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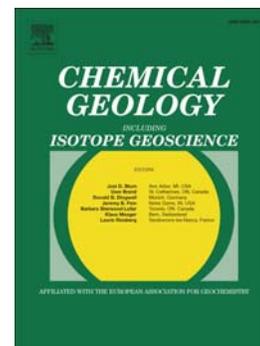
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Distributions and compound-specific isotopic signatures of sedimentary chlorins reflect the composition of photoautotrophic communities and their carbon and nitrogen sources in Swiss lakes and the Black Sea

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ABSTRACT

We examined the distributions of tetrapyrrole pigments (i.e. intact chlorophylls and bacteriochlorophylls, pheopigments) as well as their compound-specific carbon and nitrogen isotopic compositions in the sediments of three Swiss lakes (Lakes Rotsee, Cadagno and Zurich) and the Black Sea to investigate the biogeochemical cycling of carbon and nitrogen mediated by phototrophic eukaryotes (algae) and bacteria. The factors controlling chlorin isotope variations are discussed and the feasibility to use chlorins as indicators for reconstructions of surface water environments are evaluated. Chlorophyll *a* and its derivatives including pheophytin *a*, a pheophytin *a* epimer, pyropheophytin *a*, $^{13}\text{C}^2,^{17}\text{C}^3$ -cyclopheophorbide-*a*-enol, chlorophyllone *a* as well as steryl and carotenol chlorin esters were detected in all sediments. The presence of bacteriochlorophylls *e* and their derivatives confirmed the presence of brown strains of green phototrophic sulfur bacteria (*Chlorobiaceae*; GSB) in all three lakes. In the shallower Lakes Rotsee and Cadagno, purple sulfur bacteria (*Chromatiaceae*; PSB) were also present as confirmed by bacteriochlorophyll *a* derivatives. Despite the different degrees of water column hypoxia at the studied sites, the chlorins in all sediments were attributed to rapid transformation of intact tetrapyrroles and the formation of related pheopigments. The scatter of compound-specific carbon isotopic compositions of Chl *a* and its derivatives resulted from different timing of pheopigment formation, likely due to the interaction of blooms of various phytoplankton communities at different times of the year and the variable degrees of carbon limitation and/or different contributions of recycled organic matter (OM). The nitrogen isotopic composition of the chloropigments mainly derived from nitrate assimilation in Lake Zurich and the Black Sea, whereas ammonium and nitrate assimilation were predominant in Lake Rotsee. In the epilimnion of the meromictic Lake Cadagno, dissolved organic nitrogen (DON) supplied to the surface water from

ammonium assimilation in the chemocline may be the main nitrogen source. Phototrophic sulfur bacteria in Lakes Rotsee and Cadagno thrived mainly under dissolved organic carbon depleted conditions within the chemocline and in the hypolimnion. GSB may use predominantly ammonium and at least in Lake Cadagno also perform N_2 fixation. In contrast, the nitrogen source of PSB could not be reconstructed with $\delta^{15}N$ values of bacteriochlorins, because nitrogen isotopic fractionation during BChl a synthesis seems to be almost independent of the assimilated substrate.

Keywords: Pigment; chlorophyll; carbon isotope; nitrogen isotope; phytoplankton; anoxia

1. Introduction

Chlorophylls and bacteriochlorophylls are the essential pigments for oxygenic and anoxygenic photosynthesis, respectively, and represent the most abundant pigments on Earth (Falkowski, 2003; Keely, 2006; Scheer, 2006). Accordingly, chlorophyll *a* (Chl *a*; Fig. A.1) is ubiquitous and the predominant pigment in eukaryotic photoautotrophs and cyanobacteria from both marine and terrestrial environments (e.g. Scheer, 2006). In contrast, bacteriochlorophylls are the main pigments of phototrophic bacteria in aquatic environments to capture electromagnetic radiation (e.g. Pfennig, 1978). Phototrophic bacteria such as *Chromatiaceae* (purple phototrophic sulfur bacteria; PSB) and *Chlorobiaceae* (green phototrophic sulfur bacteria; GSB) thrive under euxinic (free H₂S) condition in the photic zone (e.g. Pfennig, 1978). While GSB are obligate anaerobes, PSB are facultative anaerobes (e.g. Pfennig, 1978).

Chlorophylls and bacteriochlorophylls are transformed to a large variety of degradation products, including pheopigments, porphyrins and maleimides (e.g. Grice et al., 1996; Hodgson et al., 1968; Keely, 2006; Louda et al., 2011). These compounds are formed within living or senescent cells, in the water column and during sedimentation (e.g. Barwise and Roberts, 1984; Bianchi et al., 1993; Louda et al., 1998; Louda et al., 2011). The underlying processes are autolytic degradation, photochemical, enzymatic and hydrolytic reactions, microbial or viral lysis and grazing (e.g. Chen et al., 2003; Gossauer and Engel, 1996; Louda et al., 1998; Owens and Falkowski, 1982).

Primary degradation products of chlorophylls include pheophytins, pheophorbides and pyropheophytins, which are formed by demetallation of the central Mg ion, by ester hydrolysis and by demethoxycarbonylation, respectively (e.g. Chen et al., 2003; Keely, 2006). Furthermore, they include 13²,17³-cyclopheophorbide-*a*-enol (CPhe *a*;

Fig. A.1), which has been considered to be formed by grazing (Goericke et al., 2000), but can also be derived from pyropheophorbide *a* within the sediment (Louda et al., 2000). Other derivatives are steryl and carotenol chlorin esters (SCEs and CCEs; Fig. A.1), esterified products of secondary reactions of tetrapyrroles with steroids or carotenoids, respectively (e.g. Chen et al., 2003; Furlong and Carpenter, 1988; Harradine et al., 1996; King and Repeta, 1991; Spooner et al., 1994).

Despite of these various alterations, which can occur on timescales of hours to days or weeks and continue in the sediment (Bianchi et al., 1993; Furlong and Carpenter, 1988; Goericke et al., 2000; Owens and Falkowski, 1982; SooHoo and Kiefer, 1982; Sun et al., 1993), these tetrapyrroles and their degradation products in the sedimentary record provide evidence of all photoautotrophic communities in the water column together with carotenoids (e.g. Keely, 2006; Züllig, 1985).

The preservation of the tetrapyrrole macrocycle facilitates unique biogeochemical reconstructions, as the carbon and nitrogen isotopic signatures are expected to be only minimally altered during diagenesis. This is valuable for the reconstruction of surface water environments, the composition of phototrophic communities and the biogeochemical cycling of carbon and nitrogen (Macko and Estep, 1984; 2006; Ohkouchi et al., 2005; Ohkouchi and Takano, 2014; e.g. Sachs et al., 1999; Tyler et al., 2010). Specifically, the bulk nitrogen isotopic composition is prone to significant alteration during diagenesis (e.g. Freudenthal et al., 2001; Lehmann et al., 2002; Robinson et al., 2012; Sigman et al., 1999), whereas nitrogen within the tetrapyrrole ring in chlorophylls and their derivatives remains unaffected (e.g. Higgins et al., 2011; Sachs et al., 1999; Tyler et al., 2010). Moreover, the inherent heterogeneity of bulk OM, as it is derived from various sources (i.e. bacteria and eukaryotes), prevents specific interpretations of phytoplankton communities. Therefore, the compound-specific isotope analysis of photosynthetic pigments provide a tool to delineate

biogeochemical processes of distinct community assemblages in surface water environments (e.g. Hayes et al., 1990; Ohkouchi et al., 2005; Tyler et al., 2010).

Recent developments in analytical techniques have enhanced chlorin purification using high performance liquid chromatography (HPLC) (Higgins et al., 2009; Sachs and Repeta, 2000) and the analysis of carbon and nitrogen isotopic signatures by sensitivity-improved isotope-ratio mass spectrometry (Ogawa et al., 2010; Polissar et al., 2009). However, only a small number of studies have explored the high potential of isotopic signatures of chlorins to better understand how different phototrophic assemblages mediate the biogeochemical cycling of carbon and nitrogen in oxygen rich and oxygen deficient aquatic environments and how environmental factors control isotopic signatures in tetrapyrroles (summarised by Ohkouchi and Takano, 2014). The underlying processes that lead to the observed stable isotope compositions of chlorins are still barely understood, especially in lakes with different biogeochemical characteristics (i.e. oxygen content, stratification, nutrient availability, phototrophic community assemblages).

Here, we analysed compound-specific carbon and nitrogen isotopic compositions of tetrapyrrole pigments (chlorophylls, bacteriochlorophylls, and related pheopigments) in the sediments of three Swiss lakes (Lakes Rotsee, Cadagno and Zurich) and the Black Sea (Romanian Shelf) to investigate the role of phototrophic algae and bacteria in the biogeochemical cycling of carbon and nitrogen in these systems. We discuss the impact of degradation on pigment distributions, factors that control variations in the stable isotope composition of isolated chlorins and evaluate their feasibility to reconstruct nitrogen sources of the phototrophic communities.

2. Material and methods

2.1. Study sites and sample collection

Lake Zurich (Lower Lake area of 67.3 km²) is a prealpine, mesotrophic, either monomictic or dimictic lake depending on prevailing winter conditions. The surface sediment sample (0-2cm) studied originates from a 110 cm long core (ZH-09-05) obtained with a gravity corer at the maximum depth of 137 m in November 2009 (47°17.004'N, 8°35.640'E, WGS84). The average sedimentation rate in the varved section of this core is ca. 0.28 cm yr⁻¹, identical with estimates on an equivalent core from the same site that has been studied previously (Naeher et al., 2013a). In this lake, non-N₂-fixing cyanobacterium *Planktothrix rubescens* ("burgundy blood algae") has become the dominant species during the past four decades as a result of reoligotrophication due to the associated decrease in phosphate concentrations and warmer water temperatures (Posch et al., 2012). From September to March *P. rubescens* alone represents more than 50% of the total phytoplankton biomass (Bossard et al., 2001). Co-dominating algae in the lake are diatoms and *Cryptophyceae*, whereas chlorophyta account for less than 10% of phytoplankton biomass (Bossard et al., 2001).

Lake Rotsee is a small (0.46 km²) prealpine, monomictic and eutrophic lake. The lake has a stable stratified water column with a strong chemocline between ca. 6 and 10 m depth and an anoxic hypolimnion for most of the year (Schubert et al., 2010). In this study we use surface sediment (0-4 cm) from a 65 cm long core (Rot-10-3) obtained with a gravity corer at the maximum depth of 16 m in August 2010 (47°4.251'N, 8°18.955'E, WGS84). Naeher et al. (2012) reported the average sedimentation rate of ca. 0.38 cm yr⁻¹. The lake has a high species richness and diversity with *Cryptophyceae*, *Chlorophyceae* and diatoms being the predominant algae. Cyanobacteria (mainly *Planktothrix rubescens*) present in the surface water

are only more abundant than algae during blooms in fall, whereas diatoms dominate at about 5-7 m water depth (monitoring data of the Cantonal Office of Environment and Energy; F. Schanz personal communication). N_2 fixing cyanobacteria are low in abundance (monitoring data of the Cantonal Office of Environment and Energy; F. Schanz personal communication). Also purple and green phototrophic sulfur bacteria (PSB, GSB) are present (e.g. Kohler et al., 1984; Züllig, 1985).

Lake Cadagno is a small (0.26 km²), alpine, mesotrophic and meromictic lake. The lake has a strong chemocline between ca. 10 and 13 m and is permanently stratified (Wirth et al., 2013). A sediment core was collected from the deepest part of the lake (21 m depth) in September 2009 (46°33.000'N, 8°42.000'E, WGS84; Wirth et al., 2013) using an UWITEC platform with a percussion piston-coring system. The surface sediment is not available, and we used a sediment sample with a depth of 633-638 cm of the core, which corresponds to 5000-5600 calendar years before present (cal yr BP) based on the radiocarbon dates. The sedimentation rate is ca. 2 mm yr⁻¹ (Wirth et al., 2013). In Lake Cadagno, phytoplanktonic communities are dominated by green algae (predominant chlorophyte: *Echinocoleum elegans*), diatoms, cryptophytes and dinophytes in early summer, with increasing abundances of diatoms, followed by green algae and flagellates towards late summer (Bossard et al., 2001; Schanz and Stalder, 1998). PSB and GSB are present in the chemocline and hypolimnion (e.g. Peduzzi et al., 2011; Tonolla et al., 2005; Züllig, 1985).

The Black Sea is a restricted, semi-enclosed brackish inland sea and represents the largest modern anoxic basin in the world. It is stratified permanently with the pycnocline between ca. 50 and 150 m water depth (Ozsoy and Unluata, 1997; Piper and Calvert, 2011). The studied surface sediment sample (0-2 cm) originates from a 56 cm long sediment core that was recovered at a water depth of 220 m on the Romanian Shelf in May 2010 with a multicorer (sampling site 10MA10; 43°43.905'N,

30°11.962'E, WGS84). The core covers the typical Black Sea unit 1 (Piper and Calvert, 2011; Ross and Degens, 1974). The phytoplankton communities in the western Black Sea are typically dominated by dinoflagellates in spring, and diatoms and coccolithophores in fall (Eker et al., 1999; Moncheva et al., 2001), although the Romanian Shelf is generally dominated by diatoms with exceptions of blooms comprising cyanobacteria and dinoflagellates (Vasiliu et al., 2012). GSB have been reported from the chemocline of the Black Sea (e.g. Repeta et al., 1989). The studied samples were frozen at -20°C after recovery of the cores and freeze dried, ground and homogenised prior to analyses.

2.2. Pigment extraction, isolation and analysis

Chloro- and pheopigments were analysed as reported in Tyler et al. (2010) with some modifications. In brief, freeze-dried and ground sediment (1-11 g) was extracted three times with acetone by ultrasonication for 15 min in an ice bath, followed each time by centrifugation at 777×G for 5 min. The volume of the total extract was reduced under a cold stream of argon and transferred into a *n*-hexane-MilliQ bilayer (ratio 1:3), homogenised using vortex and centrifuged at 777×G for 2 min. The *n*-hexane layer was recovered and dried under reduced pressure. All steps were carried out under careful exclusion of light irradiation where possible and dried samples were stored within an argon atmosphere at -20°C. Samples were dissolved in *N,N*-dimethylformamide (DMF) prior to analysis by reversed-phase high performance liquid chromatography (HPLC), using an Agilent Infinity 1260 series HPLC equipped with a photodiode array detector (DAD) and fraction collector. Prior to injection, the extracts were filtered by 0.2 µm centrifuge filters (GHP Nanosep® MF, Pall Co., USA) in order to remove particles.

For the determination of pigment distributions (Fig. 1) and the isolation (fraction collection) of chloro- and pheopigments from the extract, the HPLC system was equipped with an Agilent Eclipse XDB-C18 column (250 mm x 4.6 mm; 5 μ m) and an Agilent Eclipse XDB-C18 guard column (12.5 mm x 4.6 mm; 5 μ m). These pigments were eluted isocratically with 75% A and 25% B for 5 min and then with a linear gradient to 50% B for 50 min at 1.0 mL min⁻¹, where A = acetonitrile/pyridine (100:0.5; v:v) and B = ethyl acetate/pyridine (100:0.5; v:v). The oven temperature was kept constant at 30°C. Pigments were identified based on relative retention times and by comparison with published UV-VIS spectra (Fig. 1; Table 1) (Airs et al., 2001; Airs and Keely, 2003; Chen et al., 2003; Chikaraishi et al., 2007; Tyler et al., 2010). The range of recorded wavelengths of the DAD spectra was set to 250-800 nm.

To achieve high single pigment purities, a second isolation step was applied using an Agilent Eclipse PAH column (250 mm x 4.6 mm; 5 μ m) with an Agilent Eclipse PAH guard column (12.5 mm x 4.6 mm; 5 μ m). Isocratic elution of the pigments was achieved using 20% B for 5 min, followed by a linear gradient to 60% B for 30 min and then to 100% B for 13 min. The flow rate was constant at 1.0 mL min⁻¹. The column compartment was kept at 15°C for the fraction collection of peaks of BChls *a*, *e* and their derivatives, Chl *a* and Pheo *a*, whereas 30°C was used for all other pigments. Prior to this second isolation, Chl *a* was converted to Pheo *a* (Mg removal) by 2 M HCl, extracted by *n*-hexane and dried under a cold stream of argon. This conversion step was also used to separate otherwise coeluting peaks of Chl *a* and BPheo *a* in the Lake Rotsee sample (Fig. 2).

A third isolation step was required for the analysis of BChls *e* and their derivatives (Fig. 3a), using the same, first HPLC isolation procedure as described above, except using two Agilent Eclipse XDB-C18 columns (250 mm x 4.6 mm; 5 μ m) in series together with an Agilent Eclipse XDB-C18 guard column (12.5 mm x 4.6 mm; 5 μ m).

To confirm the identification of the detected BChls *e* and their related chlorins, the isolated fractions were also analysed by HPLC coupled to electrospray ionisation mass spectrometry (HPLC-ESI-MS), using an Agilent 1200 LC coupled to an Agilent 6460 ESI-MS system. The same, first HPLC isolation procedure as described above. Two Agilent Eclipse XDB-C18 columns (250 mm x 4.6 mm; 5 μm) were connected in series together with an Agilent Eclipse XDB-C18 guard column (12.5 mm x 4.6 mm; 5 μm). The MS was operated in positive ion mode, with an ion source temperature of 290°C, gas flow of 5.0 L min⁻¹, nebuliser pressure of 45 psi, sheath gas temperature of 300°C and sheath gas flow of 11 L min⁻¹. The capillary voltage was 4000 V, the nozzle voltage 1500 V and the fragmentation voltage 180 V. The acquired mass range was m/z 400-1200 in full scan mode.

2.3. Carbon and nitrogen isotope analyses

Prior to isotope analysis, potential remains of pyridine in the vials containing the isolated chlorins were removed by liquid-liquid extraction of added MilliQ water with *n*-hexane, which was then dried under a cold argon stream. Samples were dissolved in dichloromethane (DCM) and transferred to pre-cleaned Sn cups (Tyler et al., 2010), followed by careful evaporation of DCM at 35°C prior to isotope analysis. Stable carbon and nitrogen isotopic compositions of chlorins were determined on a modified FlashEA1112 automatic Elemental Analyser (EA) connected to a Thermo Finnigan Delta plus XP isotope-ratio mass spectrometer *via* a ConFlo III (Ogawa et al., 2010). The compound-specific $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are given relative to Vienna PeeDee Belemnite (VPDB) and relative to atmospheric N₂ (AIR), respectively.

The analytical errors estimated by the repeated analysis of our laboratory standard (Nickel octaethylporphyrin: $\delta^{13}\text{C} = -34.17 \pm 0.06 \text{ ‰}$, $\delta^{15}\text{N} = 0.86 \pm 0.03 \text{ ‰}$) were

within 0.5‰ and 0.8‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. The purity of the chlorins was verified by the comparison of UV-VIS spectra of samples and standards (isolated pigments obtained from higher plant leaves and sediments; Chikaraishi et al., 2007) and by comparing the measured C/N ratios with the theoretical values. The analysis of bulk sediment $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, the used instrumentation and analytical errors of the procedure have been reported in detail by Naeher et al. (2012).

Isotopic fractionation factors were expressed in the epsilon notation with $^{13}\epsilon_{a/b}$ values corresponding to $^{13}\epsilon_{a/b} = 1000 [(^{13}\text{C}/^{12}\text{C})_a / (^{13}\text{C}/^{12}\text{C})_b - 1]$ and $^{15}\epsilon_{a/b}$ values to $^{15}\epsilon_{a/b} = 1000 [(^{15}\text{N}/^{14}\text{N})_a / (^{15}\text{N}/^{14}\text{N})_b - 1]$.

3. Results and Discussion

3.1. Distributions of chlorophyll a and its derivatives in the studied sediments and the impact of pigment degradation

The pigment distributions in the surface sediment of Lake Zurich were dominated by Chl a and its derivatives including pheophytin a (Pheo a), pyropheophytin a (PPhe a), 13²,17³-cyclopheophorbide-a-enol (CPhe a) and chlorophyllone a (Chlone a), which were identified based on relative retention times and characteristic UV-VIS spectra (Figs. 1, A.1; Table 1; Section 2.3) (Airs et al., 2001; Airs and Keely, 2003; Chen et al., 2003; Chikaraishi et al., 2007; Tyler et al., 2010). Minor compounds comprise an epimer of Pheo a and steryl and carotenol chlorin esters (SCEs and CCEs; Figs. 1, A.1). These compounds were also detected in the sediment samples of the other study sites. Only a trace amount of Chl a was detected in the Lake Cadagno sample due to the high progress of Chl a degradation during the aging of the studied sample

(during the last 5000-5600 years), represented by the predominance of PPh_e *a* relative to Chl *a* and Pheo *a*. Similarly, the low abundance of Chl *a* relative to its pheopigments in the Black Sea sediment results from the long timescales of sedimentation in the Black Sea (Ross and Degens, 1974). The Black Sea sediment contained relatively higher amounts of SCEs and CCEs than in the lake sediments (King and Repeta, 1991), which are also characteristic for a higher degree of chlorophyll transformation in the water column and more intense grazing by zooplankton (e.g. Chen et al., 2003; Furlong and Carpenter, 1988; Harradine et al., 1996; King and Repeta, 1991; Spooner et al., 1994).

The chlorin index provides additional evidence for rapid transformation of chloro- to pheopigments. This index is defined as the ratio between the fluorescence intensity of a sediment extracted with acetone and treated with hydrochloric acid (1M HCl) and the original sediment extract, using 428 nm and 671 nm as excitation and emission wavelengths, respectively (Schubert et al., 2005). It is a measure of the degree of degradation of labile OM, mainly based on the transformation of Chl *a* to Pheo *a*. The values of 0.6 and 0.7 in the surface samples and the rapid increase of these values with increasing sediment depth in Lakes Rotsee and Zurich (Naeher et al., 2012; in prep) show that pheopigments are rapidly formed within the water column and the most recent sediments of these lakes. No chlorin index values were available for the Lake Cadagno and Black Sea sediments.

*3.2. Detection of bacteriochlorophyll *a* and *e* derivatives as indicators of phototrophic bacterial communities*

In contrast to Lake Zurich, the other study sites are characterised by pronounced photic zone euxinia (e.g. Naeher et al., 2013b; Repeta et al., 1989; Wirth et al., 2013;

Züllig, 1985). Aquatic systems affected by anoxic conditions with H₂S within the photic zone provide ideal habitats for phototrophic sulfur bacteria (Pfennig, 1978). In Lakes Rotsee and Cadagno previous studies reported the presence of PSB and GSB (e.g. Kohler et al., 1984; Naeher et al., 2013b; Peduzzi et al., 2011; Tonolla et al., 2005; Züllig, 1985), whereas in the Black Sea only GSB have been reported (Repeta et al., 1989).

In agreement with these observations, in Lakes Rotsee and Cadagno the presence of PSB was confirmed by two and nine BChl *a* derivatives, respectively, including bacteriopheophytin (BPhe) *a* and bacteriopheophorbide (BPhide) *a*, detected on the basis of characteristic UV-VIS spectra with absorption maxima at ca. 360 and 750 nm (Fig. 2) and relative retention times. The most abundant peak in the 750 nm chromatogram was assigned to BPhe *a* based on its diagnostic absorption maxima at 357, 524 and 746 nm (Fig. 2; Table 1). Although this compound coeluted with Chl *a*, comparison with relative retention times reported in Airs and Keely (2003) further support the identification of this pigment as BPhe *a*. In agreement with previous reports (Repeta and Simpson, 1991; Repeta et al., 1989), BChl *a* and its derivatives were not detected in the Black Sea sediment.

In Lake Cadagno, Lake Rotsee and the Black Sea, both BChls *e* and BPhes *e* were observed (Figs. 1, 3), which confirms the presence of brown strains of GSB in these systems. The vertical segregation of GSB below PSB in the water columns of Lakes Rotsee and Cadagno has been reconstructed by bacteriochlorophyll profiles (Gregersen et al., 2009; Kohler et al., 1984), with a higher abundance of PSB in the upper chemocline, whereas GSB were more abundant in the lower chemocline and monimolimnion.

BChls *e*₁₋₃ and BPhes *e*₁₋₃ were identified based on characteristic UV-VIS spectra with absorption maxima at 460 and 650 nm, and 440 and 660 nm, respectively (Fig.

3). Furthermore, the diagnostic $[M+H]^+$ ions m/z 799.5, 813.5 and 827.5 suggest BPhes with ethyl (e_1), *n*-propyl (e_2) and *iso*-butyl (e_3) groups substituted at C8 position, respectively (Chen et al., 2001). LC-MS analysis of the isolated BPhes e further revealed two isomers of both BPhes e_2 and e_3 in the Lake Cadagno samples. Although the Lake Cadagno sediment sample revealed in total seven BChls e and five BPhes e based on their characteristic absorption spectra (Fig. 3), only BChls e_1 - e_3 and related BPhes e_1 - e_3 could be assigned. Similar to these observations, Gregersen et al. (2009) detected six main BChl e homologs in the water column of this lake, which were tentatively assigned as two series of BChls e_1 - e_3 . The first three have farnesol as the side chain, whereas the latter three peaks could not be determined. Comparison of HPLC chromatograms of Lake Cadagno with the respective fractions from Lake Rotsee and the Black Sea (Fig. 1) indicated that the same BChls e_1 - e_3 and BPhes e_1 - e_3 were also present in these samples.

3.3. Evaluation of the purity of isolated chlorins

The data quality of chlorins for the analysis of their compound-specific carbon and nitrogen isotopic compositions depends crucially on the achieved purity of the isolated pigments. The pigment purity has been evaluated based on the similarity of measured UV-VIS spectra of isolated compounds obtained from samples relative to reference compounds (Section 2.3; e.g. Ohkouchi et al., 2005; Tyler et al., 2010). Furthermore, the correspondence of measured and theoretical C/N ratios on the basis of chemical structures was used as a control for sufficient purity (e.g. Ohkouchi et al., 2005; Tyler et al., 2010).

The measured molar C/N ratio of Chl *a* in Lake Rotsee (14.6) was only slightly higher than the theoretical value of 13.8 (Table 2). Similarly, the predicted molar C/N ratio of

Pheo *a* is 13.8, which is lower than values of 17.4 and 15.1 in Lakes Zurich and Rotsee, respectively. Measured C/N ratios of PPhe *a* were somewhat higher (Lake Cadagno 15.7; Lake Rotsee 13.9; Black Sea 14.8) than the predicted value of 13.3. CPhe *a* was also slightly higher (8.8 and 10.1 for Lake Rotsee and Lake Zurich, respectively) than the predicted molar C/N ratio of 8.0. BChl *a* and BPhes *a* have theoretical C/N ratio of 13.8, whereas molar C/N ratios of BChls *e* and BPhes *e* depend on the respective homolog, either 12.8, 13.0 or 13.3 for BChls and BPhes *e*₁, *e*₂ and *e*₃, respectively. While an unidentified BChl *a* derivative in Lake Cadagno had very similar C/N ratios (13.6), another unidentified BChl *a* derivative in Lake Rotsee (13.2) agreed well with the theoretical prediction.

However, although the comparison of measured UV-VIS spectra of all isolated pigments were diagnostic and suggested sufficient purity, the C/N ratios of some isolated fractions are at least 25% higher than the predicted values (Table 2). Especially the values for Chl *a* fraction in Lake Zurich (C/N ratio of 22.4 instead of 13.8), Pheo *a* and CPhe *a* fraction in the Black Sea (19.1 and 12.2 instead of 13.8 and 8.0, respectively), the Pheo *a* epimer fraction (19.0 instead of 13.8) and BPhes *a* fraction (23.7 instead of 13.8) in Lake Rotsee were significantly higher than the theoretical values. The C/N ratio from the mixture of BPhes *e*₁, *e*₂ and *e*₃ (17.4) in Lake Cadagno were significantly higher than theoretical values of 12.8, 13.0 and 13.2, respectively (Table 2).

The C/N ratios that were higher than predicted values may result predominantly from carbon derived from coeluting lipids or carotenoids, which are difficult to remove completely with our chlorin purification processes (Ohkouchi and Takano, 2014; Tyler et al., 2010). Because coelution with carotenoids was not observed in the absorption range of 500-550 nm for all samples, other lipids are potential contaminants that cannot be detected by UV-VIS spectroscopy. Therefore, due to the at least 25%

higher molar C/N ratio than expected based on structural reasons, at least one fourth of the carbon in the Chl *a* fraction in Lake Zurich, Pheo *a* and CPhe *a* fractions in the Black Sea, Pheo *a* epimer and BPhe *a* fractions in Lake Rotsee and BPhes e_1 , e_2 and e_3 in Lake Cadagno may be derived from carbon from other compounds. Therefore, the $\delta^{13}\text{C}$ values of these chlorins must be considered unreliable and were not interpreted.

However, the $\delta^{15}\text{N}$ values of these compounds in the same samples are considered to be much less biased due to the limitation of coeluting nitrogen-containing compounds (Ohkouchi and Takano, 2014; Tyler et al., 2010) and are therefore discussed in this study. However, only the lower than predicted molar C/N ratio of BPhe *a* fraction (10.6 instead of 13.8; Table 2) may be derived from significant contributions of nitrogen (and maybe also carbon) from other compounds, so its $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are disregarded in this study.

*3.4. Factors influencing the stable carbon isotopic signatures of Chl *a* and related pheopigments in the studied systems*

The $\delta^{13}\text{C}$ value of Chl *a* ($\delta^{13}\text{C}_{\text{Chl-a}}$) was -31.4‰ for Lake Rotsee, which was close to or higher than its value for the pheopigments (as low as -36.1‰; Fig. 4a). Similarly, $\delta^{13}\text{C}$ values of pheopigments in Lake Cadagno were as low as ca. -36.2‰ and -33.6‰ in Lake Zurich. In contrast, $\delta^{13}\text{C}_{\text{Pphe-a}}$ value was -24.1‰ in the Black Sea. Those values are very similar to the $\delta^{13}\text{C}_{\text{bulk}}$ values with -34.5, -32.4 and -25.8‰ in the surface sediments of Lake Zurich, Lake Rotsee and the Black Sea, respectively (Fig. 4a).

The isotopic compositions of intact chlorophylls reflect those of the used substrates, assimilation pathways and growth conditions of the phototrophic communities (e.g.

Hayes et al., 1987; Ohkouchi et al., 2005; Ohkouchi et al., 2015; Sachs et al., 1999). Chl *a* and its derivatives should have a common isotopic composition if they are derived from a homogeneous pool of the substrate. However, the deviations of $\delta^{13}\text{C}$ values between Chl *a* and related pheopigments ranged from at least 1‰ and 2‰ in Lake Cadagno and Lake Zurich, respectively, up to 5‰ in Lake Rotsee (Fig. 4a).

Such a variation in $\delta^{13}\text{C}$ values among Chl *a* derivatives has been reported previously and originates partly from relatively large analytical errors associated with pigment analyses ($1\sigma = 0.8\text{‰}$ for $\delta^{13}\text{C}$). Furthermore, the often higher measured molar C/N ratios compared to the theoretical values (Section 3.3; Table 2) also suggest that part of the scatter may be explained by different contributions from non-pigment derived carbon that could not be completely removed during pigment isolation. However, only in Lake Rotsee analytical errors and uncertainties due to pigment impurities cannot fully explain the observed scatter among chloro- and pheopigments and the underlying processes of these variations are still incompletely understood.

One explanation is lateral transport of OM in the water column, because such OM potentially contains older particles and therefore more degraded species like PPhe *a* (Junium et al., 2015; Ohkouchi and Takano, 2014). However, at least the supply of Chl *a* from the lake catchment should be minor due to the rapid (both biological and chemical) degradation of the intact pigments in soils, because of enhanced radical degradation during the senescence of leaves, which leads, for instance, to the opening of the tetrapyrrole ring (Matile et al., 1996). The contributions of the more degraded derivatives (e.g. Pheo *a* and PPhe *a*), however, should be most pronounced in Lake Cadagno due to the high supply of terrestrial OM (Wirth et al., 2013).

Alternatively, the scatter may also be explained by varying contributions of recycled carbon used as substrate, as indicated by the low $\delta^{13}\text{C}_{\text{bulk}}$ values in Lake Zurich (-34.5‰) and Lake Rotsee (-32.4‰) as well as $\delta^{13}\text{C}_{\text{DIC}}$ values as low as -8 and -13‰ in surface water of Lake Rotsee and the surface sediment of Lake Cadagno, respectively. In addition to fast carbon turnover, the active methane cycle in Lake Rotsee (Naeher et al., 2014; Schubert et al., 2010) and Lake Cadagno (Milucka et al., 2015; Schubert et al., 2011) further explains the low $\delta^{13}\text{C}_{\text{DIC}}$ values in these lakes. Lake Rotsee is further characterised by a highly diverse phytoplankton community with blooms of different communities at different times during the year. Therefore, the isotope offsets between pigments may result from Chl *a* produced at different depths (cyanobacteria at <2.5 m vs. diatoms at 5-7 m) and different seasons (diatoms in spring vs. cyanobacteria in fall). Indeed, this interpretation agrees with observations at other study sites, where stable isotope variations were interpreted to be driven by changes in algal productivity and community shifts (e.g. Kusch et al., 2010; Tyler et al., 2010). In contrast to Lake Rotsee, the low scatter of $\delta^{13}\text{C}$ values of Chl *a* and its derivatives in Lake Zurich and Lake Cadagno would therefore result from the large predominance of either cyanobacteria or algae, respectively, and low variations in the formation of the pheopigments throughout the year, despite the averaging of the accumulated sediment in each sample.

*3.5. Stable nitrogen isotopic signatures of Chl *a* and related pheopigments as indicators of the nitrogen source used by algae and cyanobacteria*

$\delta^{15}\text{N}_{\text{Chl-a}}$ values of -7.5 and -1.8‰ were lower than values for the associated pheopigments for Lake Rotsee and Lake Zurich, respectively (Fig. 4b). In contrast, the most ^{15}N -enriched Chl *a* derivative in both lakes was CPhe *a* with values of -1.0

and 4.8‰, respectively. In Lake Zurich, $\delta^{15}\text{N}_{\text{Pheo-a}}$ (0‰), $\delta^{15}\text{N}_{\text{PPhe-a}}$ (2.7‰) and $\delta^{15}\text{N}_{\text{Cphe-a}}$ (4.8‰) values were higher than in the other lakes and the Black Sea. Pheopigments in the Black Sea ranged between -5.1 and -1.0‰, whereas the $\delta^{15}\text{N}_{\text{PPhe-a}}$ value was -1.1‰ in Lake Cadagno. The low $\delta^{15}\text{N}$ values of some chlorins (as low as ca. -2‰, -8‰ and -5‰ in Lake Cadagno, Lake Rotsee and the Black Sea, respectively) are typical for chlorins produced in anaerobic water columns with values ranging between -8 and -2‰ and a typical average value of about -5‰ (Higgins et al., 2010; Ohkouchi et al., 2005; Ohkouchi and Takano, 2014; Sachs et al., 1999). For comparison, $\delta^{15}\text{N}_{\text{bulk}}$ values were 6.8 and 1.8‰ in the surface sediments of Lake Zurich and Lake Rotsee, respectively (Fig. 4b).

In Lake Zurich, the predominating non- N_2 -fixing cyanobacterium (*Planktothrix rubescens*) uses mainly nitrate as a nitrogen source (Jacquet et al., 2005), which is generally abundant in the water column (up to ca. 60 μM during full circulation in winter/spring between 1990-1999; Bossard et al., 2001). Posch et al. (2012) suggested that the low total phosphorus to nitrate ratio of lake water facilitates the *P. rubescens* blooms, which has led to the predominance of this cyanobacterium in the lake during the last decades. At least in culture experiments, Chl *a* derived from cyanobacteria is ~10‰ enriched in ^{15}N relative to whole cell (Higgins et al., 2011; Katase and Wada, 1990). Together with the high nitrate concentrations, this enrichment can explain the high $\delta^{15}\text{N}$ values of chlorins in Lake Zurich (Fig. 4b).

However, this mechanism cannot explain the $\delta^{15}\text{N}$ values of the total nitrogen in the lake, indicating that the nitrogen pool in the lake is enriched in ^{15}N relative to the other lakes. Similarly high bulk $\delta^{15}\text{N}$ values (up to ca. 8‰) have also been observed in Lake Biwa (Japan), which was explained by increased eutrophication due to higher fertiliser input into the lake and/or enhanced rates of denitrification (Ogawa et al., 2013; Ogawa et al., 2001). In Lake Zurich, Naeher et al. (in prep) showed that the

bulk $\delta^{15}\text{N}$ values remained constant (ca. 6-7‰) in the upper 25 cm of the core (since ca. 1920) and the OM supply from the lake catchment is low, suggesting that eutrophication cannot explain the high bulk $\delta^{15}\text{N}$ values. However, high denitrification rates in the anoxic hypolimnion and sediments may indeed lead to the high $\delta^{15}\text{N}$ values, as denitrification results in relative ^{14}N -depletion (e.g. Cabana and Rasmussen, 1994; Granger et al., 2011).

In Lake Cadagno, despite the high abundance of ammonium in the hypolimnion (up to 40 μM ; Halm et al., 2009), nitrate concentrations were low in the upper water column (up to 3 μM ; Halm et al., 2009). Due to the limitation of nitrogen available to phytoplankton in the epilimnion, Halm et al. (2009) suggested that N_2 fixation in the surface water must contribute to the nitrogen supply in the surface water of this lake, which may be reflected by the $\delta^{15}\text{N}_{\text{Pphe-a}}$ value of -1.1‰ in this lake. However, chlorophytes are predominant in the epilimnion (Bossard et al., 2001; Schanz and Stalder, 1998), which cannot fix N_2 and must depend on dissolved organic nitrogen (DON). This DON could be derived from ammonium assimilated by the biomass in the chemocline of the lake and supplied to the surface water.

In Lake Rotsee, nitrate is relatively abundant (up to ca. 10 μM) throughout the water column after mixing and during the first half of the year, but limited during the rest of the year (Schubert et al., 2010). In contrast, ammonium concentration reaches up to 300 μM in the hypolimnion during the stratified period in the second half of the year (Schubert et al., 2010). Therefore, in Lake Rotsee, both nitrate and ammonium are potential nitrogen sources for the phototrophic communities. Ammonium uptake by blooming algae lead to higher ^{15}N depletion (up to 15‰) than other nitrogen substrates (e.g. Ohkouchi et al., 2005), which are reflected by the low $\delta^{15}\text{N}$ values of Chl *a* and related pheopigments down to -7.5‰ (Fig. 4b). In contrast, cyanobacteria that are more abundant than algae only during blooms in fall, are dominated by *P.*

rubescens, which uses nitrate as discussed above for Lake Zurich and therefore leads to more ^{15}N enriched pigments than expected for those from purely ammonium-utilising algae. N_2 fixation is only be of minor importance, because only during blooms nitrogen availability can be limited in this lake (Bloesch, 1974) and the abundance of N_2 fixing cyanobacteria is low in the lake (Cantonal Office of Environment and Energy).

In the sediment from the Black Sea shelf edge, the low $\delta^{15}\text{N}$ values (-5 to 0‰) reported in Kusch et al. (2010) are similar to the results in the studied sample (ca. -5 to -1‰; Fig. 4b); both collected from a location near the shelf edge. The progressively higher values towards the river mouth in the former study may be interpreted to result from the higher supply of biologically available, ^{15}N enriched nitrogen derived from fertiliser through the Danube River. Kusch et al. (2010) interpreted the trend as the relative contribution of nitrate (relatively ^{15}N enriched) and N_2 fixation ($^{15}\epsilon_{\text{Chl/biomass}} = -9$ to 9‰; Higgins et al., 2011). However, Möbius and Dähnke (2015) showed that nitrate assimilation and not N_2 fixation is predominant on the Romanian Shelf, because the decreasing nitrate concentration with increasing distance off the coast is associated with progressively increasing $\delta^{15}\text{N}$ values of nitrate. Therefore, $\delta^{15}\text{N}$ values in the studied sample result predominantly from nitrate assimilation.

3.6. Biogeochemical processes mediated by phototrophic bacteria in Lakes Cadagno, Rotsee, and the Black Sea

$\delta^{13}\text{C}_{\text{BChl-a-derivatives}}$ values in Lake Rotsee and Lake Cadagno were as low as -43.9 and -42.8‰, respectively (Fig. 4c). These values agree well with the $\delta^{13}\text{C}$ value of hydrogenated okenone (-45‰) in the sediment of Lake Cadagno (Schaeffer et al., 1997).

Smith et al. (2015b) reported that BChl *a* in culture experiments is ¹³C-depleted relative to biomass (¹³ε_{BChl-a/biomass} value of ca. -4‰) and carbon dioxide (¹³ε_{BChl-a/CO₂} value of ca. -22‰). In contrast, the difference between δ¹³C_{DIC} values in the upper chemocline of Lake Rotsee (ca. -10 to -8‰; Fig. 5a; Schubert et al., 2010) and in the surface sediment of Lake Cadagno (ca. -8‰; Fig. 5b; Schubert et al., 2011) compared to the low δ¹³C_{BChl-a-derivatives} values (as low as -43.9‰; Fig. 4c) is higher than observed in cultures. The higher apparent fractionation in the lakes may be explained by the sharp gradients of δ¹³C_{DIC} values in the chemoclines of both lakes as well as the vertical migration of PSB in the water column (e.g. Pfennig, 1978), associated with DIC uptake from water depths with different δ¹³C values.

δ¹⁵N_{BChl-a-derivatives} values ranged between -1.5 and 0.1‰, and between -1.0 and 1.1‰ for Lake Cadagno and Lake Rotsee, respectively (Fig. 4d). These variations may be explained by the different timing of pheopigment formation as discussed in Section 3.4. In Lake Rotsee, these variations may also be the result of seasonal changes in water column stratification, which cannot explain the variations observed in the meromictic Lake Cadagno. The vertical migration of PSB (e.g. Overmann, 2008) further complicates the interpretation of these data, because these bacteria may assimilate different nitrogen sources from different water depths in both epi- and hypolimnion.

BChl *a* derived from the phototrophic purple nonsulfur bacterium *Rhodobacter capsulatus* is depleted in ¹⁵N relative to biomass with average ¹⁵ε_{BChl-a/biomass} values of ca. -8‰ for N₂ fixation and -10‰ for ammonium assimilation (Beaumont et al., 2000). Similarly, average ¹⁵ε_{BChl-a/biomass} values of the purple nonsulfur bacterium *Rhodospseudomonas palustris* were ca. 12‰ and also independent on the substrate (Higgins et al., 2011). In these bacteria nitrogen isotopic fractionation during BChl *a* synthesis seems to be almost independent of the assimilated substrate, whereas at

least in *R. capsulatus* the fractionation of ^{15}N of the biomass relative to the substrate ranged from $^{15}\epsilon_{\text{biomass}/\text{N}_2}$ values of -2 to -1‰ and from $^{15}\epsilon_{\text{biomass}/\text{ammonium}}$ values of -12 to -10‰ (Beaumont et al., 2000). Beaumont et al. (2000) and Higgins et al. (2011) consider another type of organisms and their data originate from a different environment, so more research is required to understand the nitrogen isotopic fractionation during BChl *a* synthesis of PSB. However, their data may explain the similarity of $\delta^{15}\text{N}_{\text{BChl-}a\text{-derivatives}}$ derived from PSB in both lakes, which indicates that the nitrogen isotopic composition of BChl *a* derivatives does not reflect the $\delta^{15}\text{N}$ signature of the nitrogen source pool and is therefore not diagnostic of the assimilated nitrogen source of PSB.

The origin of BChls *e* from GSB suggests that the related chlorins are ~10‰ enriched in ^{13}C relative to algae and PSB derived pigments, which can be explained by the fact that GSB fix carbon dioxide through the reverse tricarboxylic acid cycle and not the Calvin cycle (Ohkouchi et al., 2005; Quandt et al., 1977; Sirevåg et al., 1977; Smith et al., 2015a). However, the relative ^{13}C enrichment of these pigments depends on the $\delta^{13}\text{C}_{\text{DIC}}$ values of the hypolimnion of the anoxic lakes (Fig. 5) and the Black Sea.

In Lake Cadagno, the BChl *e* derivatives were at least 6‰ depleted in ^{15}N relative to BChl *a* derivatives, ranging between -9.8 to -8.2‰ (Fig. 4d). Further, BChls *e* were at least 7‰ depleted in ^{15}N relative to PPhe *a* (Figs. 4b, 4d). This result agrees with observations in Lake Kaiike, where BChls *e* were up to 8‰ depleted in ^{15}N relative to Chl *a* (Ohkouchi et al., 2005). GSB thrive only in the deeper part of the chemocline and in the hypolimnion, where the availability of ammonium is much higher than for PSB in the upper chemocline. Therefore, the slow ammonium assimilation resulting from the slow growth rate of GSB can explain the relatively ^{15}N -depleted values of the BChl *e* derivatives. Moreover, Halm et al. (2009) could, for the first time in

environmental samples, link N₂ fixation in Lake Cadagno directly to GSB. Indeed, the low $\delta^{15}\text{N}$ values (-9.8 to -8.2‰) of the BChl *e* derivatives (Fig. 4d) agree well with a value of -9‰ observed for N₂ fixation in Lake Karike (Ohkouchi et al., 2005).

4. Summary

The pigment distributions comprised at all study sites Chl *a* and its derivatives Pheo *a*, Pheo *a* epimer, PPh_e *a*, CPh_e *a*, Chlone *a* as well as steryl and carotenol chlorin esters. Furthermore, Lake Cadagno, Lake Rotsee and the Black Sea are characterised by photic zone euxinia, explaining the presence of a series of BChls *e* and BPhes *e* derived by brown strains of GSB. In both Lakes Cadagno and Rotsee, BChl *a* derivatives indicated the presence of PSB. The pigment distributions in the surface sediments of all four systems illustrate rapid transformation of intact chloro- and bacteriochlorophylls to their related pheopigments.

Apart from the analytical error of pigment analysis, the scatter of $\delta^{13}\text{C}$ values of chlorins results from the different timing of pheopigment formation, the predominance of various phytoplankton communities producing Chl *a* in the lakes with blooms at different times (associated with varying degrees of carbon limitation), different contributions of recycled OM, or a combination of these factors.

In Lake Zurich, the $\delta^{15}\text{N}$ values of the chlorins suggested that the nitrogen substrate for the primary producers (dominated by non-N₂-fixing cyanobacteria) was nitrate. In Lake Cadagno, dissolved organic nitrogen (DON) supplied to the surface water from ammonium assimilation in the chemocline may be the predominant nitrogen source for the phytoplankton, whereas in Lake Rotsee ammonium and nitrate assimilation by algae and cyanobacteria, respectively, explain the observed $\delta^{15}\text{N}$ signatures of Chl *a* and related pheopigments. At the Romanian Black Sea Shelf edge, the predominant

nitrogen source of phytoplankton seems nitrate. The low $\delta^{13}\text{C}$ values (-44 to -43‰) of the BChl *a* derivatives in Lakes Cadagno and Rotsee agree with a source from PSB in both lakes, whereas the $\delta^{15}\text{N}$ values of the BChl *a* derivatives could not be used to reconstruct their nitrogen source due to the very similar nitrogen isotope fractionation for different nitrogen sources during BChl *a* synthesis. In contrast, the ^{15}N depletion of BChl *e* derivatives relative to Chl *a* derivatives in Lake Cadagno may be due to the very slow growth rates of GSB, resulting from the slow assimilation of nitrogen from ammonium, as well as from N_2 fixation GSB in this lake.

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Figure captions

Fig. 1. High performance liquid chromatography - photodiode array detection (HPLC-DAD) chromatograms (660 nm signal in green, 750 nm signal in red) showing pigment distributions analysed in the total lipid extracts obtained from sediments of a) Lake Zurich (0-2 cm depth), b) Lake Rotsee (0-4 cm depth), c) Lake Cadagno (633-648 cm depth), d) Romanian Black Sea Shelf (0-2 cm depth). Abbreviations indicate identified compounds comprising chlorophyll *a* (Chl *a*), pheophytin *a* (Pheo *a*), pheophytin *a* epimer (Pheo *a* epimer), pyropheophytin *a* (PPhe *a*), 13²,17³-cyclopheophorbide-*a*-enol (CPhe *a*), chlorophyllone *a* (Chlone *a*), bacteriochlorophylls *a* and *e* (BChls *a*, *e*) and bacteriopheophytins *a* and *e* (BPhe *a*, *e*), steryl and carotenol chlorin esters (SCEs and CCEs). Circles indicate parts of the chromatograms where BChls *e* and BPhe *e* were detected.

Fig. 2. High performance liquid chromatography - photodiode array detection (HPLC-DAD) chromatogram of the second isolation (fraction collection) of the collected chlorophyll *a* (Chl *a*) peak in Figure 1 b. This chromatogram comprises the signals at wavelength of 660 nm (green) and 750 nm (red), illustrating the separation of the coeluting peaks of bacteriopheophytin *a* (BPhe *a*) and chlorophyll *a* (Chl *a*). Prior to analysis, Chl *a* was transformed to pheophytin *a* (Pheo *a*) by hydrochloric acid both compounds collected as separate fractions (details in Section 2.2). The UV-VIS spectrum of the isolated BPhe *a* is also shown, normalised to the highest peak of the spectrum. The numbers correspond to the wavelengths (nm) of the absorption maxima.

Fig. 3. High performance liquid chromatography - photodiode array detection (HPLC-DAD) chromatogram (660 nm signal) corresponding to the third isolation (fraction collection) of bacteriochlorophylls *e* (BChls *e*) and bacteriopheophytins *e* (BPhes *e*) from the fraction collected between 3 and 6 min in the original chromatogram (Fig. 1) of the Lake Cadagno sediment sample (details in Section 2.2). Numbers in a) refer to detected BChls *e* (1-7) and BPhes *e* (8-12) homologues.

UV-VIS spectra (range of 400-800 nm) of b) bacteriochlorophylls *e* (BChls *e*) and c) bacteriopheophytins *e* (BPhes *e*) in the Lake Cadagno sample with peak numbers correspond to the numbers in the chromatogram shown in a). The spectra are normalised to the peak with the highest absorption intensity (460 nm or 440 nm). Numbers in b) and c) correspond to the wavelengths of the diagnostic absorption maxima of BChls *e* and BPhes *e*.

Fig. 4. a, c) $\delta^{13}\text{C}$ values (‰ vs. VPDB) and b, d) $\delta^{15}\text{N}$ values (‰ vs. AIR) of isolated chlorophyll *a* (Chl *a*) and its pheopigments and derivatives of bacteriochlorophylls (BChls) *a* and *e* analysed in the samples from Lake Cadagno, Lake Rotsee, Lake Zurich and the Black Sea.

Fig. 5. a) Water column $\delta^{13}\text{C}_{\text{DIC}}$ profile (‰ vs. VPDB) of Lake Rotsee adapted from Schubert et al. (2010), also published in Naeher et al. (2014). The shaded area illustrates the depth variability of the chemocline in Lake Rotsee according to Schubert et al. (2010). b) $\delta^{13}\text{C}_{\text{DIC}}$ profile (‰ vs. VPDB) of Lake Cadagno sediments adapted from Schubert et al. (2011).

Appendix

Fig. A1. Structures of chlorins discussed in this study.

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Table 1

Retention times (min) and diagnostic, maximum UV-VIS absorption bands (nm) of chlorophyll *a* and its derivatives chlorophyllone (Chlone *a*), 13²,17³-cyclopheophorbide-*a*-enol (CPhe *a*), pheophytin *a* (Phe *a*), pheophytin *a* epimer (Phe *a* epimer), pyropheophytin *a* (PPhe *a*) as well as those of bacteriopheophytin *a* and other bacteriochlorophyll *a* (BChl *a*) derivatives. The retention times indicated with * correspond to the second isolation step (Section 2.2).

| Retention time (min) | Pigment | UV-VIS absorption bands (nm) |
|----------------------|--------------------------|------------------------------|
| 3.9 | Chlone <i>a</i> | 409, 609, 666 |
| 11.2 | CPhe <i>a</i> | 359, 425, 451, 628, 686 |
| 15.8 | Chl <i>a</i> | 430, 617, 663 |
| 22.6 | Pheo <i>a</i> | 408, 505, 608, 665 |
| 22.7 | Pheo <i>a</i> epimer | 409, 505, 608, 665 |
| 30.8 | PPhe <i>a</i> | 410, 507, 609, 666 |
| 15.8 | BPhe <i>a</i> | 357, 524, 746 |
| 10.5* | BChl <i>a</i> derivative | 358, 523, 748 |
| 22.5* | BChl <i>a</i> derivative | 358, 523, 748 |

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Table 2

$\delta^{13}\text{C}$ (‰ vs. VPDB), $\delta^{15}\text{N}$ values (‰ vs. AIR), measured and theoretical molar carbon to nitrogen (C/N) ratios of chlorins analysed in the sediments of Lake Cadagno, Lake Rotsee, Lake Zurich and the Romanian Black Sea shelf. Chlorin names are abbreviated as chlorophyll *a* (Chl *a*), pheophytin *a* (Phe *a*), pheophytin *a* epimer (Phe *a* epimer), pyropheophytin *a* (PPhe *a*), 13²,17³-cyclophosphoride-*a*-enol (CPhe *a*), bacteriochlorophylls *a* and *e* (BChls *a*, *e*) and bacteriopheophytins *a* and *e* (BPhes *a*, *e*).

| Study site | Chlorin name | $\delta^{15}\text{N}$ (‰ AIR) | $\delta^{13}\text{C}$ (‰ VPDB) | Measured molar C/N ratio (-) | Theoretical molar C/N ratio (-) |
|--------------|--|----------------------------------|-----------------------------------|---------------------------------|--|
| Lake Cadagno | PPhe <i>a</i> | -1.1 | -36.2 | 15.7 | 13.3 |
| | BChl <i>a</i> derivative (elution after Phe <i>a</i>) | 0.1 | -42.8 | 13.6 | 13.8 |
| | BChl <i>e</i> derivative | -8.2 | | 21.0 | 12.8 |
| | BPhe <i>e</i> ₁ + <i>e</i> ₂ + <i>e</i> ₃ | -9.8 | | 17.4 | 12.8 (<i>e</i> ₁), 13.0 (<i>e</i> ₂), 13.2 (<i>e</i> ₃) |
| Lake Rotsee | Chl <i>a</i> | -7.5 | -31.4 | 14.6 | 13.8 |
| | Phe <i>a</i> | -6.4 | -36.1 | 17.4 | 13.8 |
| | Phe <i>a</i> epimer | -3.9 | | 19.0 | 13.8 |
| | PPhe <i>a</i> | -2.8 | -35.5 | 13.9 | 13.3 |
| | CPhe <i>a</i> | -1.0 | -31.6 | 8.8 | 8.0 |
| | BPhe <i>a</i> | -1.0 | | 23.7 | 13.8 |
| | BChl <i>a</i> derivative (elution after Chl <i>a</i>) | 1.1 | -43.9 | 13.2 | 13.8 |
| Lake Zurich | Chl <i>a</i> | -1.8 | | 22.4 | 13.8 |
| | Phe <i>a</i> | 0.0 | -33.6 | 15.1 | 13.8 |
| | PPhe <i>a</i> | 2.7 | | | 13.3 |
| | CPhe <i>a</i> | 4.8 | -33.6 | 10.1 | 8.0 |
| Black Sea | Phe <i>a</i> | -1.0 | | 19.1 | 13.8 |
| | PPhe <i>a</i> | -5.0 | -24.1 | 14.8 | 13.3 |
| | CPhe <i>a</i> | -5.1 | | 12.2 | 8.0 |

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Figure 1

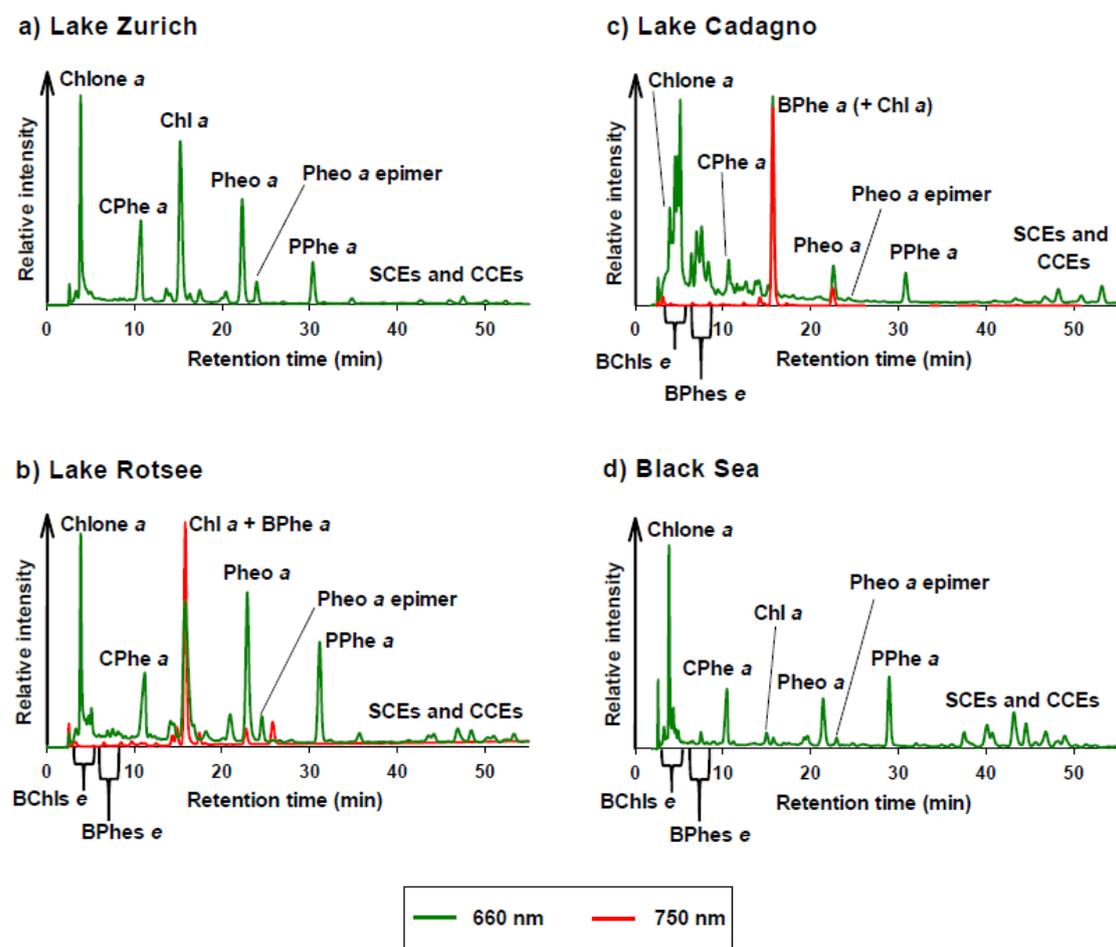


Figure 2

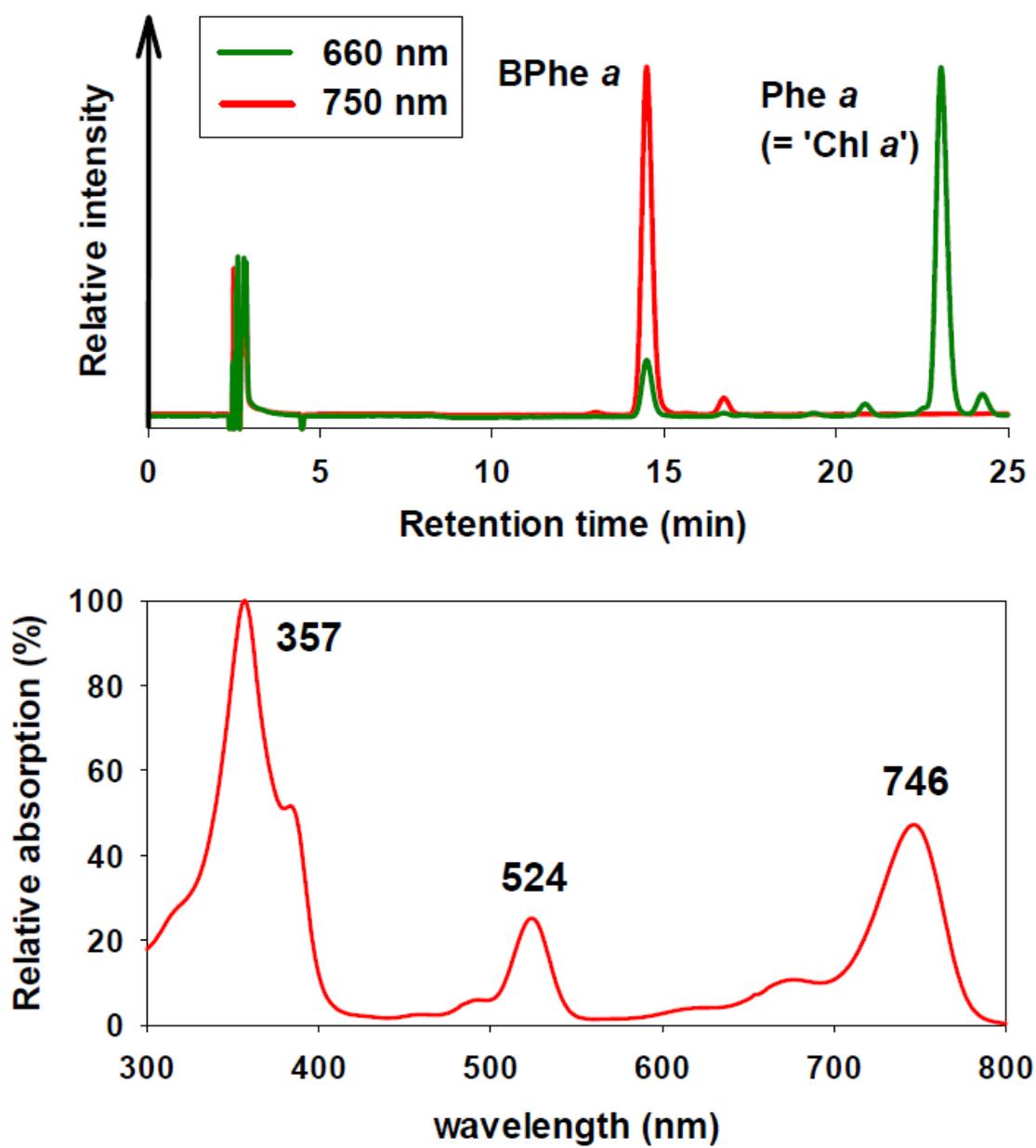


Figure 3

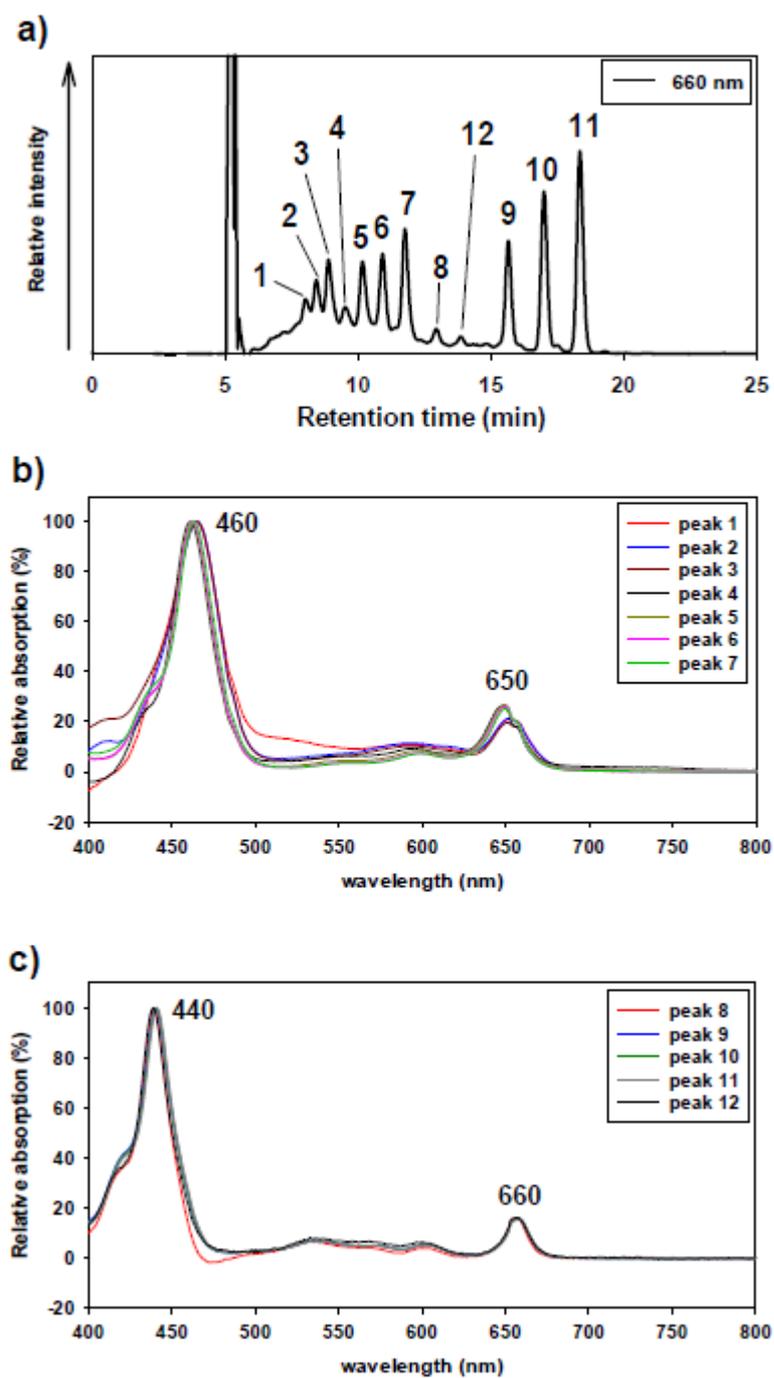
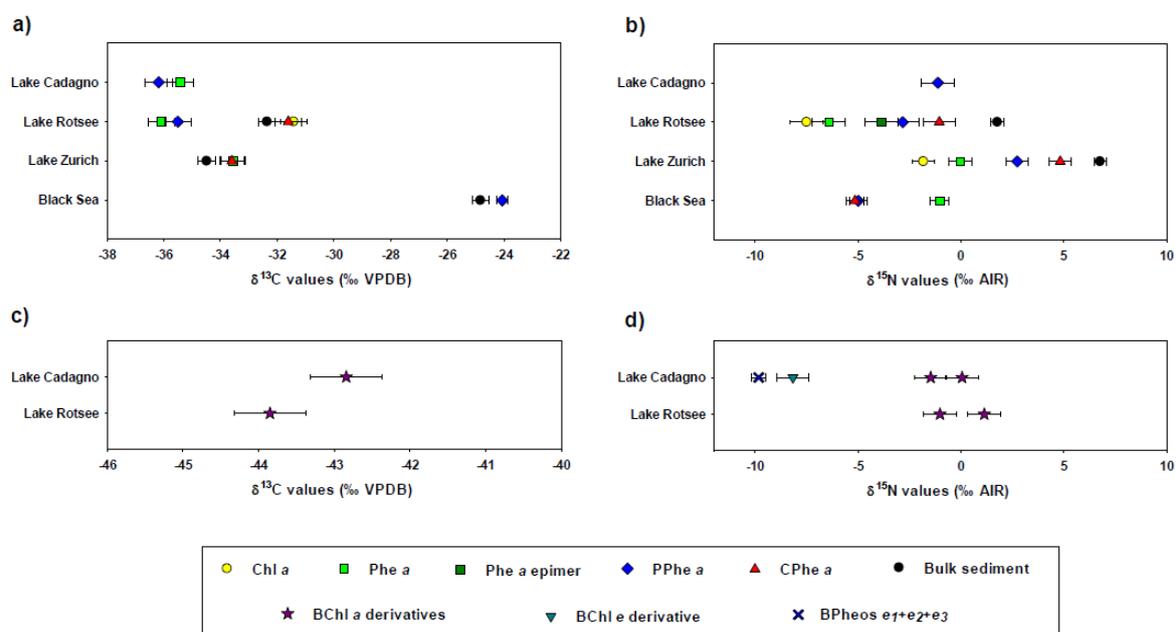


Figure 4



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Figure 5

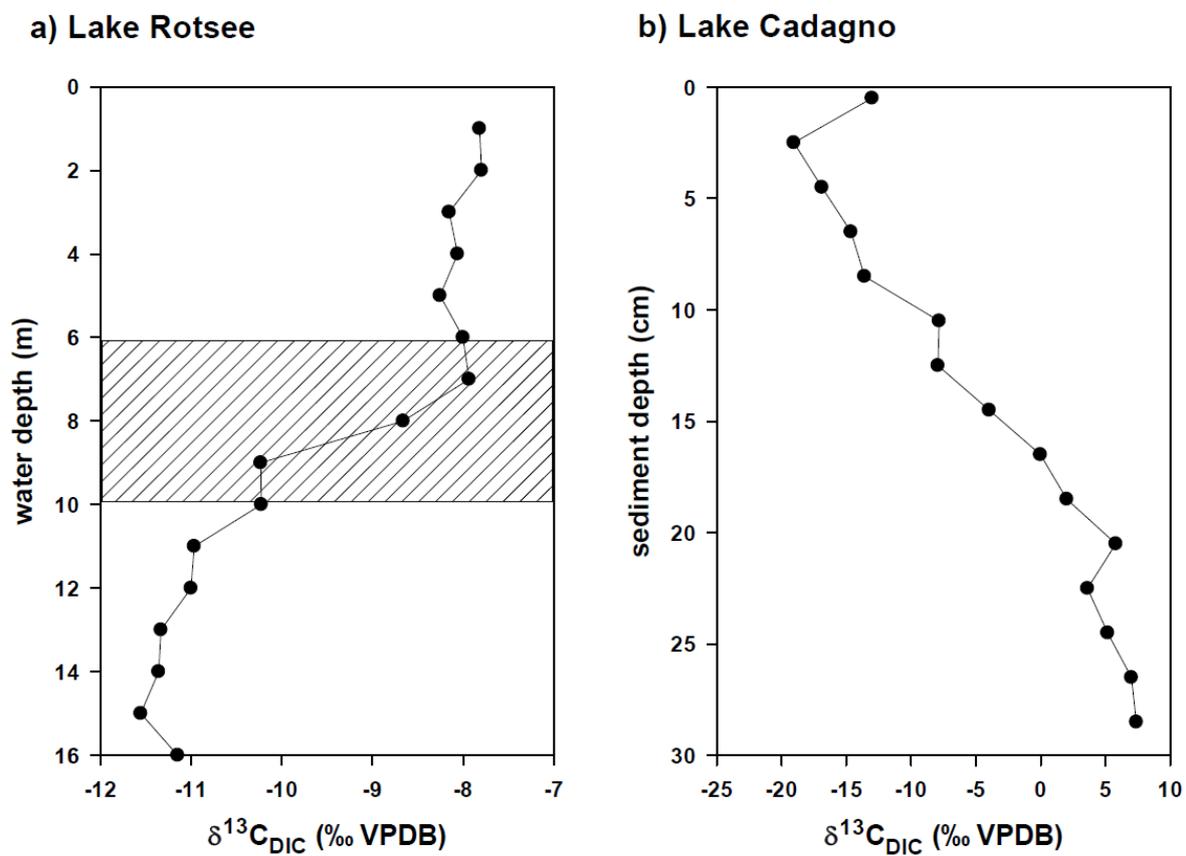
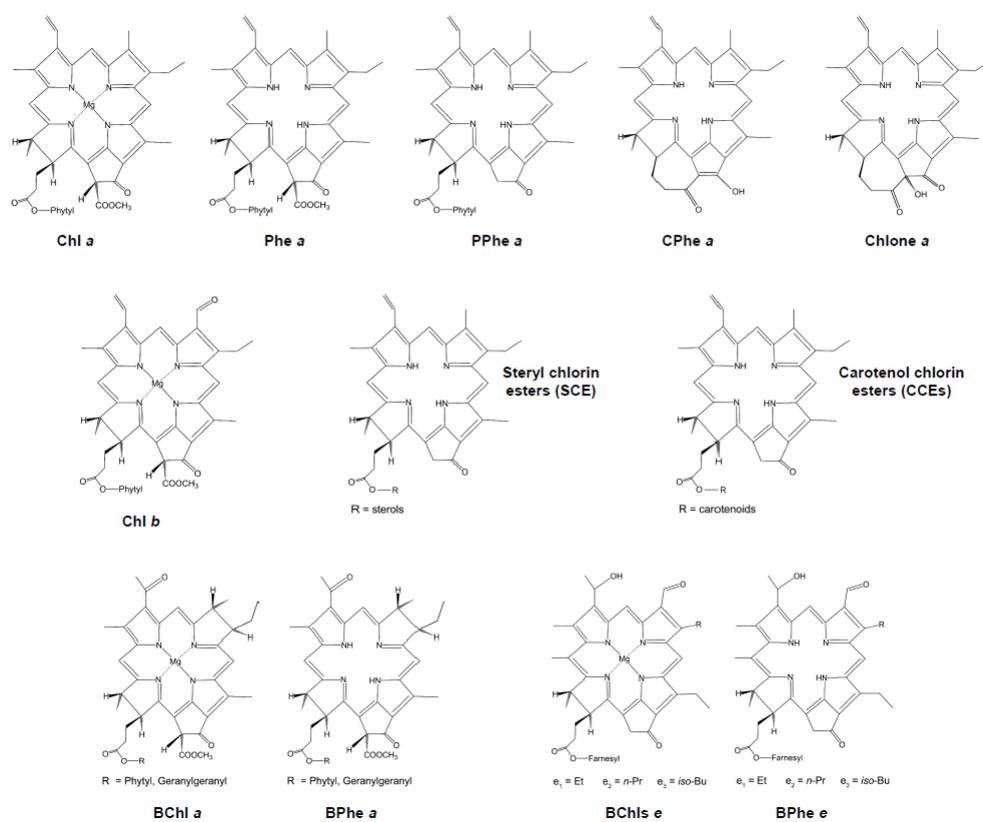


Fig. A1.



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