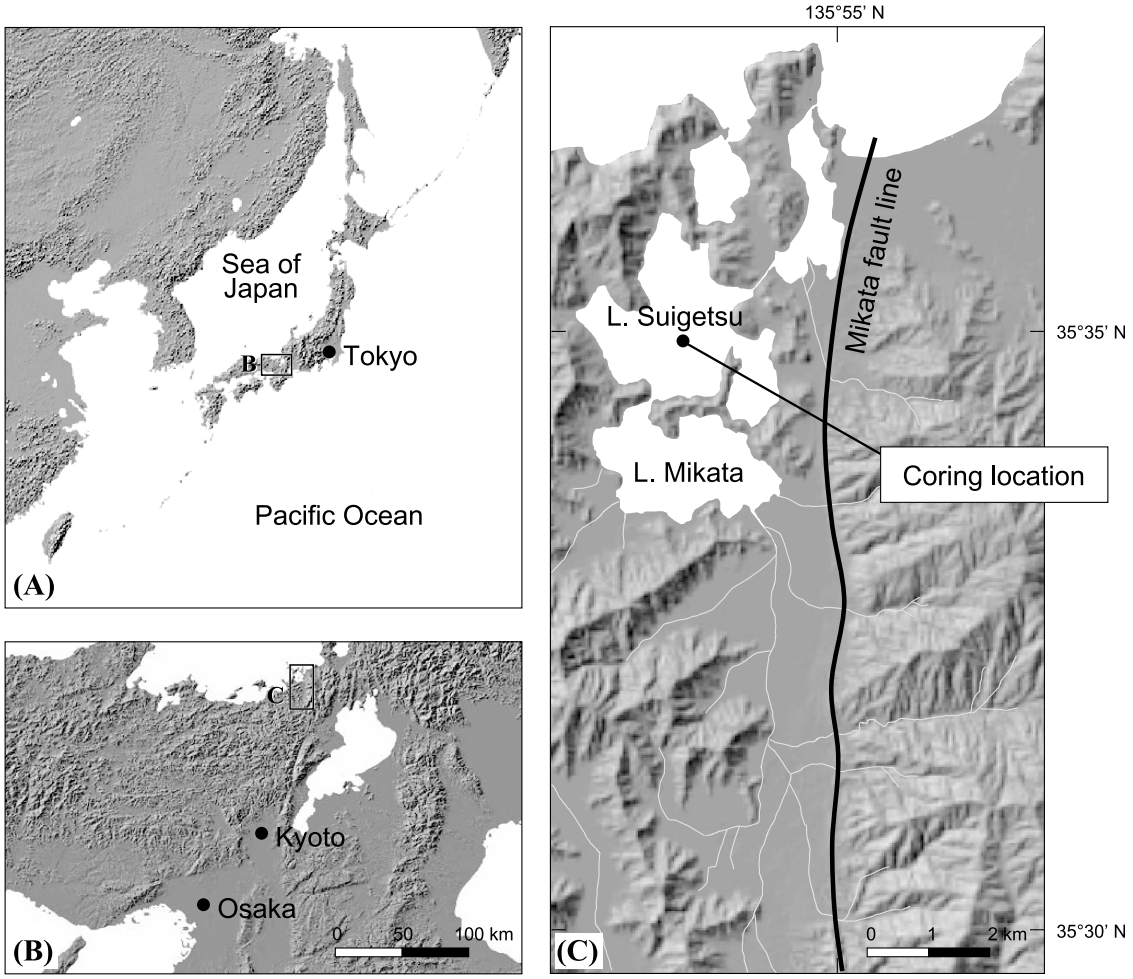

Figure 1. Map of Japan showing location of Lake Suigetsu.

during the transition from glacial to interglacial (~17–10 ka) and during the early interglacial period (~10–6 ka). In order to reduce light induced chlorin degradation, sediments were wrapped in Al foil immediately after subsampling and refrigerated, with care taken to minimize light exposure during subsequent sample handling. Sediment samples were freeze-dried prior to ultrasonic assisted extraction using anhydrous acetone (five times). The extracts were mixed with Milli-Q water and *n*-hexane to a 3:5:1 ratio (acetone:water:hexane, v/v/v). This mixture was then shaken, centrifuged and the hexane decanted to a separate vessel, with the procedure repeated until the hexane remained colorless after centrifugation. The hexane fraction was then concentrated and filtered through Na_2SO_4 and glass fiber to remove water and particulate matter. A volume of 0.2 mL *N*,*N*-dimethylformamide (DMF) was added and stored overnight at −40°C. Hexane and DMF do not mix and chlorins concentrate within the DMF fraction, which was decanted to a separate vial. The resultant DMF fraction was eluted twice through reversed phase HPLC. The first HPLC step entailed a ZORBAX SB-C18 column (4.6 x 250 mm, 5 mm silica particle size) and a methanol versus acetone mobile phase following *Sachs and Repeta* [2000]. Distinctive Chl *a*, Phe *a* and Ppe *a* peaks were identified and quantified by comparison with laboratory standards, based on UV-vis absorbance spectra and elution times (Figure 3). The second HPLC step utilized a ZORBAX Eclipse PAH column (4.6 x 250 mm, 5 mm silica particle size) and an acetonitrile versus ethyl acetate (plus 0.5% pyridine) mobile phase, ramped from 25% ethyl acetate to 50% over 30 min. Prior to the second HPLC injection, the initial Chl *a* fraction, dissolved in acetone, was converted to Phe *a* by adding a drop of 1 M HCl, immediately diluted with Milli-Q water and the chlorin fraction separated to hexane, as above [*Sachs and Repeta*, 2000]. Purified chlorin fractions were dried under a N_2 stream and stored at −40°C. Prior to isotope analy-

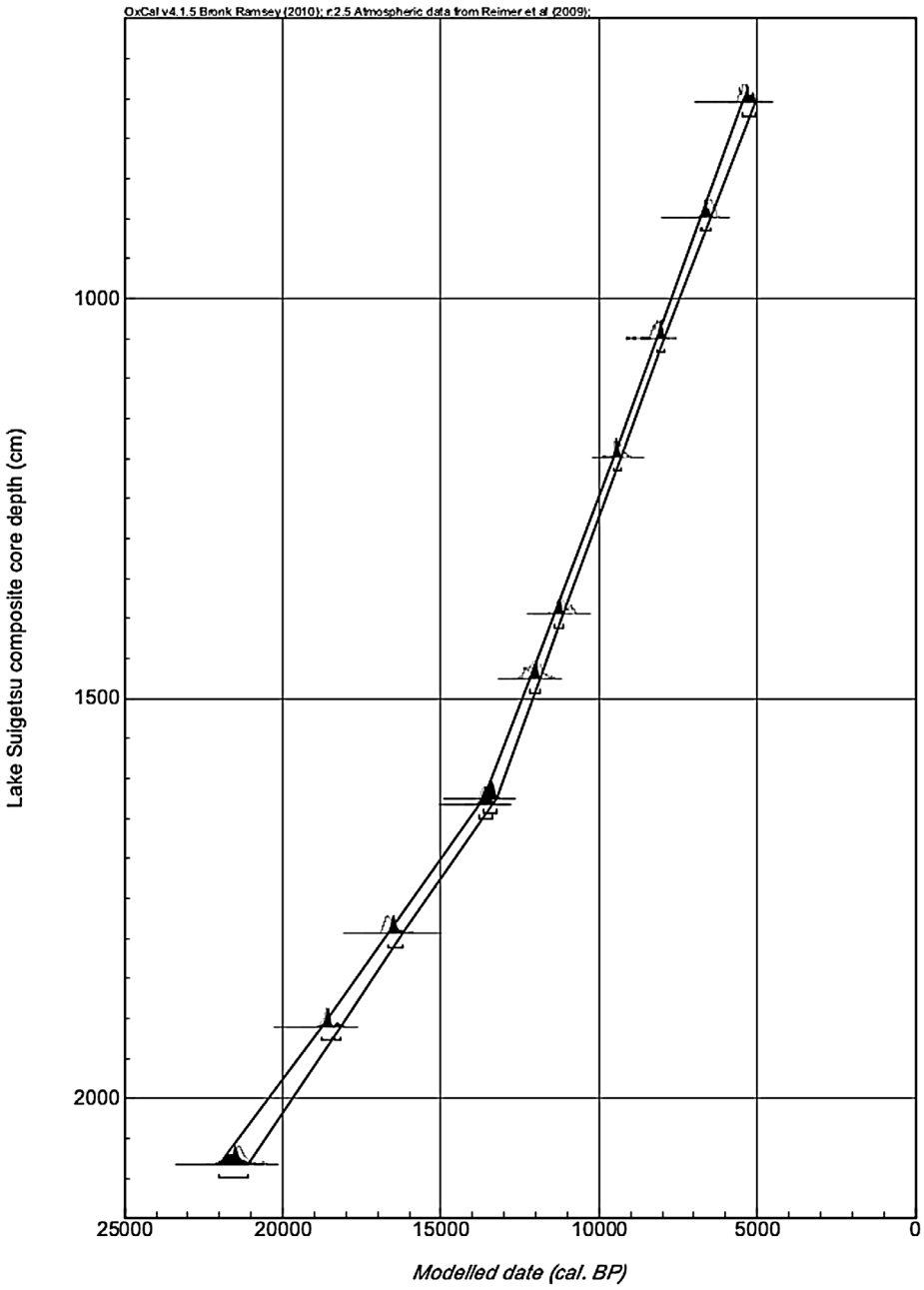

Figure 2. Age-depth model (at 2 sigma uncertainty range) based on 10 preliminary AMS radiocarbon dates plotting Lake Suigetsu composite core depth (ver. 24 August 2009) (cm) against modeled age (cal B.P.). A 'P-sequence' deposition model has been applied [*Bronk Ramsey*, 2008] using the calibration software OxCal (ver. 4.1) [*Bronk Ramsey*, 2009] and the IntCal 2009 [*Reimer et al.*, 2009] calibration curve.

sis, the samples were dissolved in dichloromethane (DCM) and transferred to pre-cleaned Sn cups for analysis. The Sn cups were pre-cleaned by soaking in 1:1 MeOH/DCM solution overnight, before rinsing in clean MeOH/DCM. Carbon and nitrogen concentrations and stable isotope ratios were measured by a Flash EA 1112 Automatic Elemental Analyzer coupled via a ConFlo III interface to a Delta Plus XP isotope-ratio mass spectrometer [*Ohkouchi et al.*, 2005; *Ogawa et al.*, 2010]. Samples were measured alongside internal standards (tyrosine, alanine and Ni-chelated octaethylporphyrin) calibrated against the international standards VPDB for C and AIR for N. In this paper we refer to $\delta^{13}C$ and $\delta^{15}N$, whereby $\delta_{Samp} = (R_{Samp} - R_{Std})/R_{Std}$ x 1000, R_{Samp} is the ratio of two isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) within a given sample and R_{Std} is the ratio within the standard. The analytical errors of our laboratory

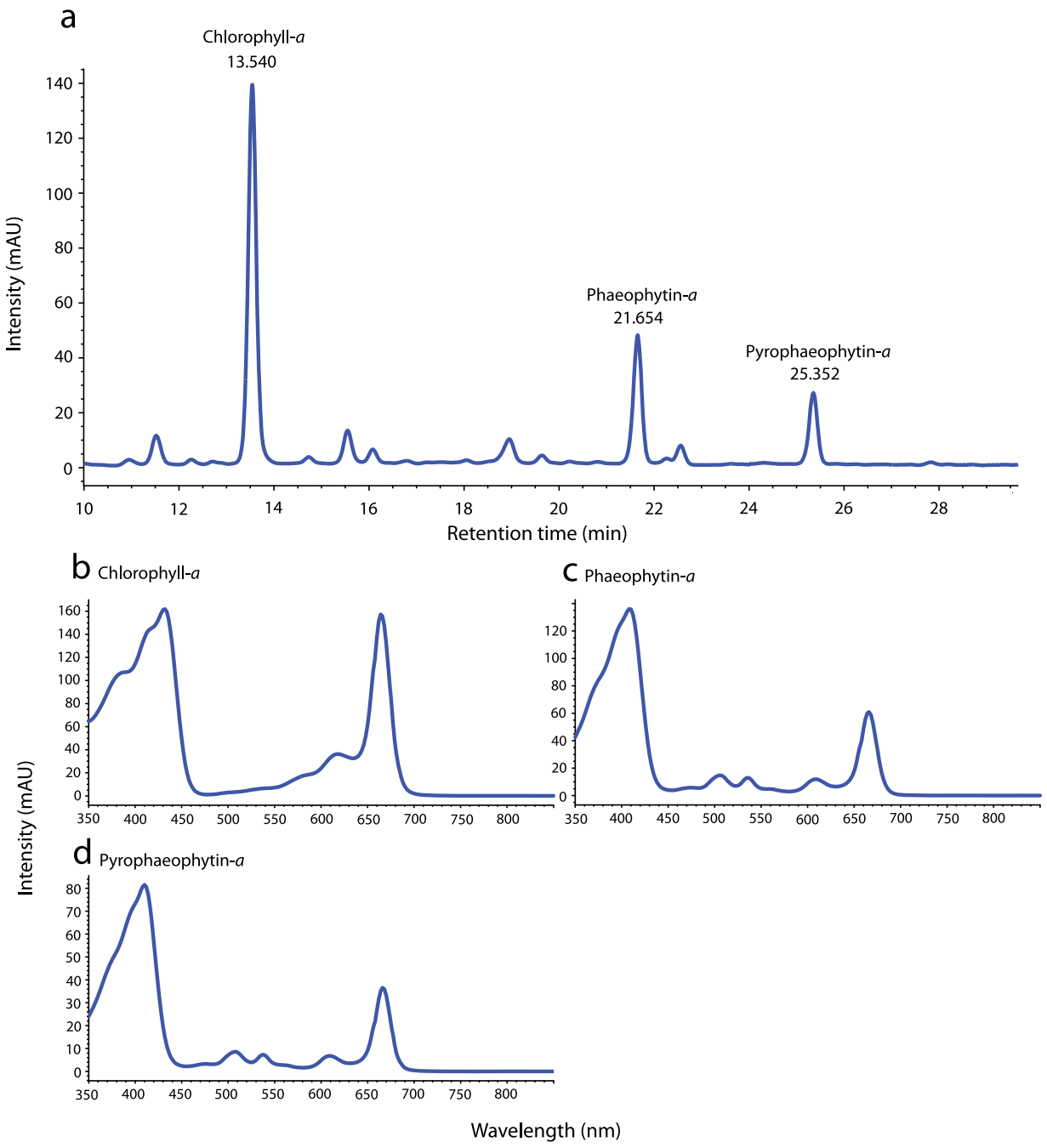

Figure 3. (a) Typical HPLC chromatogram for 660 nm and (b–d) UV-VIS spectra of chlorins within Lake Suigetsu sediment (sample 793.2–798.2 cm composite depth).

standards (Ni octaethylporphyrin and tyrosine) are 0.4‰ for nitrogen and 0.2‰ for carbon. The purity of chlorin samples analyzed by EA-IRMS can be partly verified by checking elemental C/N ratios and comparing with the known composition of the chlorins analyzed. Theoretically, the C/N weight ratios of Chl *a*, Phe *a* and Ppe *a* are 11.8, 11.8 and Ppe *a* 11.4, respectively.

2.4. Bulk Organic Matter Analyses

[9] 90 volumetric subsamples 9 mm in diameter were taken at ~15 cm intervals throughout the section analyzed. Sediments were prepared for EA-IRMS by drying, powdering and acidification (20% HCl) within Sn cups to remove carbonate traces. C and N concentrations, $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were measured using a Fisons NA 1500 CHN ana-

lyzer online to a Finnigan-MAT Delta Plus isotope ratio mass spectrometer at the Center for Advanced Marine Core Research, Kochi University. C and N concentrations were calibrated against a sulphanilamide standard and isotope ratios were calibrated against histidine, in addition to an in-house standard (marine sediment). C and N concentrations have a reproducibility of ±0.5%, $\delta^{13}C$ and $\delta^{15}N$ of ±0.3‰.

3. Interpreting Isotopes in Chlorins

[10] The Chl *a* molecule consists of two components: the chlorophyllide and the phytol chain. The chlorophyllide, which contains all four nitrogen atoms within Chl *a*, originates entirely from the condensation of eight 5-aminolevulinate molecules which in turn are derived solely from glutamate [*Beale*, 1995]. Thus, the $\delta^{15}N$ of chlorophyll *a* ($\delta^{15}N_{Chla}$) is also inherited from glutamate. Phytol is synthesized from isopentenyl diphosphate (IPP) [*Kleinig*, 1989; *Rohmer*, 1993] thus the $\delta^{13}C$ of chlorophyll *a* ($\delta^{13}C_{Chla}$) reflects the mass balance of both glutamate (chlorophyllide) and IPP (phytol) carbon [*Hayes*, 1993]. Accurate interpretation of the isotope composition of chlorins should therefore invoke consideration of the biochemical response of amino acid synthesis in response to environmental change [*Ohkouchi et al.*, 2008]. However, to date such synthetic pathways and their isotopic fingerprint are poorly understood. Instead, the isotopic composition of chlorins have been reported relative to total cellular organic matter, and empirical transfer functions have been derived in order to relate changes in the $\delta^{13}C$ and $\delta^{15}N$ of chlorins ($\delta^{13}C_{Chlorin}$ and $\delta^{15}N_{Chlorin}$) back to the cell [*Laws et al.*, 1995; *Sachs et al.*, 1999; *Beaumont et al.*, 2000].

[11] The empirical relationship between $\delta^{13}C_{Chla}$ and cellular $\delta^{13}C$ ($\delta^{13}C_{cell}$) has been examined in detail [*Laws et al.*, 1995; *Sachs et al.*, 1999], however a full understanding of the discrimination of carbon isotopes between chlorophyll and the cell remains elusive [*Sachs et al.*, 1999]. Whereas *Laws et al.* [1995] observed a constant +2.74‰ offset between $\delta^{13}C_{Chla}$ and $\delta^{13}C_{cell}$ ($r^2 = 0.91$, n = 19) in a compilation of higher plant data including two phytoplankton samples, subsequent research using 7 marine phytoplankton species indicated a smaller +0.32 ± 1.61‰ (n = 12) offset [*Sachs et al.*, 1999]. There is no evidence for a significant inter-species difference in $\delta^{13}C_{Chla} - \delta^{13}C_{cell}$, however marked intraspecies variability was attributed to a negative correlation between $\delta^{13}C_{Chla} - \delta^{13}C_{cell}$ and phytoplankton growth rate ($r^2 = 0.60$, n = 6) [*Sachs et al.*, 1999]. This phenomenon is interpreted to reflect changes in the source of carbon to the tricarboxylic acid (TCA) cycle, which alternates between ^{13}C-enriched oxaloacetic acid [*O'Leary et al.*, 1981] during rapid cell growth, and ^{13}C-depleted acetyl-Coenzyme A [*DeNiro and Epstein*, 1977] when cell growth is slow [*Sachs et al.*, 1999].

[12] Previous research into nitrogen isotope discrimination between cellular tissue and Chl *a* suggest a correlation between $\delta^{15}N_{Chla}$ and cellular $\delta^{15}N$ ($\delta^{15}N_{cell}$) within phytoplankton [*Sachs et al.*, 1999], higher plants [*Kennicutt et al.*, 1992; *Chikaraishi et al.*, 2005] and photosynthetic bacteria [*Beaumont et al.*, 2000]. *Sachs et al.* [1999] reported that the $\delta^{15}N_{Chla}$ of marine phytoplankton is 5.1 ± 1.8‰ (n = 15) depleted relative to the cell. With the addition of data collected by Goericke and Montoya (reported by *Sachs et al.* [1999]), an offset of 4.8 ± 1.5‰ (n = 20) can be calculated [*Ohkouchi et al.*, 2006], whereas *Beaumont et al.* [2000] reported a depletion of 8.7 ± 1.6‰ in the $\delta^{15}N$ of bacteriochlorophyll *a* extracted from purple nonsulphur bacteria. In previous studies of $\delta^{15}N_{Chlorin}$, *Ohkouchi et al.* [2005] used the mean of the two published offsets (5.1‰ and 8.4‰) to estimate $\delta^{15}N_{cell}$ of lacustrine photautotrophic bacteria, whereas *Enders et al.* [2008] and *Higgins et al.* [2010] used a 5.1‰ offset for freshwater and marine phytoplankton. However, while a ~5‰ offset would appear to be a robust mean estimate for algal species, *Sachs et al.* [1999] observed an inter-species range of 2.5‰ within phytoplankton cultures. Thus it is possible that changes in contribution from different species may also affect $\delta^{15}N_{Chlorin}$.

4. Results

[13] Figure 3 illustrates a typical HPLC chromatogram for Lake Suigetsu sediments. The most abundant chlorins in all samples were identified as Chl *a*, Phe *a* and Ppe *a* (Figure 3), the concentration of which decrease with increasing depth down-core (Figure 4c). Phe *a* is most abundant within the majority of samples, however Chl *a* is also found in high concentrations within the younger sediments (8–6 ka, 13–15 $\mu g\ g^{-1}$) whereas in the older sediments (21–15 ka) Chl *a* concentrations are low (0.05–0.5 $\mu g\ g^{-1}$) and Phe *a* and Ppe *a* are most abundant (Figure 4c).

[14] The carbon and nitrogen isotopic compositions of purified chlorins are given in Table 1. In a number of cases, analysis of isotopes in all three compounds was not possible due to issues related to progressive

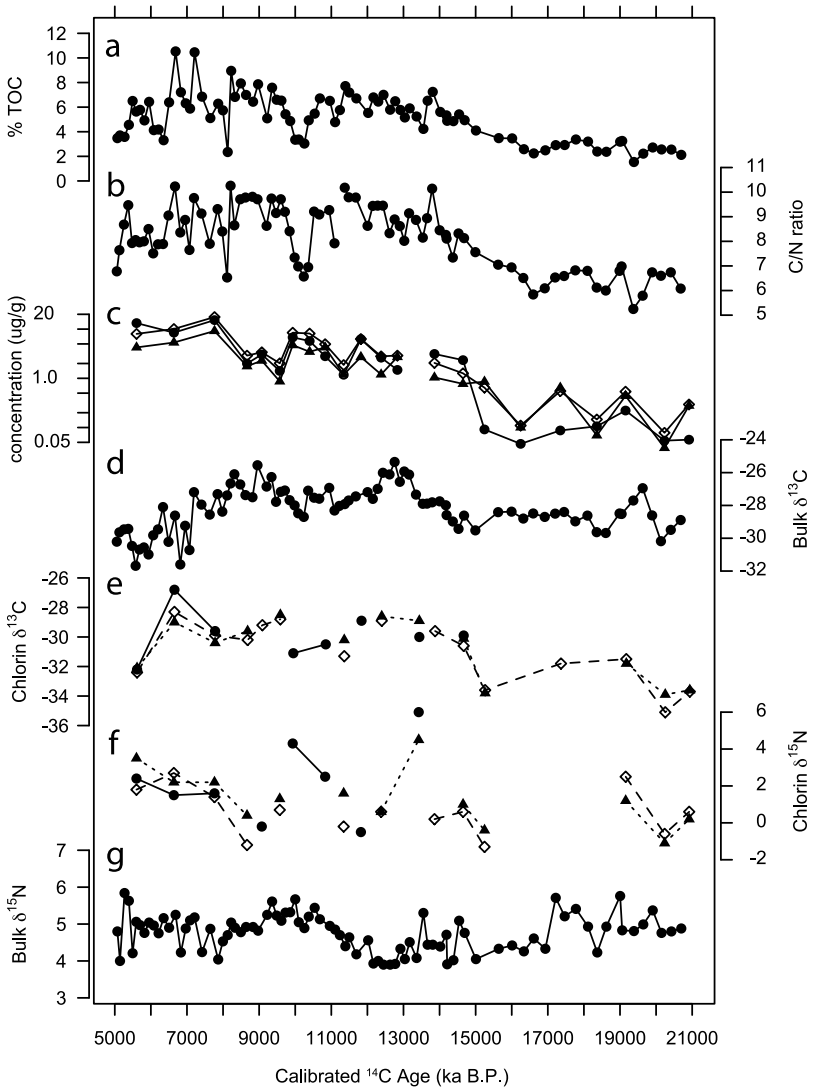

Figure 4. Comparison between (a) bulk organic matter % total organic matter content (%TOC), (b) bulk organic C/N ratio, (c) chlorin concentration (μg per g of sediment) plotted against a logarithmic scale, (d) bulk organic matter δ^{13}C, chlorin-specific (e) δ^{13}C and (f) δ^{15}N, and (g) bulk organic matter δ^{15}N versus time (calibrated ^{14}C years before present (AD 1950)). For chlorin data, filled circles and solid lines indicate chlorophyll *a*, open diamonds and dashed lines indicate phaeophytin *a*, and filled triangles and dotted lines indicate pyrophaeophytin *a*.

method development during the project, coupled with low initial Chl *a* concentration in some samples (particularly samples between 1650 and 2060 cm depth). However, where possible the isotopic composition of the three chlorins are compared in Figures 5 and 6. In most cases, the number of data points are too few to undertake a reliable regression exercise, however the limited data do facilitate some preliminary observations. While the four available $\delta^{13}C_{Chla}$ and Phe *a* δ^{13}C ($\delta^{13}C_{Phea}$) data points appear to correlate (Figure 5a), there is no significant correlation between $\delta^{13}C_{Chla}$ and Ppe *a* δ^{13}C ($\delta^{13}C_{Ppea}$) (Figure 5b) and a significant correlation between $\delta^{13}C_{Phea}$ and $\delta^{13}C_{Ppea}$ (Figure 5c). When plotted together, δ^{13}C values for all three chlorins appear to correlate well and the majority of samples fall about a near unity slope. There is no significant correlation between either $\delta^{15}N_{Chla}$ and Phe *a* δ^{15}N ($\delta^{15}N_{Phea}$) or between $\delta^{15}N_{Chla}$ and Ppe *a* δ^{15}N ($\delta^{15}N_{Ppea}$). However, $\delta^{15}N_{Phea}$ and $\delta^{15}N_{Ppea}$ do correlate ($r^2 = 0.49$; Figure 6c) and again, when all data are plotted together, the correlation appears more robust about a near-unity slope, albeit with a degree of scatter (Figure 6d).

[15] The majority of chlorin C/N ratios are higher than would be expected based on their molecular structure (Table 1 and Appendix A). This suggests

Table 1. Carbon and Nitrogen Isotope Compositions; C/N Weight Ratios; and Masses of Chl *a*, Phe *a*, and Ppe *a* Samples[a]

Depth: Upper (cm)	Depth: Lower (cm)	Mean Depth (cm)	Mean Age (cal. ka B.P.)	Chlorophyll-a				Phaeophytin-a				Pyrophaeophytin-a			
				$d^{15}N$	$d^{13}C$	Mass (μg)	C/N	$d^{15}N$	$d^{13}C$	Mass (μg)	C/N	$d^{15}N$	$d^{13}C$	Mass (μg)	C/N
793.20	798.20	795.70	5.62	2.4	−32.2	5.0	12.0	1.8	−32.4	3.1	12.0	3.5	−32.1	5.2	14.6
909.50	914.50	912.50	6.65	1.5	−26.8	6.9	12.9	2.7	−28.3	4.0	12.2	2.2	−29.0	6.8	17.0
1009.50	1014.50	1012.50	7.78	1.6	−29.6	4.8	19.0	1.4	−29.9	4.6	12.4	2.2	−30.4	6.8	17.2
1109.60	1114.60	1112.10	8.68					−1.2	−30.2	4.0	16.4	0.4	−29.6	2.2	16.0
1149.60	1159.60	1154.60	9.09	−0.2		2.9	12.6		−29.2	3.0	12.4				
1209.51	1214.34	1211.93	9.59					0.7	−28.8	3.0	15.1	1.3	−28.5	3.9	15.0
1248.78	1258.57	1253.68	9.95	4.3	−31.1	5.5	12.6				14.0				
1351.90	1361.90	1356.90	10.85	2.5	−30.5	8.0	13.1								
1409.90	1414.90	1412.40	11.36					−0.2	−31.3	3.6	13.7	1.6	−30.2	2.9	14.6
1452.23	1462.05	1457.14	11.84	−0.5	−28.9	5.2	12.4								
1510.54	1515.09	1512.82	12.40					0.6	−28.9	3.2	13.6	0.6	−28.6	2.5	14.2
1610.10	1615.10	1612.60	13.44	6.0	−30.0	3.4	14.0					4.5	−28.9	3.3	14.5
1650.29	1659.96	1655.13	13.88					0.2	−29.6	4.1	15.1				
1715.32	1719.93	1717.63	14.67		−29.9	2.7	13.1	0.6	−30.6	3.9	16.1	1.0	−30.1	4.2	12.8
1750.00	1760.00	1755.00	15.26					−1.3	−33.6	7.7	14.2	−0.4	−33.8	3.8	13.3
1850.00	1858.50	1854.25	17.37						−31.8	2.8	12.0				
1950.00	1960.00	1955.00	19.17					2.5	−31.5	3.8	13.2	1.2	−31.8	3.3	12.6
2015.00	2020.00	2017.50	20.25					−0.6	−35.1	2.7	15.3	−1.1	−33.9	2.2	13.0
2050.39	2060.67	2055.53	20.93					0.6	−33.7	3.4	14.4	0.2	−33.6	3.7	13.2

[a]Sample depths represent composite depth below sediment-water interface, calculated by cross-calibration of each individual core via marker varve and other stratigraphic horizons (e.g., tephra layers, turbidites). Sample ages are preliminary AMS radiocarbon dates calibrated against IntCal 2009 [*Reimer et al.*, 2009].

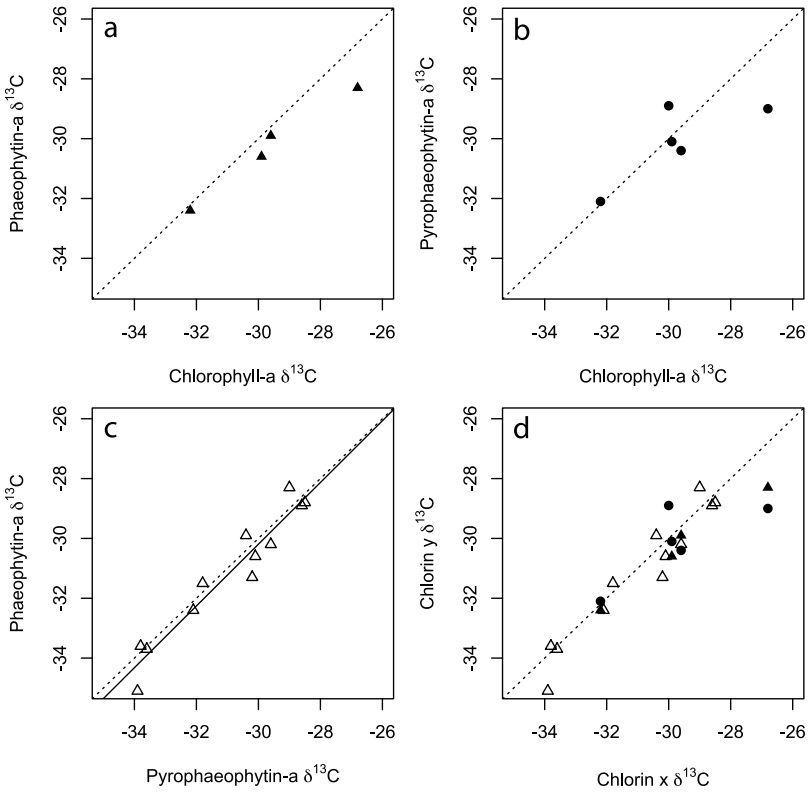

Figure 5. Plots comparing $\delta^{13}C$ between chlorins: (a) $\delta^{13}C_{Chla}$ versus $\delta^{13}C_{Phea}$ (filled triangles); (b) $\delta^{13}C_{Chla}$ versus $\delta^{13}C_{Ppea}$ (filled circles); (c) $\delta^{13}C_{Phea}$ versus $\delta^{13}C_{Ppea}$ (open triangles); and (d) all data combined, where *x* axis = $\delta^{13}C_{Chla}$ and $\delta^{13}C_{Ppea}$ and *y* axis = $\delta^{13}C_{Phea}$ and $\delta^{13}C_{Ppea}$. Dotted lines indicate 1:1, and the solid line in Figure 5c is the regression line ($p = 5.4 \times 10^{-7}$; $y = 1.03 + 0.76$; $r^2 = 0.93$; $n = 12$).

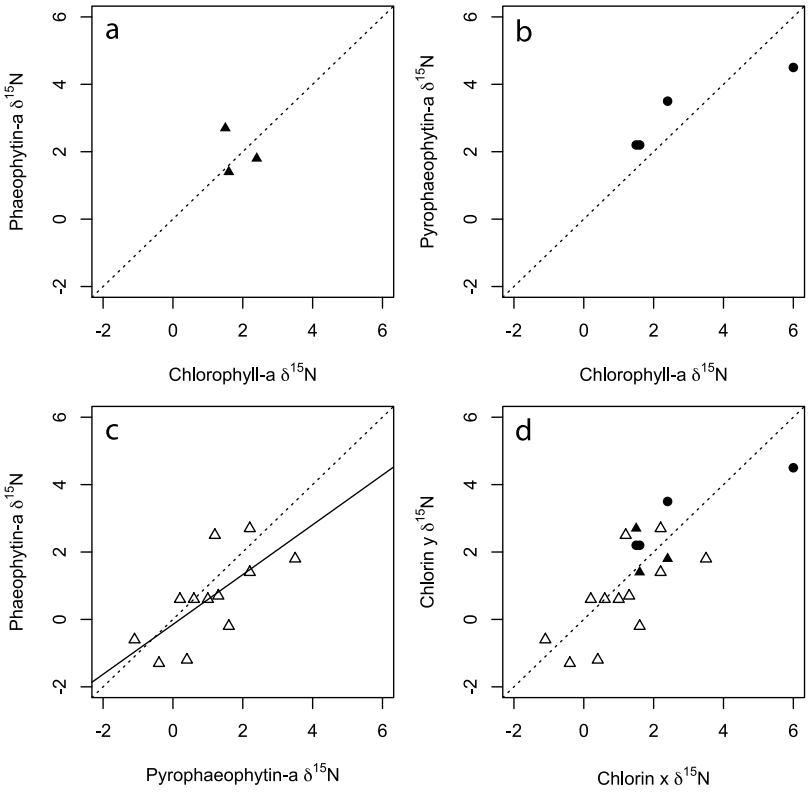

Figure 6. Plots comparing $\delta^{15}N$ between chlorins: (a) $\delta^{15}N_{Chla}$ versus $\delta^{15}N_{Phea}$ (filled triangles); (b) $\delta^{15}N_{Chla}$ versus $\delta^{15}N_{Ppea}$ (filled circles); (c) $\delta^{15}N_{Phea}$ versus $\delta^{15}N_{Ppea}$ (open triangles); and (d) all data combined, where x axis = $\delta^{15}N_{Chla}$ and $\delta^{15}N_{Ppea}$ and y axis = $\delta^{15}N_{Phea}$ and $\delta^{15}N_{Ppea}$. Dotted lines indicate 1:1, and the solid line indicates the regression line (where p = 0.011; y = 0.74x − 0.15; r^2 = 0.49; n = 12).

that some of the isotope ratios reported here were subject to an influence of contaminating carbon, which may be responsible for some of the scatter observed in $\delta^{13}C$ (Figure 5) but which less likely influences $\delta^{15}N$. The most probable source of contaminating carbon is co-elution with unknown carotenoids, which in part share the synthetic pathway of phytol and thus chlorophyll. Therefore, the isotopic composition of contaminating carotenoid would not be expected to differ markedly from Chl *a*. However, careful inspection of UV/vis absorbance spectra (Figure 3) does not indicate any clear evidence of carotenoid contamination, which would manifest as broad peaks within the 450–500 nm wavelength. Other potential contaminants are lipid, whose significance as contaminants is unknown. However, the extraction procedure is designed to remove lipids, and potential outliers in Figures 5 and 6 do not correspond with samples with higher C/N ratios suggesting that contamination effects were not responsible for these values.

[16] Coherent $\delta^{13}C$ and $\delta^{15}N$ patterns are apparent between the different chlorins when viewed in a temporal context (Figures 4e and 4f). This is particularly evident in $\delta^{13}C$ throughout the record (Figure 4e), and concurrent trends in $\delta^{15}N$ can be observed between 21 and 19 ka and 10–6 ka (Figure 4f). Values of $\delta^{13}C_{Chlorin}$ fall within a range between −36 and −27‰ (Figure 4e). Low $\delta^{13}C_{Chlorin}$ values occur at 21–20 ka, 15 ka, 12–10 ka, 9–8 ka and notably at ~5.5 ka. Maxima occur between 19 and 16 ka, between 15 and 12 ka, at 10 ka and at ~7 ka (Figure 4e). Values of $\delta^{15}N_{Chlorin}$ fall within a range from −1.5 to +6.0‰, with minima occurring on three occasions (at ~20, ~15, and ~8 ka), in addition to lows of between −1 and +1‰ at ~12 ka and between 10 and 9 ka (Figure 4f). High $\delta^{15}N_{Chlorin}$ values occur at ~13.5 ka (4.5–6‰), between 11 and 10 ka (2–4‰) and 8–5 ka (2–4‰) (Figure 4f).

[17] Bulk sediment total organic carbon content (% TOC) and C/N ratio correlate throughout the sediments analyzed (Figures 4a and 4b). Both TOC and C/N values are low between 21 and 16.5 ka (~3% and ~6 respectively), increase to maxima of 8% and 10 between 16.5 and 11 ka and fluctuate about these

values between 11 and 5 ka, with notable minima at ~10 ka and 7 ka. The $\delta^{13}C$ of bulk organic matter ($\delta^{13}C_{bulk}$) and $\delta^{13}C_{Chlorin}$ show similar patterns throughout the sediments analyzed. Between 21 and 15 ka $\delta^{13}C_{bulk}$ generally falls between −31.5 and −28.5‰, whereas $\delta^{13}C_{Chlorin}$ ranges from −34 to −31.5‰, i.e., $\delta^{13}C_{bulk}$ is roughly 2.5 to 3‰ higher than $\delta^{13}C_{Chlorin}$ (Figure 4d). At ~15 ka, $\delta^{13}C_{bulk}$ increases to a maximum of ~−25.5‰ while $\delta^{13}C_{Chlorin}$ increases to −27.5‰. Both $\delta^{13}C$ data sets decrease toward 10 ka, whereupon $\delta^{13}C_{bulk}$ drops to ~−27‰ and $\delta^{13}C_{Chlorin}$ drops to ~−30‰. However, the two signals diverge at 7 ka, whereby $\delta^{13}C_{bulk}$ decreases to −29.5‰ and $\delta^{13}C_{Chla}$ increases to −32‰. Bulk organic $\delta^{15}N$ ($\delta^{15}N_{bulk}$) values vary within a narrow range of 4–6‰ throughout the section, with the majority of values fluctuating around 5‰ but for minima between 17 and 15 ka and 13–12 ka. The marked variability in $\delta^{15}N_{Chlorin}$ is not manifest in $\delta^{15}N_{Bulk}$ and overall comparison between the two data sets suggests little correspondence either in absolute values or temporal trends (Figure 4g).

5. Discussion

5.1. Concentration and Isotopic Composition of Different Chlorins

[18] The concentration of Chl *a* in lake waters and sediments has long been used as a proxy for photosynthetic biomass and primary productivity [*Sanger*, 1988; *Leavitt and Hodgson*, 2001]. The carbon and nitrogen isotopic composition of Chl *a* offers valuable additional information concerning the type of photoautotrophs responsible for Chl *a* synthesis, their physiological state and nutrient source. In cases where Chl *a* is not preserved in sufficient amount for isotope analysis, phaeopigments (e.g., Phe *a* and Ppe *a*) can provide useful alternatives [e.g., *Sachs and Repeta*, 1999, 2000; *Enders et al.*, 2008], however this relies on the assumption that phaeopigment $\delta^{13}C$ and $\delta^{15}N$ faithfully represent the original chlorophyll values. Therefore, in addition to providing information on past changes in aquatic environmental change, comparison of isotopic compositions between Chl *a* and phaeopigments can shed light on the sources and pathways of these compounds in aquatic systems and sediments and validate the use of phaeopigments as proxies for Chl *a* isotope composition [e.g., *Chikaraishi et al.*, 2007].

[19] At Lake Suigetsu the transition from glacial to postglacial conditions (20–10 ka) is marked by an increase in the absolute concentration of all chlorins and a shift in the relative concentration of Chl *a* over Phe *a* and Ppe *a* whereby Chl *a* is found in low concentrations relative to Phe *a* and Ppe *a* within the organic-poor, clay-rich sediments of the last glacial maximum (LGM) and in higher concentrations in the organic-rich Holocene sediments, where Phe *a* remains most abundant but Ppe *a* concentration is relatively low (Figure 4). This increase in chlorin concentration may represent either an increase in flux to the sediments due to increased productivity of photoautotrophs, or a decrease in dilution by minerogenic clays and silts, although the change in the relative abundance of Chl *a* suggests that a portion of this change is also a function of reduced diagenetic loss of Chl *a* during sedimentation. However, the diagenetic pathways to form phaeophytin and pyrophaeophytin are not fully understood [*Keely*, 2006]. Phaeophytin *a* is known to be actively synthesized within the cell, playing a part in electron transport during photosynthesis [*Klimov*, 2003], but in some cases Phe *a* and especially Ppe *a* have been observed as products of grazing activity within aquatic systems [*Head and Harris*, 1992]. It is possible that the higher relative abundance of Ppe *a* within the older sediments reflects higher grazing activity during this period, relative to the production of chlorophyll. Therefore, although the increased relative abundance of Chl *a* and total chlorin concentration within the more recent sediments of Lake Suigetsu implies an elevated productivity, interpretation of this change is not unambiguous.

[20] Comparison between the $\delta^{13}C$ and $\delta^{15}N$ of different chlorins enables a preliminary assessment of the extent to which the isotopic composition of Phe *a* and Ppe *a* in lake sediments reflect that of Chl *a*. Comparisons between $\delta^{13}C_{Chla}$ and either $\delta^{13}C_{Phea}$ or $\delta^{13}C_{Ppea}$ are limited by the small number of data points attainable for $\delta^{13}C_{Chla}$. However, $\delta^{13}C_{Phea}$ and $\delta^{13}C_{Ppea}$ significantly correlate (r^2 = 0.91, n = 12) close to a 1:1 relationship (Figure 5c) and this pattern remains when all $\delta^{13}C_{Chlorin}$ data are included within a single plot (Figure 5d). Similarly, although we cannot confidently determine the correlation between $\delta^{15}N_{Chla}$ and either $\delta^{15}N_{Phea}$ or $\delta^{15}N_{Ppea}$ (Figures 6a and 6b), there is a weak correlation (r^2 = 0.49) between $\delta^{15}N_{Phea}$ and $\delta^{15}N_{Ppea}$ (Figure 6c) which is strengthened by the combination of all $\delta^{15}N_{Chlorin}$ data within a single plot (Figure 6d). Transformation of Chl *a* to Phe *a* is relatively simple, involving demetallation through the loss of a single Mg^{2+} ion [*Keely*, 2006]. Because Mg^{2+} is bound to N within the tetrapyrrole ring, and not C, it is possible that demetallation is associated

with a N isotope fractionation but we would expect a limited effect on $\delta^{13}C$. The number and range of either $\delta^{13}C_{Chla}$ or $\delta^{15}N_{Chla}$ data are insufficient to conclusively test this hypothesis, however the $\delta^{15}N$ and $\delta^{13}C$ offsets between Chl *a* and Phe *a* fall within the range of scatter between Phe *a* and Ppe *a* (Figure 6d), suggesting that demetallation does not have a significant effect on either $\delta^{15}N_{Phea}$ or $\delta^{13}C_{Phea}$. Transformation from Phe *a* to Ppe *a* involves decarbomethoxylation, which may lead to a C isotope fractionation, however the significant correlation between $\delta^{13}C_{Phea}$ and $\delta^{13}C_{Ppea}$ suggests that this is also not the case (Figure 5c). Therefore, our data tentatively suggest that there is no systematic offset between the $\delta^{13}C$ and $\delta^{15}N$ of Chl *a*, Phe *a* and Ppe *a*, and that the transformation between these compounds is not associated with a systematic C or N isotopic fractionation. However, although the data suggest that there is some justification in using the isotopic composition of phaeopigments as a proxy for $\delta^{13}C_{Chla}$ and $\delta^{15}N_{Chla}$, the degree of scatter between $\delta^{13}C$ and particularly $\delta^{15}N$ of the three chlorins analyzed indicates that a strong degree of precision cannot be assumed. It is possible that the scatter between $\delta^{13}C$ and $\delta^{15}N$ between chlorins relates to the transformation of Chl *a* to Phe *a* or Ppe *a* being biased according to a particular source or season. The rate of transformation of Chl *a* to Phe *a* and Ppe *a* is unlikely to be constant across the range of potential sources, thus the flux of various chlorins to the sediments will be biased according to individual sources and synthesis at particular times. It is important to recognize that the sediment samples analyzed here represent an amalgamation of material accumulated over approximately 60–120 years, thus the range of potential chlorin sources and transformational pathways is large. The greater amount of scatter between $\delta^{15}N$ of different chlorins, compared to $\delta^{13}C$, implies that that the seasonal and inter-species variability in nitrogen sources and cell-chlorin isotope fractionation was greater than that associated with carbon utilization in Lake Suigetsu. It can also be inferred that the isotopic composition of Phe *a* and Ppe *a* more likely represent average conditions within the photosynthetic community, whereas Chl *a* may be biased toward taxa or conditions which promote preservation – such as rapidly sedimenting phytoplankton, those growing among peak seasonal biomass or those with more robust cell walls. Such factors are unlikely to remain constant with time, and may lead to variability in sedimentary $\delta^{13}C$ or $\delta^{15}N$ values irrespective of changes in the isotopic composition of Chl *a*. To date, studies into the rate of chlorin transformation in aquatic environments are scarce and further research is required in order to better understand such processes. However, it is clear that caution is required when interpreting the isotopic composition of phaeopigments or amalgamated chlorins as an unambiguous proxy for the isotopic composition of Chl *a*.

5.2. Comparing Bulk and Compound-Specific Isotope Data

[21] Compound-specific isotope analysis of chlorins is a potentially powerful means to access the isotopic composition of past photoautotrophic communities, however current analyses are difficult to make and time consuming. By contrast, bulk organic matter isotope analysis is relatively straightforward and quick to obtain data [*Meyers and Lallier-Verges*, 1999]. In cases where the majority of sedimentary organic matter is derived from aquatic sources, $\delta^{13}C_{bulk}$ and $\delta^{15}N_{bulk}$ can also be useful tracers of aquatic photoautotrophs. However, this approach is seriously limited by the inability to determine the relative contribution of 'contaminating' terrestrial organic detritus and unknown effects of diagenetic processes within the water column and the sediments. At Lake Suigetsu, bulk organic matter C/N ratios > 10 (Figure 4b) suggest a predominant aquatic source of organic matter [*Meyers and Lallier-Verges*, 1999]. Therefore we would expect a certain degree of correlation between the isotope composition of chlorins and the bulk isotope signal. For $\delta^{13}C$, this is largely true: $\delta^{13}C_{bulk}$ captures much of the long-term pattern in $\delta^{13}C_{Chlorin}$, however, there are also occasions where $\delta^{13}C_{bulk}$ appears to be affected by the contribution of detrital carbon (Figure 4). In particular, between 7 and 6 ka (~9 m depth), $\delta^{13}C_{bulk}$ reaches minimal values (~−30‰), whereas $\delta^{13}C_{Chla}$ and $\delta^{13}C_{Phea}$ indicate maxima (Figure 4). Sediments of this age are also marked by peaks in total organic carbon concentration, suggestive of detrital organic matter flux. Interestingly, these samples do not have distinctive C/N ratios, and thus may indicate reworked aquatic organic matter, possibly from the neighboring Lake Mikata (Figure 1). However, with the exception of such events, it appears that $\delta^{13}C_{bulk}$ can be used as a coarse indicator for the $\delta^{13}C$ of aquatic photoautotrophs at Lake Suigetsu.

[22] By contrast, $\delta^{15}N_{bulk}$ demonstrates little correspondence with $\delta^{15}N_{Chlorin}$. $\delta^{15}N_{bulk}$ values are systematically higher than $\delta^{15}N_{Chlorin}$, opposite to the relationship usually observed between $\delta^{15}N_{cell}$ and $\delta^{15}N_{Chla}$ [*Sachs et al.*, 1999; *Beaumont et al.*, 2000]. In addition, the magnitude of $\delta^{15}N$ variability throughout the core is small, contrasting with the

large changes in $\delta^{15}N_{Chlorin}$. Given that organic nitrogen in lake sediments is predominantly derived from aquatic sources, these data suggest that diagenetic processes may be responsible in significantly altering the $\delta^{15}N_{bulk}$ signal, to the extent that a rigorous interpretation of $\delta^{15}N_{bulk}$ in terms of aquatic change at Lake Suigetsu would be compromised. In particular, it has long been recognized that ammonium can adhere to clay minerals and contribute to the bulk nitrogen isotope composition of sediments [*Müller*, 1977]. In addition, breakdown of organic matter within the water column and surface sediments involves deamination that can potentially alter the $\delta^{15}N$ of sedimenting material [*Macko and Estep*, 1984]. *Lehmann et al.* [2002] observed decreases in $\delta^{15}N$ of 1.5–3‰ associated with diagenesis of organic matter during both laboratory experiments and monitoring at Lake Lugano, Switzerland, which they ascribe to the accumulation of ^{15}N-depleted anaerobic bacterial biomass. The relatively lower $\delta^{15}N_{bulk}$ compared to $\delta^{15}N_{Chlorin}$ derived estimates of $\delta^{15}N_{cell}$, alongside the comparably muted $\delta^{15}N_{bulk}$ signal suggests that accumulation of bacterial biomass may have played an important role in determining $\delta^{15}N_{bulk}$ at Lake Suigetsu.

5.3. Implications for Paleolimnology and Paleoclimatology at Lake Suigetsu

[23] Despite the variability in $\delta^{13}C$ and $\delta^{15}N$ between chorins in individual samples, the coherent temporal trends in both $\delta^{13}C$ and $\delta^{15}N$ of the three chlorins (Figure 4) suggests that $\delta^{13}C_{Chlorin}$ and $\delta^{15}N_{Chlorin}$ reflect changes in the aquatic productivity and nutrient utilization during the last glacial-interglacial transition at Lake Suigetsu, with differences between the individual chlorin isotope values a useful marker for within- sample variability. Interpreting the carbon isotopic composition of algal organic matter remains a subject of continued debate, however in the majority of cases, $\delta^{13}C_{cell}$ is used as a proxy for past change in algal growth rate or productivity. For the most part, field and laboratory evidence suggests that the fractionation of carbon isotopes during assimilation (ε_p) is an inverse function of the ratio of growth rate to concentration of dissolved inorganic carbon (DIC) [*Calder and Parker*, 1973; *Pardue et al.*, 1976; *Takahashi et al.*, 1990; *Schelske and Hodell*, 1991; *Laws et al.*, 1995; *Schelske and Hodell*, 1995; *Bidigare et al.*, 1997; *Laws et al.*, 2002; *Moschen et al.*, 2009]. On this basis, increased growth rate will lead to a reduction in ε_p and a consequent increase in $\delta^{13}C_{cell}$. In addition, the removal of CO_2 and concomitant increase in pH both increase the relative abundance and relative uptake by phytoplankton of HCO_3^-, which is enriched in ^{13}C by 9–12‰ and may also contribute to increasing $\delta^{13}C_{cell}$ [*Badger and Gallagher*, 1987; *Badger et al.*, 1994; *Korb et al.*, 1997; *Laws et al.*, 1998; *Burkhardt et al.*, 2001]. However, fractionation of carbon isotopes between DIC and cellular organic carbon can also be influenced by a number of factors including cell morphology [*Popp et al.*, 1998] the exact compounds synthesized and their position within the reaction chain [*Hayes*, 1993] and species-specific physiological factors such as carbon transport mechanisms, rate of diffusion and growth limiting factors [*Burkhardt et al.*, 1999b, 1999a; *Riebesell et al.*, 2000]. To date, the extent to which these factors influence the fractionation between inorganic and chlorin-bound carbon has yet to be investigated and a rigorous interpretation of the environmental significance of $\delta^{13}C_{Chlorin}$ cannot yet be applied. However, if we assume that $\delta^{13}C_{Chlorin}$ reflects $\delta^{13}C_{cell}$ with negligible offset [*Sachs et al.*, 1999] then it is possible that $\delta^{13}C_{Chlorin}$ is a function of changing primary productivity at Lake Suigetsu.

[24] Understanding the nitrogen isotope ratio of past photoautotrophs is equally complicated First, dissolved inorganic nitrogen (DIN) exists as a number of different species in water (i.e., nitrate, nitrite, ammonium, urea), the isotopic composition of which vary according to water column microbial processes such as nitrification, denitrification and ammonium oxidation. Isotopic fractionation occurs during assimilation of nitrogen within algal tissue, which is a function of the DIN species, the organism concerned and the extent of DIN assimilation [*Wada and Hattori*, 1978; *Waser et al.*, 1998]. Considering the discrimination in nitrogen isotopes between cellular and chlorophyll nitrogen, the range of $\delta^{15}N_{Chlorin}$ throughout the last glacial-interglacial transition at Lake Suigetsu (Figure 4f) suggest a variability in $\delta^{15}N_{cell}$ between +5 and +12‰ which implies a predominance of nitrate or ammonium assimilation over N_2 fixation. Values of $\delta^{15}N_{cell} <$ 0‰ are usually associated with N_2 fixation by cyanobacteria, or nitrate assimilation by some cyanobacteria species [*Bauersachs et al.*, 2009]. Therefore, lower $\delta^{15}N_{Chlorin}$ values may be interpreted as reflecting a relatively higher contribution of cyanobacteria derived chlorins to the sediments. Although it is impossible to fully constrain the nitrogen isotope dynamics within Lake Suigetsu, two extreme end-members can be visualized – a

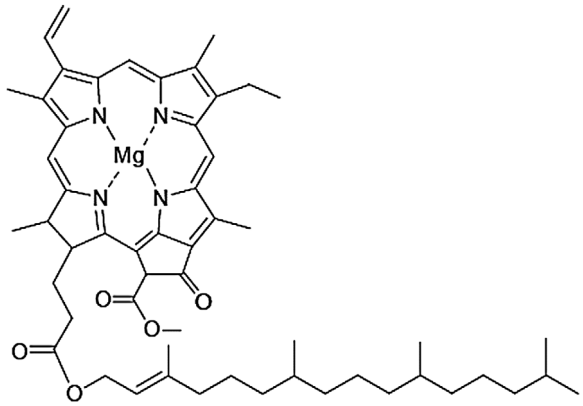

Figure A1. Chemical structure of chlorophyll *a*.

physically stable, nutrient-poor, cyanobacteria rich lake causing lower $\delta^{15}N$ and an effectively mixed, nutrient-rich, productive lake where assimilation of ammonium or nitrate leads to higher $\delta^{15}N$.

[25] The combined $\delta^{13}C_{Chlorin}$ and $\delta^{15}N_{Chlorin}$ records suggest marked change in the photoautotrophic productivity of Lake Suigetsu during the last glacial-interglacial transition, characterized by low productivity during glacial conditions (21–16 ka) and an increase in productivity after ~15 ka, initially reflected in $\delta^{13}C_{Chlorin}$, followed by maximum $\delta^{15}N_{Chlorin}$ at ~13.5 ka. The subsequent decline in $\delta^{15}N_{Chlorin}$ to a notable minimum at ~12 ka, which contrasts with rising $\delta^{13}C_{Chlorin}$ (Figure 4e) is difficult to decipher, however this may relate to changes in the photoautotrophic community composition, or changes in nutrient source or availability in Lake Suigetsu. However, at present the paleoecological and environmental significance of these changes is unclear and further research is required using additional organic biomarkers, diatom and pollen remains and inorganic geochemical techniques in order to unravel these questions.

6. Conclusion

[26] The carbon and nitrogen isotope composition of Chl *a* offers a potentially valuable proxy for the isotopic composition of photoautrophs in paleo-lake waters. Where Chl *a* is not preserved, the isotopic composition of the phaeopigment derivatives Phe *a* and Ppe *a* can be useful alternatives, assuming that the transformation from Chl *a* is not associated with an isotopic fractionation. Analyses of the $\delta^{13}C$ and $\delta^{15}N$ of Chl *a*, Phe *a* and Ppe *a* from the last glacial-interglacial transition sediments of Lake Suigetsu, Japan, support this assumption, and generally coherent isotopic patterns are apparent between the three chlorins. However, the data suggest that the isotopic composition of phaeopigments is an imprecise proxy for $\delta^{13}C_{Chla}$ and particularly $\delta^{15}N_{Chla}$, possibly as a consequence of biases in synthetic flux and diagenesis according to different sources and seasons. Comparison between chlorin-specific $\delta^{13}C$ and $\delta^{15}N$ and the isotope composition of bulk organic matter suggests that $\delta^{13}C_{bulk}$ can be used as a coarse tracer of aquatic change, however $\delta^{15}N_{bulk}$ appears subject to diagenetic alteration which prohibits interpretation in an environmental context. Temporal patterns in $\delta^{13}C_{Chlorin}$ and $\delta^{15}N_{Chlorin}$ at Lake Suigetsu suggest changes in aquatic primary productivity, in particular an increase associated with the transition from glacial conditions at ~20 ka, through to postglacial conditions after ~15 ka. However, further research within a multiproxy framework is required in order to fully understand this transition and it's ecological and environmental significance. Nevertheless, these first combined $\delta^{13}C$ and $\delta^{15}N$ measurements from lake

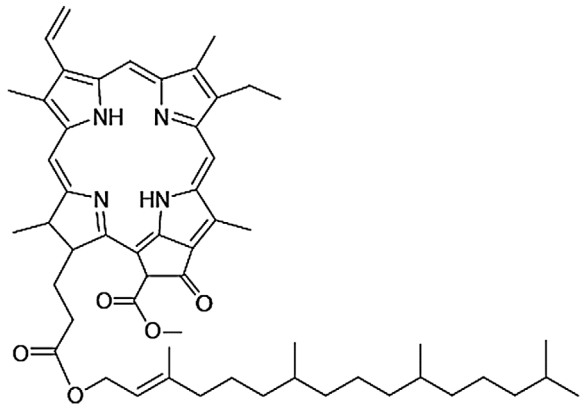

Figure A2. Chemical structure of phaeophytin *a*.

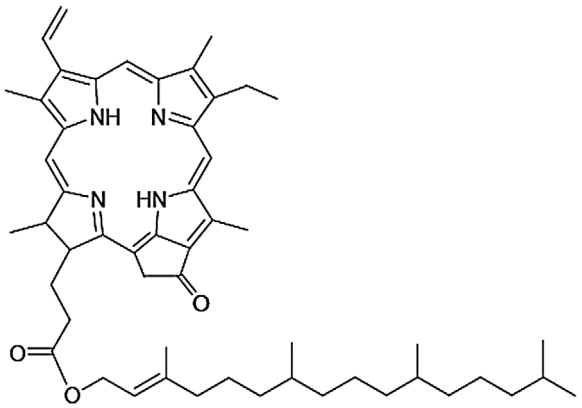

Figure A3. Chemical structure of pyrophaeophytin *a*.

sedimentary chlorins underline the potential this tool for tracking primary productivity in both marine and freshwater aquatic systems. However, further laboratory and field based research is required to understand the mechanisms and isotope fractionation associated with synthesis and transformation of chlorins within the water column in order to exploit this potential.

Appendix A

[27] The sedimentary chlorins chlorophyll *a* (Figure A1), phaeophytin *a* (Figure A2) and phaeophytin *a* (Figure A3) were identified based on HPLC retention times and UV-vis spectra in comparison with laboratory standards. Knowledge of their chemical structure enables an assessment of sample purity based on C/N ratios measured using an elemental analyzer.

Acknowledgments

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