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Lipid biomarkers in Holocene and glacial sediments from ancient Lake Ohrid (Macedonia, Albania)

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Abstract

Organic matter preserved in Lake Ohrid sediments originates from aquatic and terrestrial sources. Its variable composition reflects climate-controlled changes in the lake basin's hydrology and related organic matter export, i.e. changes in primary productivity, terrestrial plant matter input and soil erosion. Here, we present first results from lipid biomarker investigations of Lake Ohrid sediments from two near-shore settings: Site Lz1120 near the southern shore, with flat lands nearby and probably influenced by river discharge, and site Co1202 which is close to the steep eastern slopes. Variable proportions of terrestrial *n*-alkanoic acids and *n*-alkanols as well as compositional changes of ω-hydroxy acids document differences in soil organic matter supply between the sites and during different climate stages (glacial, Holocene, 8.2 ka cooling event). Changes in the vegetation cover are suggested by changes in the dominant chain length of terrestrial *n*-alkanols. Effective microbial degradation of labile organic matter and in situ contribution of organic matter derived from the microbes themselves are both evident in the sediments. We found evidence for anoxic conditions within the photic zone by detecting epicholestanol from sulphur-oxidising phototrophic bacteria and for the influence of an early human community from the occurrence of coprostanol, a biomarker for human and cattle faeces, in an early Holocene sample. This study illustrates the potential of lipid biomarkers for future environmental reconstructions using one of Europe's oldest continental climate archives, Lake Ohrid.

1 Introduction

Ancient Lake Ohrid is special for a number of reasons. As one of the oldest lakes in the World it potentially preserves the oldest continuous archive of environmental change in Europe, dating back 3–5 million years (Wagner et al., 2008, 2009). It hosts more than 200 endemic species which makes it a unique ecosystem in Europe and, taking its size into account, is the most diverse lake in the world (Albrecht and Wilke, 2008).

Finally, it is situated in an intramontaneous basin that belongs to one of only three Mediterranean refugia that were vital for the survival of important groups of plants such as deciduous trees through the hostile climate conditions of the Pleistocene glaciations, the other two being the southern Iberian Peninsula and southern Italy (Brewer et al., 2002). It is, therefore, of value for the understanding of the variability of the dominant continental and Mediterranean climate regimes in the southern Balkans over time and the hydrological conditions required for a refuge as well as the evolution of endemic species. The sediments of Lake Ohrid provide a unique archive to obtain a maximum of information on environmental change in high-resolution.

Organic geochemistry provides powerful tools to reconstruct environmental change based on the variable supply of organic matter (OM) from aquatic and terrestrial sources such as phytoplankton, bacteria, macrophytes, land plants and soils as well as anthropogenic sources. This is particularly helpful in settings where the preservation of microfossils is poor. In Lake Ohrid calcifying ostracods were rarely or not at all preserved, for example, during the last glacial period (Wagner et al., 2009; Belmecheri et al., 2009). Changing proportions of OM from the various autochthonous (aquatic) and allochthonous (terrestrial) sources in Lake Ohrid sediments are driven by the hydrological dynamics of the Ohrid Basin. Production of aquatic biomass, for instance, depends on the availability of nutrients that are introduced to the lake mainly through terrestrial run-off. Input from adjacent Lake Prespa, to which Lake Ohrid is connected via karst systems, is negligible since karstic and groundwater sources are depleted in nutrients (Matzinger et al., 2006). Without any major river entering the basin, supply of allochthonous OM depends entirely on the hydrology of the Ohrid Basin itself. Export of plant litter, humus and soil OM from the terrestrial biosphere is controlled by surface drainage and thus depends on local precipitation patterns, vegetation cover, evaporation, moisture storage capacities and soil stability. Here, we provide evidence for changing contributions from the various OM pools to the bulk sedimentary OM under contrasting climatic stages using source-specific lipids.

2 Material and methods

Two sediment cores, Lz1120 and Co1202, were taken in 2005 and 2007 from a floating platform using a gravity corer for undisturbed surface sediments and a piston corer for deeper sediments (see Fig. 1 for core positions). Site Lz1120 is situated in the southeastern area of the lake at 105 m water depth. The southern shores are less steep than the mountain ranges to the east and include flat areas that once presumably have been flooded. Also nearby are the karst springs of Tushemisht and Sveti Naum (Fig. 1). These and further subaquatic karst springs in the area are fed by overflow from neighbouring Lake Prespa and account for about 50% of the hydrological inflow of the lake. The Cerava River entering the lake to the south of the site contributes less than 1% to the total inflow today (Matzinger et al., 2007). Site Co1202 is located in the northeast in 145 m water depth. Sedimentation rates at site Lz1120 (39 cm/1000a) are generally higher than at site Co1202 (22 cm/1000a) due to the fact that wind-induced surface currents rotating counter-clockwise (cf. Vogel et al., 2010) increase the supply of terrestrial material from the southern shores towards Lz1120 but prevent terrestrial material entering the lake in the Northeast, e.g. by the Koselska River, from reaching site Co1202 (Fig. 1). Composite sediment successions have been obtained by overlapping 3 m core sections, resulting in contiguous sediment sequences of 11.51 m (Lz1120) and 14.94 m (Co1202), respectively. Stratigraphy based on tephrochronology and radiocarbon dates (^{14}C) has been established for core Lz1120 (Wagner et al., 2009) as well as for core Co1202 (Vogel et al., 2010b). Accordingly, core Lz1120 dates back to ~40 ka with a hiatus between 14.6 and 9.4 ka. Core Co1202 dates back to 135 ka, with a hiatus between 82 and 97 ka.

We have chosen a limited sample set for this study in order to understand how natural variability generally affects biomarker composition in Lake Ohrid. The criteria of sample selection were based on the existing data of carbonate and OM sedimentation (Fig. 2, data from Wagner et al., 2009). The records of carbonate ($CaCO_3$) and total organic carbon (TOC) of Lz1120 and Co1202 show climatically controlled environmental

changes: almost carbonate-free sediments during the glacial and generally carbonate-rich sediments during the Holocene (Fig. 2). Climate changes of shorter duration and moderate intensity such as the prominent 8.2 ka cooling event also appear in the carbonate and TOC records of both cores. Even though the $CaCO_3$ and TOC minimum around 8 ka occurs somewhat later under the present stratigraphic model at Co1202 than at Lz1120, the almost identical patterns, with a small $CaCO_3$ peak just before the minimum and a strong subsequent increase, suggest that they derive essentially from the same event. Accordingly, we have chosen six samples from site Lz1120: one surface sample, four samples from the Holocene core sections, including one corresponding to the 8.2 ka event, and one glacial sample. Three samples were investigated from core Co1202: one that presumably represents the 8.2 ka event and two from the immediately predating and following Holocene sediment sections. The sediment samples were freeze-dried and homogenised. Total carbon (TC), total nitrogen (TN) and total sulphur (TS) contents were measured using a Vario Micro Cube. TOC was determined after acid digestion of the carbonate fraction (with hydrochloric acid, 16%). Weight percentages of $CaCO_3$ were then calculated from the difference between TC and TOC measurements using the equation $CaCO_3$=(TC-TOC)$\times M_{CaCO3}/M_C$; $M_{CaCO3}/M_C \approx 8.33$, M = molar mass.

Lipids were extracted from an aliquot of sediment (1–2 g) which was sonicated in a solvent mix of dichloromethane and methanol (9:1; 45 min). The total lipid extract (TLE) was concentrated and passed through sodium sulphate (anhydrous) to remove any remaining water. Free and bound acids were then trans-methylated by adding a solution of acetyl chloride in methanol (1:30) and leaving the samples at 45 °C (12 h). The extracts were then passed through potassium carbonate which removes excess acids (acetic acid, hydrochloric acid). Finally, compounds containing hydroxy groups were derivatised by adding N,O-bis(trimethyl-silyl)trifluoroacetamide (with 1% trimethylchlorosilane) and leaving the samples at 65 °C for 30 min.

GC-MS analyses of the TLE were carried out using a Trace 2000 Series gas chromatograph (GC) fitted with a J&W Scientific DB-5MS capillary column (60 m, 0.25 mm

i.d.; 5% phenyl/95% methyl polysiloxane equivalent phase, 0.1 µm film thickness; carrier gas: helium at 1.6 ml min^{-1}; on-column injector). The oven temperature was programmed from 60 °C to 170 °C at 6 °C min^{-1} after 1 min, then to 315 °C at 2.5 °C min^{-1} and held for 10 min. The column was fed directly into a Thermoquest Finnigan TSQ 7000 mass spectrometer (MS). Typical operating conditions were: ionisation potential 70 eV; source temperature 215 °C; trap current 300 µA. Mass data were collected at a resolution of 600, cycling every second from 50–600 Thompsons. Organic compounds were identified according to their mass spectrum and relative retention time compared to reference standards. They were then quantified by relating their peak area to the peak area of the internal standard, 5α-(H)-cholestane, of which a known amount was added to the samples prior to extraction. The relative response factors of the analytes were determined individually for 35 representative alkanoic acids, *n*-alkanols and sterols using authentic standards. Response factors for analytes where standards were unavailable were assumed to be identical to those of available compounds of the same class.

3 Results

3.1 Elemental analysis

TOC contents at site Lz1120 range from 0.5 to 2.9% over the past 20 ka (Fig. 2, data according to Wagner et al., 2009). Sediment samples from the cold climate stage prior to the hiatus (20–17.4 ka) generally contain <1% TOC. Highest TOC contents of ~3% are observed in early Holocene samples (9.4–8.8 ka). Carbonate contents range from 0 to 81% with values close to zero prior to the hiatus (Fig. 2). The Holocene section shows generally high values, with two remarkable drops, one around 8.2 ka and another around 4 ka (Fig. 2). At site Co1202 both TOC and carbonate contents are in a very similar range and show a similar pattern compared to Lz1120. Even though there are some leads and lags between both records, which most likely result from

uncertainties of the age models of both cores, changes in carbonate sedimentation can clearly be correlated. In contrast to Lz1120, however, there is no early Holocene maximum in TOC contents at site Co1202 (Fig. 2; Vogel et al., 2010a).

The elemental data of the investigated samples is summarised in Table 1. The TOC/TN ratios are generally higher at Lz1120 (average: 9) compared to Co1202 (average: 7) suggesting that terrestrial input is higher at the southern position. The lowest values at both sites are observed in the samples representing the 8.2 ka event and the glacial, presumably due to the fact that these climate stages were drier and terrestrial run-off reduced. The TOC/TS ratio reveals a very similar pattern, with generally lower values at site Co1202 and minimum values during the drier climate stages, which may result from both changing OM quantities supplied to the sediments and different proportions of terrestrial and aquatic OM as well as different levels of OM degradation.

3.2 Lipid biomarkers

We quantified straight, branched, mono-unsaturated and hydroxy fatty acids/alkanoic acids (as fatty acid methyl esters), *n*-alkanols (alcohols), *n*-alkanes, sterols and some miscellaneous compounds such as C_{15} branched alkanol, β-amyrin, 17β(H),21β(H)-bishomohopanoic acid and 17β(H),21β(H)-bishomohopanol. The amounts of compounds and groups of compounds relevant for detailed discussion as well as their sources are listed in Table 2 (incl. references). A detailed list of all individually quantified compounds is available as supplementary data. In the following, amounts of lipid compounds will be given as percentages either of the TOC content ($\%_{TOC}$) or of the TLE ($\%_{lipids}$).

The amounts of extracted lipids from the Holocene samples of Co1202 are lower (0.8–1.5 $\mu g_{lipids}/g_{Sed}$) than those from Lz1120 (0.9–6.3 $\mu g_{lipids}/g_{Sed}$) mirroring the differences in TOC contents. The lowest concentration of lipids (0.5 $\mu g_{lipids}/g_{Sed}$), however, was found in the glacial sample from Lz1120 (Table 2). The samples presumed to represent the 8.2 ka event from both cores also reveal lower concentrations of lipids. The overall lower percentages of extractable lipids relative to the TOC content in samples

from Co1202 suggest a higher proportion of non-extractable organic carbon in stable biopolymers such as lignin or cellulose in terrestrial plant tissues or black carbon. High amounts of extractable lipids in the surface sample of Lz1120 (3.2%$_{TOC}$), on the other hand, result from a high amount of labile organic compounds such as the mono-unsaturated alkanoic acids (0.4%$_{TOC}$) and branched alkanoic acids (0.1%$_{TOC}$) that are not completely degraded by microbial consumption yet although this process is most intense near the sediment-water interface.

The major compound classes of the TLE's from Lz1120 and Co1202 are *n*-alkanoic acids and *n*-alkanols (Fig. 3), together accounting from 49%$_{lipids}$ (surface sample, Lz1120) to 91%$_{lipids}$ (7.9 ka, Co1202). Differences in their proportions are evident between the two sites as well as between the Holocene samples and the samples representing the glacial and the 8.2 ka event (Fig. 3). However, there were more subtle differences in the contribution of minor compounds and, more important, in the distribution of single compounds within the various compound classes (e.g., long-chain vs. short-chain alkanoic acids).

3.2.1 *n*-Alkanoic acids (fatty acids, FA)

Highest proportions of saturated *n*-alkanoic acids (*n*-fatty acids, C_x FA) occur in the Holocene samples 252 and 246 of core Co1202 accounting for 62 and 63% of the total lipids, respectively (Fig. 3, Table 2). By far lowest proportions in each core were found in the samples representing the 8.2 ka event at both sites, 23%$_{lipids}$ (Lz1120, sample 505) and 27%$_{lipids}$ (Co1202, sample 248), as well as in the glacial sample from Lz1120 (sample 643) with 25%$_{lipids}$ saturated *n*-alkanoic acids (Fig. 3). The observed chain lengths of the *n*-alkanoic acids range from C_{14} to C_{32}. Most samples show a bimodal *n*-alkanoic acid distribution with maxima for *n*-C_{16} and *n*-C_{24} or *n*-C_{26} FA (Fig. 4). Short-chain FA's, in particular *n*-C_{16} and *n*-C_{18} FA, derive mainly from phytoplankton whereas long-chain *n*-alkanoic acids (C_{22}-C_{32} FA) derive from leaf waxes of terrestrial plants (e.g., Meyers and Ishiwatari, 1993; Ficken et al., 2002). Highest proportions of phytoplankton-derived C_{16} and C_{18} FA relative to terrestrial C_{24}-C_{28} FA's

were observed in sample 505 from Lz1120 and sample 248 from Co1202, representing the 8.2 ka event (Fig. 4). Terrestrial FA's appear strongly depleted in these samples. Among the long-chain terrestrial fraction, where present, the dominating *n*-alkanoic acids are *n*-C_{24} and *n*-C_{26} FA. While *n*-C_{24} dominates in the surface sample (Lz1120) and the samples representing the 8.2 ka event, *n*-C_{26} FA either dominates or is present in equal concentrations in the remaining samples. This quasi alternating behaviour suggests two terrestrial organic matter sources with slightly different maxima in their *n*-alkanoic acid distributions.

3.2.2 Branched alkanoic acids (branched FA)

Branched alkanoic acids were detected in relatively high amounts in the surface sample of Lz1120 and in small amounts in the Holocene samples. They were absent, however, in the glacial sample and in the three samples from site Co1202 (Table 2). Iso- and anteiso-C_{15} FA are the most abundant and the only branched FA found in samples 399 (5.3 ka) and 483 (7.5 ka). The surface sample furthermore contains iso- and anteiso-C_{17} FA, some anteiso-C_{16} FA and a small amount of C_{18} branched FA together accounting for 38% of the total branched FA's. Apart from this, only the early Holocene sample (517, 8.5 ka) contains a small amount of iso-C_{16} FA. Branched FA, in particular branched iso- and anteiso-C_{15} FA, derive from bacterial living in sediments as well as in soils (Cranwell, 1973; Goosens et al., 1989; Amblès et al., 1994; van Bergen et al., 1998). Accordingly, microbial organic matter of either sediment or soil origin as indicated by iso- and anteiso-C_{15} FA is abundant in the surface sample of Lz1120, still detectable in most of the Holocene samples but absent at Co1202.

3.2.3 Mono-unsaturated alkanoic acids ($C_{n:1}$ FA)

Mono-unsaturated fatty acids, namely various isomers of $C_{16:1}$ and $C_{18:1}$ FA, were detected in considerable amounts (12% of total lipids) only in the surface sample of Lz1120. Both compounds are abundant in phytoplankton. Small amounts of $C_{18:1}$ (*cis*)

mono-unsaturated FA were also present in sample 517 from Lz1120. In this case, it probably does not derive from phytoplankton but from bacteria living in the sediment (Bobbie and White, 1980). Unsaturated lipids are generally more susceptible to microbial degradation than their saturated counterparts (Haddad et al., 1991; Meyers and Ishiwatari, 1993). The complete absence of any other unsaturated compounds illustrates effective microbial consumption of labile organic matter in the near surface sediments.

3.2.4 Hydroxy acids (OH-FA)

Hydroxy acids were found in the sediments at both sites. However, their proportion was clearly higher at Lz1120: 1–6%$_{lipids}$ compared to 0.2–1.3%$_{lipids}$ at Co1202. Hydroxy acids are major constituents of the biopolyesters cutin and suberin. Cutin makes up the protective layers of the aerial parts of plants (plant waxes) and contains mainly C_{16} and C_{18} hydroxy acids (Kolattukudy, 1980) while suberin is found in root material and contains large quantities of C_{22} and C_{24} ω-hydroxy acids as well as C_{16} ω-hydroxy acid (Bull et al., 2000; Nierop et al., 2005). In contrast, α-hydroxy acids are assumed to be of microbial origin (Fukushima et al., 1992). At Lz1120, ω-hydroxy acids account for 90–100% of all hydroxy acids except in sample 505 (8.2 ka) where 58% of the hydroxy acids are α-hydroxy acids. Apart from sample 505, the highest proportion of α-hydroxy acids of ~10% is found in the early Holocene sample 517 (8.5 ka). Notably, these two samples also contain small amounts of $C_{18:1}$ α-hydroxy acid (0.05 and 0.1%$_{lipids}$, respectively). The presence of this labile compound and of $C_{18:1}$ FA in these samples suggests in situ contribution from bacterial biomass. At site Co1202, α-hydroxy acids were present in samples 246 and 248. Their proportion appears to be higher than in Lz1120. In sample 248 representing the 8.2 ka event, they account for 41% of all hydroxy acids.

Shifts in the dominant chain lengths of the ω-hydroxy acids suggest enhanced contribution of suberin-derived material in the glacial sample while increased proportions of cutin-derived material occur prior to the 8.2 ka event at both sites.

3.2.5 *n*-Alkanols

The second-most abundant compound class in the investigated sediments is *n*-alkanols (saturated alcohols) which make up 18–45% at site Lz1120 and 26–56% at Co1202, respectively. At both sites, alkanols range from *n*-C_{12} to *n*-C_{32} alkanols but are clearly dominated by the long-chain *n*-C_{24} and *n*-C_{26} alkanols suggesting that the alkanol fraction is overwhelmingly of terrestrial origin.

The *n*-C_{24} alkanol is the major alkanol in Holocene samples at Lz1120 except in sample 517 (ka) where the *n*-C_{28} alkanol dominates (25% of total alkanol fraction) and the surface sample where the *n*-C_{22} alkanol is most abundant (26% of total alkanols). Volkman et al. (1999) found the *n*-C_{22} alkanol to dominate in eustigmatophytes, phototrophic marine and freshwater microalgae. Since the surface sample furthermore reveals slightly higher contribution of short-chain *n*-C_{16} and *n*-C_{18} alkanols (7 and 2.5%, respectively) as well as mono-unsaturated (12%$_{lipids}$) and branched alkanoic acids (2.8%$_{lipids}$), i.e. labile organic matter of algal and microbial sources, it appears most likely that the *n*-C_{22} alkanol in this sample actually derives from relatively fresh/non-degraded lacustrine OM. In the glacial sample the *n*-C_{22} alkanol also is the dominating alkanol. The distribution patterns of the *n*-alkanols in the surface sample and the glacial sample appear similar. In both cases *n*-C_{22} alkanol appears superimposed as a singular compound from a different source on a nearly Gaussian distribution of plant-derived *n*-alkanols with the *n*-C_{26} alkanol as the dominating compound. Thus, increased contribution from lacustrine algal sources appears to be still recorded in the alkanol fraction even though short-chain, branched or mono-unsaturated fatty acids that could confirm this source have been partly or completely degraded.

At site Co1202, the amount of short-chain *n*-C_{16} and *n*-C_{18} alkanols is ~3–5 times higher relative to the dominating *n*-C_{26} and *n*-C_{24} alkanols than at Lz1120 (without surface sample) suggesting that contribution from autochthonous, lacustrine organic matter was slightly higher. Nevertheless, the terrestrial long-chain alkanols clearly dominate.

3.2.6 *n*-Alkanes

n-Alkanes represent a minor fraction of the total extractable lipids contributing an average of 2.4% to the TLE's from Holocene sediments of both cores. At Lz1120 their proportion is lowest in sample 517 from the early Holocene (1.4%$_{lipids}$) and slightly higher in the surface sample (2.7%$_{lipids}$). A higher percentage is observed only in the glacial sample of core Lz1120: 8.9%$_{lipids}$. Proportions are slightly lower at site Co1202, with the maximum percentage observed in the sample representing the 8.2 ka event (2.3%$_{lipids}$). As for *n*-alkanoic acids, the chain length of *n*-alkanes indicates their major sources, with short-chain *n*-alkanes indicating algal input, mid-chain *n*-alkanes deriving from macrophytes and long-chain *n*-alkanes indicating terrestrial plant matter (Ficken et al., 2000). The *n*-alkanes detected in sediments of Lake Ohrid are mid- and long-chain odd-numbered compounds. The dominant compounds in most samples are the *n*-C_{29} and *n*-C_{31} alkanes with a significant contribution from *n*-C_{27} and minor proportions of *n*-C_{25}, *n*-C_{23} and *n*-C_{21} alkanes. In the surface sample from Lz1120, the *n*-C_{21} alkane dominates accounting for 54% of the total *n*-alkane fraction. In contrast, only *n*-C_{27}, *n*-C_{29} and *n*-C_{31} alkanes were detected in sample 248 from site Co1202 representing the 8.2 event, suggesting purely terrestrial plant matter as a source for the *n*-alkanes in this sample.

3.2.7 Sterols

Sterols are far more abundant at Lz1120 than at Co1202. At Lz1120 they account for 11–26% of the total extracted lipids whereas they reach a maximum of only 10% at Co1202. Sterols are membrane lipids that occur ubiquitously in eukaryotic organisms (i.e. in animals, plants, fungi and microorganisms with a nucleus inside the cell membranes) but tend to be absent in prokaryotes (bacteria) with some exceptions such as methylotrophic bacteria (Volkman, 2005). The most abundant sterols in the Ohrid sediments

are lanosterol (4,4′,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol), stigmastanol (24-ethyl-5α(H)-cholest-22-en-3β-ol), sitosterol (24-ethylcholest-5-en-3β-ol), cholesterol (cholest-5-en-3β-ol) and cholestanol (5α(H)-cholestan-3β-ol). At site Lz1120, these compounds account for all sterols in the glacial sample, though lanosterol was present only in trace amounts. Although the sterol fractions of the remaining samples at Lz1120 are also dominated by these compounds they furthermore contain 7 to 26% of other sterols including dinosterol (4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol) and dinostanol (as two distinct isomers, presumably 4α,23**R**,24R-Trimethyl-5α(H)-cholestan-3β-ol and 4α,23**S**,24R-Trimethyl-5α(H)-cholestan-3β-ol) that together account for 5–20% of the total sterols in samples 1 (surface), 483, 505 and 517. Furthermore, stigmasterol makes up 2–9% of the sterols in almost all samples from Lz1120 except the glacial one (643, 18.9 ka), coprostanol (5β-cholestan-3β-ol) and epicoprostanol (5β-cholestan-3α-ol) represent 6% in the surface sample and 1% in sample 517 and, finally, epicholestanol contributes 2–3% to the total sterols except in the glacial sample.

In contrast to site Lz1120, the dominant sterols at site Co1202 are the two isomers of dinostanol that, together, account for 21–24% of the total sterols. Only in sample 252 sitosterol is more abundant (34%). Second-most abundant compounds are stigmastanol in sample 246 (20%) and cholesterol in sample 248 (19%). Lanosterol is less abundant at this site and ranges from 7 to 13% of the total sterols, similar to cholesterol and cholestanol (4–12%). Dinosterol (4α,23,24-trimethyl-5α-cholest-22*E*-en-3β-ol) is present in all samples (4–7%). Other than at site Lz1120, epicholestanol is present only in a very small amount (~1% of total lipids) in sample 248 while coprostanol, epicoprostanol and stigmasterol are absent in samples from Co1202, or at least below the detection limit.

Cholesterol and cholestanol are very common in living organisms and not very specific (e.g. Goosens et al., 1989). Cholesterol may derive from zooplankton such as ostracods or zoobenthos such as gastropods (Thiel et al., 1997), however, it is also found in dinoflagellates and some diatoms (Volkman, 1986). Sitosterol is the major sterol in higher plants but can also occur in diatoms and microalgae (Volkman, 1986;

Volkman et al., 2005). More specific are compounds such as dinosterol and dinostanol that derive from dinoflagellates (e.g., Volkman, 1986; Mouradian et al., 2007).

Lanosterol is synthesised mainly by animals and fungi (Volkman, 2005). Like dinosterol it is also found, in smaller amounts, in dinoflagellates (Al-Mutlaq et al., 2008). Some sterols, however, have very specific sources. Coprostanol (5β-cholestan-3β-ol) and epicoprostanol (5β-cholestan-3α-ol), for example, are typically formed in the intestines of higher mammals including humans. Coprostanol is actually the dominant sterol in human faeces while epicoprostanol is absent. Both compounds are therefore often used as indicators for manure or sewage contamination in soils and sediments (Sherwin et al., 1993; Bull et al., 2000; Cordeiro et al., 2008). We observed both coprostanol and epicoprostanol in the surface sample of Lz1120 where they make up 6% of the sterol fraction, thus reflecting modern inputs of faecal material from human and animal sources. Apart from the surface sample we found coprostanol in small amounts (<1% of total sterols) in sample 517 from the early Holocene. Epicoprostanol, in contrast, is absent from all remaining investigated samples. However, epicholestanol (5α-cholestan-3α-ol) was present in substantial amounts in almost every sample from site Lz1120 apart from the glacial sample (643) as well as in sample 248 from Co1202. This compound has been reported relatively rarely. It has been found, for example, by Cordeiro et al. (2008) to be the second-most abundant faecal sterol after coprostanone and even more abundant than coprostanol in sediments of the Iguaçu River estuary (Brazil). These authors deduced that epicholestanol is produced in situ from the changing relative amounts of cholestanol, coprostanol and epicoprostanol. Actually, epicholestanol is produced from cholesterol by bacteria under highly anoxic conditions (Robinson et al., 1984; Mermoud et al., 1985). Robinson et al. (1984) found high amounts of epicholestanol in *Chlorobium*, a phototrophic sulphide-oxidising bacterium living near the sediment-water interface under anoxic conditions. In fact, those samples with low proportions of cholesterol also show low proportions of epicholestanol. The ratios of epicholestanol to cholestanol and of epicholestanol to coprostanol found in Lake Leman (Switzerland/France) by Mermoud et al. (1985) are very close to those

found in *Chlorobium* by Robinson et al. (1984). This led Mermoud et al. (1985) to conclude that epicholestanol derives from anoxic bacteria contributing to the organic matter in surface sediments of Lake Leman. The ratios of epicholestanol to coprostanol in samples from Lz1120, where available, show similar values to those observed in Lake Leman (0.86 and 1.88 vs. 0.81 to 1.58) while the ratios of epicholestanol to cholestanol are slightly lower: 0.17–0.24 in Lake Ohrid vs. 0.32–0.63 in Lake Leman. Regarding these similarities, anoxic bacteria such as *Chlorobium* could actually be considered as a source of epicholestanol and cholestanol in Lake Ohrid sediments. At least, these compounds are likely to share a common source since they show an excellent correlation in the Holocene sediments of Lz1120 (r^2=0.97, n=5). Contribution from microbial biomass formed in situ is confirmed by the presence of branched fatty acids (iso- and anteiso-C_{15} FA). Like epicholestanol, these are absent in the glacial sample and the samples from site Co1202.

3.2.8 Others (triterpenoids, branched alkanols)

Other quantified compounds include 17β(H),21β(H)-bishomohopanoic acid and 17β(H),21β(H)-bishomohopanol, both triterpenoids that derive directly from bacteriohopanepolyols (BHP's) of bacteria in sediments (e.g., Innes et al., 1997) and soils (e.g., Ries-Kautt and Albrecht, 1989). At site Lz1120, 17β(H),21β(H)-bishomohopanoic acid accounts for up to 9% of the total extracted lipids, e.g., in sample 483 (7.5 ka). Lowest amounts of 2.5 and 2.8%$_{lipids}$ are observed in sample 505 (8.2 ka event) and in the glacial sample. The concentrations of 17β(H),21β(H)-bishomohopanoic acid and 17β(H),21β(H)-bishomohopanol do not correlate at site Lz1120 which suggests that they do not derive in similar proportions from the same source, i.e. from bacterial OM in sediments or in soils. In contrast, maximum values of 17β(H),21β(H)-bishomohopanoic acid and 17β(H),21β(H)-bishomohopanol do occur contemporaneously in sample 248 representing the 8.2 ka event at site Co1202. There, however, amounts of both compounds are clearly lower than at site Lz1120 and ranges from 1 and 2%$_{lipids}$, and 0.1 to 0.4%$_{lipids}$, respectively.

β-Amyrin which derives from higher plants (Volkman et al., 2005) was detected in small amounts (0.2–0.9%$_{lipids}$) in almost all samples except sample 252 (7.9 ka) from Co1202. As for the 17β(H),21β(H)-bishomohopanoic acid, highest concentrations are observed in the samples representing the 8.2 ka event. Furthermore, we found iso- and anteiso-branched C_{15} alkanol in samples 1 (surface), 399 (5.3 ka) and 483 (7.5 ka) as well as branched C_{17} alkanol (iso and anteiso) in the surface sample of Lz1120. Similar to the iso- and anteiso-C_{15} branched alkanoic acids, these compounds derive from bacteria (Cranwell, 1980). Branched C_{17} alkanol has been identified by Thiel et al. (1997) in microbial mats from the Florida Everglades. None of these compounds, however, could be detected in the samples from site Co1202.

4 Discussion

The results of our study of Lake Ohrid extractable lipid biomarkers have revealed a number of compositional differences between the two sites but also between samples of different levels of organic matter degradation (surface sample vs. the rest) and from different climate conditions (Holocene vs. glacial and presumed 8.2 ka event). For a reconstruction of environmental changes based on sedimentary OM composition, the detection of changes in the contributions from various sources is crucial. One problem in this context is the preservation potential of the source-specific compounds which can differ considerably. For example, unsaturated compounds are generally more labile than their saturated counterparts and branched compounds more labile than straight-chain compounds (Haddad et al., 1992). Equally important, however, is the matrix in which the biomarkers are incorporated at the time of supply to the sedimentary system as well as the sedimentary regime itself, i.e. whether anoxic, suboxic or oxic conditions prevail, and determine the mode of microbial consumption of the sedimentary OM. Long-chain terrestrial fatty acids and *n*-alkanols, for example, are per se not more resistant towards microbial attack than short-chain autochthonous alkanoic acids and alkanols. However, when introduced into an aquatic system they are incorporated in

plant tissues or particles largely consisting of relatively recalcitrant organic substances such as lignin or cellulose that usually have already been under microbial attack and, hence, represent the debris from terrestrial degradation (e.g., Meyers, 1997). They can also be firmly associated with clay minerals as a result of soil formation processes, which equally makes them relatively inaccessible for microbes in the water column and the sediment and thus increases their preservation potential compared to easily accessible autochthonous organic matter (Goosens et al., 1989; Haddad et al., 1992; Meyers and Ishiwatari, 1993). Another factor controlling the preservation potential of sedimentary organic matter is the availability of oxygen. Under oxygen-depleted (suboxic) or oxygen-free (anoxic) conditions anaerobic bacteria replace the aerobic micro-organisms and gain energy, e.g., from sulphate reduction. Anaerobic OM decay, however, is considerably slower and thus increases the OM preservation potential. Precipitation of sulphides is a common side effect of anaerobic organic matter degradation. The finding that TOC/TS ratios at Co1202 are lower than at site Lz1120 might result from the fact that Co1202 is situated in deeper water just below the summer thermocline (Matzinger et al., 2007) that is less frequently mixed and hence exhibits a greater chance of oxygen depletion. Finally, we also have to consider variable degrees of degradation for the terrestrial organic input for a correct interpretation of the biomarker data. Terrestrial OM is delivered as relatively fresh plant litter from various types of vegetation, e.g., deciduous/pine forests or grasslands, or as soil OM in a whole range of possible stages of degradation.

4.1 Evidence for soil organic matter supply

There is evidence for the contribution of soil OM to the sediments of Lake Ohrid when considering the fact that soil OM has often been found depleted in *n*-alkanoic acids relative to *n*-alkanols and hydroxy acids (e.g., Bull et al., 2000; Nierop et al., 2005, 2009). While terrestrial *n*-alkanols are present throughout the cores as a major organic fraction, the proportion of terrestrially derived *n*-alkanoic acids strongly varies. In those samples where terrestrial *n*-alkanols are the dominating compounds, i.e. in the glacial

sample and the samples representing the 8.2 ka event from both sites, long-chain terrestrially derived *n*-alkanoic acids ($>C_{24}$) are almost absent. This suggests two distinct terrestrial OM sources, one of which is strongly depleted in long-chain *n*-alkanoic acids relative to long-chain *n*-alkanols and therefore most likely represents soil OM. Further evidence for the contribution of soil OM comes from the bimodal distribution of C_{16}, C_{22} and $C_{24}\omega$-hydroxy acids. Such a pattern is described for lake sediments (Goosens et al., 1989; Fukushima et al., 1992) but also by for soils (Nierop et al., 2005). Fukushima et al. (1992) regard plant matter and eroded soils as the source at least for the long-chain hydroxy acids. With the exception of the surface sample, C_{22} and $C_{24}\omega$-hydroxy acids correlate very well in samples of Lz1120 (r^2=0.99; n=5) whereas $C_{16}\omega$-hydroxy acid does not correlate to these compounds. This suggests that C_{22} and $C_{24}\omega$-hydroxy acid share a common source that might not necessarily be identical to the source of $C_{16}\omega$-hydroxy acid. Based on their investigations of soil OM profiles and plant litter Nierop et al. (2009) suggest that higher ratios of $C_{22}/C_{16}\omega$-hydroxy acid reflect enhanced proportions of suberin-derived root material relative to cutin-derived aerial plant material, i.e. plant litter. They also found that a substantial proportion of soil OM derives from root material. Slightly higher values of the $C_{22}/C_{16}\omega$-hydroxy acid ratio at Lz1120 compared to Co1202 would thus indicate higher soil OM proportions relative to plant litter. It would also suggest that soil OM proportions are highest during the glacial and elevated in the surface sample and sample 399 from the mid-Holocene (5.3 ka). Soil OM is likely to be depleted overall in extractable lipids and enriched in more stable OM and black carbon. Since the glacial sample shows the lowest amount of extractable lipids relative to the TOC it can be assumed that high levels of soil OM contribute to the bulk sedimentary OM.

According to the above observations on *n*-alkanol/*n*-alkanoic acid and hydroxy acids distributions, soil OM input is generally higher at Lz1120 compared to Co1202 since all samples from the southern site reveal higher proportions of long-chain *n*-alcohols and hydroxy acids. It also appears to be higher in the samples from the 8.2 event and the glacial. However, this does not necessarily indicate higher soil erosion during

these periods. According to a number of paleoclimatological studies regional climate was considerably drier during the glacial and the 8.2 ka event (Bordon et al., 2009; Fouache et al., 2010). If the long-chain *n*-alkanoic acids derive primarily from less degraded plant litter it is rather likely that their contribution was diminished in response to precipitation-controlled changes in the vegetation cover and lower biomass productivity. Accordingly, concentrations of *n*-alkanoic acids per gram sediment at both sites are lower by a factor of 13 on average in the samples representing the 8.2 ka event relative to the previous and the following samples. Concentrations of *n*-alkanols, however, remain roughly the same. This implies that mineral and soil OM supplies are associated and depend on the same transport mechanism, i.e. surface run-off. Although precipitation was generally reduced soil erosion rates might have been increased due to higher soil exposure and lower stabilising soil moisture, thus, compensating for lower run-off and creating a rather constant background signal of soil supply through the 8.2 ka event at both sites. Greater sedimentation rates and soil OM contributions at site Lz1120 might be related to the proximity of the Cerava River mouth. Although its today's discharge is very small ($0.2\,m^3\,s^{-1}$) compared to the total input from rivers and streams to the lake ($8.9\,m^3\,s^{-1}$; Matzinger et al., 2006) the river might have exported very different amounts of mineral and soil organic matter at times depending on the precipitation intensity. The discharge of the karst springs of Tushemisht and Sveti Naum, which are responsible for about a quarter of the total inflow into Lake Ohrid ($10\,m^3\,s^{-1}$), responded directly to changes in precipitation and is likely to have affected the availability of nutrients such as phosphorus (Matzinger et al., 2006). However, it does not deliver any significant amounts of mineral and soil organic matter.

4.2 Evidence for vegetation changes

The changing predominance of long-chain terrestrial *n*-alkanols, *n*-alkanoic acids and *n*-alkanes in the sediments from both sites can potentially ascribed to the type of vegetation they derive from. Results of studies comparing plant matter and soil organic

matter from different types of vegetation by van Bergen et al. (1997, 1998), Bull et al. (2000) and Nierop et al. (2005) suggest that *n*-alkanols of leaf tissue from trees are dominated by the *n*-C_{24} alkanol whereas grassy vegetation results in *n*-alkanol distributions dominated by the *n*-C_{26} or *n*-C_{28} alkanols. Accordingly, contribution of *n*-alkanols from grasses and herbs dominates in the early Holocene samples and the samples representing the 8.2 ka event from both sites (Lz1120: 517, 505, Co1202: 252, 248), while *n*-alkanols from leaves dominate in the remaining samples except the surface sample and the glacial sample which are dominated by algal-derived *n*-C_{22} alkanol. However, the *n*-alkanol distributions of these two samples are, notably, almost bimodal and show the *n*-C_{26} alkanol as the second most important compound suggesting significant contribution from grasses and herbs. This finding is largely consistent with the pollen record of Lz1120 (Wagner et al., 2009) which indicates strongly increased proportions of pollen from herbs during the glacial and after 3.5 ka and slightly increased proportions for the 8.2 ka event.

So far, no general pattern has emerged for the distribution of long-chain *n*-alkanoic acids in relation to vegetation types. Regarding the *n*-alkanes, however, Ficken et al. (2000) defined a proxy for contribution from emergent and submerged plants to lacustrine organic matter as $P_{aq}=(n\text{-}C_{23}+n\text{-}C_{25})/(n\text{-}C_{23}+n\text{-}C_{25}+n\text{-}C_{29}+n\text{-}C_{31})$. Accordingly, $P_{aq}<0.1$ indicates pure terrestrial origin, $P_{aq}=0.1$–0.4 indicates dominating contribution from emergent plants and $P_{aq}=0.4$–1 indicates dominating contribution from submerged plants. The P_{aq} values of most of the Lake Ohrid samples range between 0.1 and 0.2 apart from sample 246 from Co1202 and, particularly, the surface sample from Lz1120 with slightly higher values of 0.31 and 0.38, respectively. This suggests that most of the *n*-alkanes in Lake Ohrid sediments derive from terrestrial plant material either with considerable contribution from emergent plants or minor contribution from macrophytes. Notably, Almendros et al. (1996) found the *n*-C_{25} alkane as the dominating compound in plant tissue from Mediterranean pines (needles and little stems). With the presence of pines in the Lake Ohrid Basin today and a much stronger presence inferred from the palynological record for the past (Wagner et al., 2009), there

is a good chance of overlap between the two possible sources for the *n*-C_{25} alkane, macrophytes and pines, which we cannot disentangle based on our current data.

The described changes in chain-lengths of *n*-alkanols, *n*-alkanoic acids and *n*-alkanes suggesting variable contribution from different types of vegetation illustrate the complexity but also the potential of biomarker distributions for reconstructions of ecosystem changes. However, similar to the reconstruction of soil input this approach requires a sound knowledge on the mechanisms controlling the supply of specific biomarkers from various sources and thus high-resolution investigations of dynamic climate stages.

4.3 Further implications from sterol and triterpenoid composition

Many of the sterols identified in Lake Ohrid sediment samples such as cholesterol, lanosterol or sitosterol are not very specific. However, they can still indicate ecosystem changes when investigated in relation to each other or to biomarkers of other compound classes. Generally, terrestrial sterols appear to have a much greater preservation potential than their autochthonous counterpart (Volkman, 1986).

Lanosterol, one of the most abundant sterols at site Lz1120, is synthesised by fungi from oxidosqualene (e.g., Volkman, 2005 and references therein) but may also derive from dinoflagellates (Al-Mutlaq et al., 2008). However, it does not show any correlation with dinosterol and dinostanol. In contrast, a fairly well, linear correlation (r^2=0.87, n=5) is observed with plant-derived β-amyrin in the Holocene samples from Lz1120. We may therefore assume a fungal origin for lanosterol. Consequently, it is not surprising to find only traces of lanosterol in the glacial sample when fungal activity was either suppressed by the considerably drier conditions or the substrate, i.e. humus, was not abundant. Also, bacteria-derived 17β(H),21β(H)-bishomohopanoic acid shows lowest concentrations in the glacial sample at Lz1120 as well as in sample 505 from the 8.2 ka event suggesting that bacterial activity was reduced under drier and cooler conditions, as well. In the remaining samples, 17β(H),21β(H)-bishomohopanoic acid correlates very well with lanosterol and β-amyrin (r^2=0.95 in both cases, n=4). These

relations suggest that lanosterol, β-amyrin and 17β(H),21β(H)-bishomohopanoic acid at times either share a common source or that their contemporaneous supply is controlled by the same mechanism, at least under relatively humid climate conditions. It is not unlikely that all of these compounds are part of a terrestrial OM fraction that combines plant matter (β-amyrin), compounds synthesised by fungi (lanosterol) and bacterial markers (17β(H),21β(H)-bishomohopanoic acid), i.e. plant litter decomposing under moist conditions as present, e.g., in humus layers of the top soils. In sample 505 from the 8.2 ka event we find the lanosterol concentration remaining high but 17β(H),21β(H)-bishomohopanoic acid concentration on a considerably lower level comparable to that of the glacial sample. This might result from the formation of substantial humus layers in the early Holocene that still contributed terrestrial OM during the 8.2 ka event, although drier and with reduced bacterial activity. This scenario, however, would require fungal-derived lanosterol to be better preserved in the top soil than bacterial-derived 17β(H),21β(H)-bishomohopanoic acid. Furthermore, the latter would have to be primarily derived from bacteria living in soils rather than the sediments. However, Winkler et al. (2006) found that concentrations of hopanoids in forest soils are far lower than in lacustrine sediments investigated by Farrimond et al. (2000) and Innes et al. (1997, 1998). Actually, the concentrations of 17β(H),21β(H)-bishomohopanoic acid and of 17β(H),21β(H)-bishomohopanol measured in Lake Ohrid samples show ranges that are remarkably similar to the ones observed by Innes et al. (1998) in anoxic sediments of Lake Pollen (Norway): 122–1841 µg/gTOC (Ohrid) vs. 194–3912 µg/gTOC (Pollen) for 17β(H),21β(H)-bishomohopanoic acid and 13–426 µg/gTOC (Ohrid) vs. 35–546 µg/gTOC (Pollen) for 17β(H),21β(H)-bishomohopanol. On the other hand, origin of 17β(H),21β(H)-bishomohopanoic acid from soils is supported by the fact that other bacterial markers such as branched alkanoic acids do not correlate with 17β(H),21β(H)-bishomohopanoic acid, or are even absent as is the case at Co1202.

In a similar way we can relate the presence of some sterol markers to proxies such as the TOC/TS ratio. Notably, epicholestanol occurs at site Co1202 only in the sample

with the lowest TOC/TS ratio (11), i.e. in sample 428 representing the 8.2 ka event. At site Lz1120, epicholestanol is present in all samples apart from the glacial one and TOC/TS ratios are considerably higher. If higher TOC/TS ratios mainly reflect higher input of terrestrial organic carbon and nutrients, oxygen consumption in the surface waters of the southern areas of Lake Ohrid might have been more efficient, particularly during humid climate stages, resulting in a shallower chemocline in the catchment of Lz1120. There, oxygen-depleted conditions apparently always established within the photic zone, except during the glacial, supporting phototrophic sulphide-oxidisers such as *Chlorobium* that synthesise epicholestanol. At Co1202, on the other hand, the chemocline might have been too deep for phototrophic anaerobic bacteria to thrive except, it appears, during the drier conditions of the 8.2 ka event. A possible explanation for this seemingly opposing pattern could be that phototrophic sulphur bacteria could live in the oxygen-depleted waters at and below the (deeper) chemocline at site Co1202 only at times of increased light penetration, e.g., times of reduced terrestrial run-off and clearer surface water. Thus, our results offer the perspective of reconstructing past changes in lake chemistry and the variable levels of the chemocline based on a specific sterol marker, epicholestanol.

The identification of coprostanol in a sample from the early Holocene (8.5 ka) is quite remarkable since it suggests the presence of some significant human population other than the odd hunter gatherer defecating at the lake's shore. Notably, human occupation is not documented until the onset of the Neolithic, i.e. after the 8.2 ka event, in the neighbouring Maliq Basin (Fouache et al., 2010). Nevertheless, this compound could clearly be identified in sample 517 although the finding needs confirmation from higher resolution studies.

5 Conclusions

Our study presents a first inventory of lipid biomarkers present in total lipid extracts from Holocene and glacial sediments of ancient Lake Ohrid. A small set of samples from two sites, Lz1120 near the southern shoreline and Co1202 near the eastern shoreline, has been investigated. Gross composition of major lipid compounds reveals significant differences between the sites reflecting the different settings in their individual catchments, i.e. morphology and associated drainage. Changes in the proportions of long-chain terrestrial *n*-alkanoic acids and *n*-alkanols and shifts in chain length of the dominant ω-hydroxy acids indicate higher proportions of soil-derived OM at Lz1120 than at site Co1202. The relative amount of soil OM also appears increased under the dry climate conditions of the 8.2 ka event at both sites. During the glacial, aquatic productivity appears to have been lower, probably due to minimum nutrient supply, resulting in overall higher proportions of both terrestrial plant and soil OM in the sediments. Changes in the chain-length of the dominating long-chain-terrestrial *n*-alkanols, particularly the switch from *n*-C_{26} and *n*-C_{28} alkanol to *n*-C_{24} alkanol after the 8.2 ka event, might indicate changes in the vegetation cover from vegetation with considerable proportions of grass and herbs to mostly trees. Epicholestanol deriving from sulphide-oxidising phototrophic bacteria has been identified and could provide a marker for vertical shifts of the chemocline. Finally, we found evidence for human faeces through the detection of coprostanol in the surface sample, where it could be expected, but also in a sample from the early Holocene where it might indicate the presence of early human communities. We could thus document the effects of spatial and temporal changes in soil organic matter supply, possibly changes in vegetation, definitely early organic matter diagenesis, furthermore anoxia in the photic zone and anthropogenic influence on the composition of total lipid extracts from ancient Lake Ohrid.

Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/7/4607/2010/bgd-7-4607-2010-supplement.pdf.

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References

Ahlgren, G., Gustafsson, I.-B., and Boberg, M.: Fatty acid content and chemical composition of freshwater microalgae, J. Phycol., 28, 37–50, 1992.

Albrecht, C. and Wilke, T.: Ancient Lake Ohrid: biodiversity and evolution, Hydrobiologia, 615, 113–140, 2008.

Almendros, G., Sanz, J., and Velasco, F.: Signatures of lipid assemblages in soils under continental Mediterranean forests, Eur. J. Soil Sci., 41, 183–196, 1996.

Al-Mutlaq, K. F., Laurel, J., Standley, L. J., and Simoneit, B. R. T.: Composition and sources of extractable organic matter from a sediment core in Lake Kivu, East African rift valley, Appl. Geochem., 23, 1023–1040, 2008.

Amblès, A., Jambu, P., Parlanti, E., Joffre, J., and Riffe, C.: Incorporation of natural monoacids from plant residues into an hydromorphic forest podzol, Eur. J. Soil Sci., 45, 115–182, 1994.

Belmecheri, S., Namiotko, T., Robert, C., von Grafenstein, U., and Danielopol, D. L.: Climate controlled ostracod preservation in Lake Ohrid (Albania, Macedonia), Palaeogeogr. Palaeoclim. Palaeoecol., 277, 236–245, 2009.

Bobbie, R. J. and White, D. C.: Characterization of benthic microbial community structure by high-resolution gas chromatography of fatty acid methyl esters, Appl. Environ. Microbiol., 39, 1212–1222, 1980.

Bordon, A., Peyron, O., Lézine, A.-M., Brewer, S., and Fouache, E.: Pollen-inferred Late-Glacial and Holocene climate in southern Balkans (Lake Maliq), Quatern. Int., 200, 19–30, 2009.

Brewer, S., Cheddadi, R., de Beaulieu, J. L., Reille, M., and data contributors: The spread of deciduous Quercus throughout Europe since the last glacial period, Forest Ecol. Manag., 156, 27–48, 2002.

Bull, I. D., van Bergen, P. F., Nott, C. J., Poulton, P. R., and Evershed, R. P.: Organic geochemical studies of soils from the Rothamsted classical experiments – V. The fate of lipids in different long-term experiments, Org. Geochem., 31, 389–408, 2000.

Cordeiro, L. G. S. M., Carreira, R. S., and Wagener, A. L. R.: Geochemistry of fecal sterols in a contaminated estuary in southeastern Brazil, Org. Geochem., 39, 1097–1103, 2008.

Cranwell, P.: Branched/cyclic alkanols in lacustrine sediments (Great Britain): recognition of *iso*- and *anteiso*-branching and stereochemical analysis of homologous alkan-2-ols, Chem. Geol., 30, 15–26, 1980.

Cranwell, P. A.: Branched-chain and cyclopropanoid acids in a recent sediment, Chem. Geol., 11, 307–313, 1973.

Farrimond, P., Head, I. M., and Innes, H. E.: Environmental influence on the biohopanoid composition of recent sediments, Geochim. Cosmochim. Ac., 64, 2985–2992, 2000.

Ficken, K. J., Wooller, M. J., Swain, D. L., Street-Perrott, F. A., and Eglinton, G.: Reconstruction of a subalpine grass-dominated ecosystem, Lake Rutundu, Mount Kenya: a novel multi-proxy approach, Palaeogeogr. Palaeoclim. Palaeoecol., 177, 137–149, 2002.

Ficken, K. J., Li, B., Swain, D. L., and Eglinton, G.: An *n*-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes, Org. Geochem., 31, 745–749, 2000.

Fouache, E., Desruelles, S., Magny, M., Bordon, A., Oberweiler, C., Coussot, C., Touchais, G., Lera, P., Lézine, A.-M., Fadin, L., and Roger, R.: Palaeogeographical reconstructions of Lake Maliq (Korça Basin, Albania) between 14,000 BP and 2000 BP, J. Archaeol. Sci., 37, 525–535, 2010.

Fukushima, K., Kondo, H., and Sakata, S.: Geochemistry of hydroxy acids in sediments – I. Some freshwater and brackish water lakes in Japan, Org. Geochem., 18, 913–922, 1992.

Goosens, H., Düren, R. R., de Leeuw, J. W., and Schenck, P. A.: Lipids and their mode of occurrence in bacteria and sediments – II. Lipids in the sediment of a stratified freshwater lake, Org. Geochem., 14, 27–41, 1989.

Haddad, R. I., Martens, C. S., and Farrington, J. W.: Quantifying early diagenesis of fatty acids in a rapidly accumulating coastal marine sediment, Org. Geochem., 19, 205–216, 1992.

Hartmann, M.-A.: Plant sterols and the membrane environment, Trends Plant Sci., 3, 170–175, 1998.

Innes, H. E., Bishop, A. N., Fox, P. A., Head, I. M., and Farrimond, P.: Early diagenesis of bacteriohopanoids in Recent sediments of Lake Pollen, Norway, Org. Geochem., 29, 1285–1295, 1998.

Innes, H. E., Bishop, A. N., Head, I. M., and Farrimond, P.: Preservation and diagenesis of hopanoids in Recent lacustrine sediments of Priest Pot, England, Org. Geochem., 26, 565–576, 1997.

Kattner, G., Gercken, G., and Hammer, K. D.: Development of lipids during a spring bloom in the northern North Sea – II. Dissolved lipids and fatty acids, Mar. Chem., 14, 163–173, 1983.

Kolattukudy, P. E.: Biopolyester membranes of plants: cutin and suberin, Science, 208, 990–1000, 1980.

Matzinger, A., Schmid, M., Veljanoska-Sarafiloska, E., Patceva, S., Guseska, D., Wagner, B., Müller, B., Sturm, M., and Wüest, A.: Eutrophication of ancient Lake Ohrid: global warming amplifies detrimental effects of increased nutrient inputs, Limnol. Oceanogr., 52, 338–353, 2007.

Matzinger, A., Jordanoski, M., Veljanoska-Sarafiloska, E., Sturm, M., Müller, B., and Wüest, A.: Is Lake Prespa jeopardizing the ecosystem of ancient Lake Ohrid?, Hydrobiologia, 553, 89–109, 2006.

Mermoud, F., Gülaçar, F. O., and Buchs, A.: 5α(H)-Cholestan-3α-ol in sediments: Characterization and geochemical significance, Geochim. Cosmochim. Ac., 49, 459–462, 1985.

Meyers, P. A.: Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes, Org. Geochem., 27, 213–250, 1997.

Meyers, P. A. and Ishiwatari, R.: Lacustrine organic geochemistry – an overview of indicators of organic matter sources and diagenesis in lake sediments, Org. Geochem., 20, 867–900, 1993.

Mouradian, M., Panetta, R. J., de Vernal, A., and Gélinas, Y.: Dinosterols or dinocysts to estimate dinoflagellate contributions to marine sedimentary organic matter?, Limnol. Oceanogr., 52, 2569–2581, 2007.

Nierop, K. G. J. and Jansen, B.: Extensive transformation of organic matter and excellent lipid preservation at the upper, superhumid Guandera páramo, Geoderma, 151, 357–369, 2009.

Nierop, K. G. J., Naafs, D. F. W., and van Bergen, P. F.: Origin, occurrence and fate of extractable lipids in Dutch coastal dune soils along a pH gradient, Org. Geochem., 36, 555–566, 2005.

Ries-Kautt, M. and Albrecht, P.: Hopane-derived triterpenoids in soils, Chem. Geol., 76, 143–151, 1989.

Robinson, N., Cranwell, P. A., Finlay, B. J., and Eglinton, G.: Lipids of aquatic organisms as potential contributors to lacustrine sediments, Org. Geochem., 6, 143–152, 1984.

Sherwin, M. R., Vanvleet, E. S., Fossato, V. U., and Dolci, F.: Coprostanol (5β-cholestan-3-β-ol) in lagoonal sediments and mussels of Venice, Italy, Mar. Pollut. Bull., 26, 501–507, 1993.

Thiel, V., Merz-Preiss, M., Reitner, J., and Michaelis, W.: Biomarker studies on microbial carbonates: extractable lipids of a calcifying cyanobacterial mat (Everglades, USA), Facies, 36, 163–172, 1997.

Vogel, H., Wagner, B., Zanchetta, G., Sulpizio, R., and Rosén, P.: A paleoclimate record with tephrochronological age control for the last glacial-interglacial cycle from Lake Ohrid, Albania and Macedonia, J. Paleolimnol., 44, 295–310, 2010a.

Vogel, H., Zanchetta, G., Sulpizio, R., Wagner, B., and Nowaczyk, N.: A tephrostratigraphic record for the last glacial-interglacial cycle from Lake Ohrid, Albania and Macedonia, J. Quaternary Sci., 25, 320–338, 2010b.

Vogel, H., Wessels, M., Albrecht, C., Stich, H.-B., and Wagner, B.: Spatial variability of recent sedimentation in Lake Ohrid (Albania/Macedonia) - a complex interplay of natural and anthropogenic factors and their possible impact on biodiversity patterns, Biogeosciences Discuss., 7, 3911–3930, doi:10.5194/bgd-7-3911-2010, 2010.

Volkman, J. K.: Sterols and other triterpenoids: source specificity and evolution of biosynthetic pathways, Org. Geochem., 36, 139–159, 2005.

Volkman, J. K., Barrett, S. M., Blackburn, S. I.: Eustigmatophyte microalgae are potential sources of C_{29} sterols, C_{22}–C_{28} *n*-alcohols and C_{28}–C_{32} *n*-alkyl diols in freshwater environments, Org. Geochem., 30, 307–318, 1999.

Volkman, J. K.: A review of sterol markers for marine and terrigenous organic matter, Org. Geochem., 9, 83–99, 1986.

Wagner, B., Reicherter, K., Daut, G., Wessels, M., Matzinger, A., Schwalb, A., Spirkovski, Z., and Sanxhaku, M.: The potential of Lake Ohrid for long-term palaeoenvironmental reconstructions, Palaeogeogr. Palaeoclimatol. Palaeoecol., 259, 341–356, 2008.

Wagner, B., Lotter, A. F., Nowaczyk, N., Reed, J. M., Schwalb, A., Sulpizio, R., Valsecchi, V., Wessels, M., and Zanchetta, G.: A 40,000-year record of environmental change from ancient Lake Ohrid (Albania and Macedonia), J. Paleolimnol., 41, 407–430, 2009.

Winkler, A., Haumaier, L., and Zech, W.: Variation in hopanoid composition and abundance in forest soils during litter decomposition and humification, Org. Geochem., 32, 1375–1385, 2001.

Table 1. Elemental data and concentrations of total lipids per gram sample and as percentage of the total organic carbon content ($\%_{TOC}$); ages according to Wagner et al. (2008) and Vogel et al. (2010b). Based on the correlation of the carbonate and TOC records we assign sample 248 at Co1202 to the 8.2 ka event.

	ID/depth (cm)	age (cal. ka BP)	stage/ event	TOC (%)	$CaCO_3$ (%)	TOC/ TN	TOC/ TS	lipids ($\mu g/g_{Sed}$)	lipids ($\%_{TOC}$)
Lz1120	1	−0.050	surface	2.0	4.9	9	59	6.5	3.2
	399	5.330	Holocene	2.1	61	12	62	6.4	3.0
	483	7.530	”	2.0	59	10	77	3.9	1.9
	505	8.167	8.2 event	1.7	35	8	24	2.0	1.2
	517	8.526	Holocene	2.2	48	10	83	4.6	2.1
	643	18.900	Glacial	0.6	0	6	33	0.3	0.4
Co1202	246	7.723	Holocene	1.6	39	9	28	1.4	0.9
	248	7.797	8.2 event	0.6	1	6	11	0.6	1.4
	252	7.945	Holocene	0.8	9	7	17	1.0	1.3

Table 2. Compounds quantified in total lipid extracts from sites Lz1120 and Co1202 as percentages of the total quantified lipids.

core	Lz1120	Lz1120	Lz1120	Lz1120	Lz1120	Lz1120	Co1202	Co1202	Co1202	sources	references
sample	1	399	483	505	517	643	246	248	252		
age (cal ka BP)	−0.05	5.330	7.530	8.167	8.526	18.900	7.723	7.797	7.945		
lipid fractions (%)											
n-alkanoic acids (FA)	30.6	39.9	33.0	22.9	41.7	24.9	63.5	27.0	62.5		
hydroxy acids (OH-FA)	1.0	4.7	3.8	2.7	3.7	6.0	1.3	1.1	0.2		
branched fatty acids	2.8	0.6	0.4	1.0	0.9	–	–	–	–		
mono-unsaturated fatty acids	12.4	0.2	–	0.04	0.2	-	–	–	–		
n-alkanols	18.3	31.5	23.1	45.4	28.4	43.8	25.9	56.5	28.3		
n-alkanes	2.7	2.3	2.4	2.2	1.5	8.8	2.0	2.3	2.1		
sterols	25.2	12.1	25.7	20.5	14.3	11.1	5.4	9.9	4.7		
others	7.1	8.6	11.6	5.4	9.2	5.4	1.9	3.2	2.2		
n-alkanoic acids											
Σ C_{14}–C_{19} FA (short-chain)	12.2	13.9	5.4	14.0	10.6	2.9	26.6	15.8	6.7	phytoplankton, bacteria	Meyers and Ishiwatari (1993),
Σ C_{20}–C_{23} FA (mid-chain)	5.6	3.8	5.7	4.3	7.0	3.6	9.8	4.7	8.1	variable	Meyers (1997)
Σ C_{24}–C_{32} FA (long-chain)	12.8	22.1	22.0	4.6	24.0	18.4	27.1	6.4	47.7	higher plants	
hydroxy acids											
ω-C_{16} OH-FA	0.32	0.83	0.83	0.40	0.97	0.49	0.21	0.30	0.10	cutin, suberin	Kolattukudy (1980), Bull et
ω-C_{18} OH-FA	–	0.05	0.05	–	0.04	0.14	–	–	–	cutin	al. (2000)
ω-C_{22} OH-FA	0.50	1.07	0.79	0.20	0.49	1.44	0.17	0.15	0.04	suberin	Bull et al. (2000), Nierop et
ω-C_{24} OH-FA	–	1.22	0.81	0.11	0.45	1.78	0.24	0.08	–	suberin	al. (2005)
$\Sigma\alpha$-hydroxy fatty acids	–	–	0.13	1.56	0.38	–	0.13	0.44	–	bacteria	Fukushima et al. (1992)
$\Sigma\omega$-hydroxy fatty acids	1.00	4.74	3.58	1.13	3.34	6.01	1.14	0.63	0.24		
ω-C_{22} OH-FA/ω-C_{16} OH-FA	*1.6*	*1.3*	*0.9*	*0.5*	*0.5*	*2.9*	*0.8*	*0.5*	*0.4*		Nierop et al. (2009)
branched fatty acids											
ΣC_{15}–C_{18} branched FA (iso- and anteiso-)	2.80	0.62	0.39	0.95	0.95	–	–	–	–	bacteria	Cranwell (1980), Goosens et al. (1989), van Bergen et al. (1998)
mono-unsaturated fatty acids											
$C_{16:1}$, $C_{18:1}$ FA	12.4	0.2	–	0.04	0.2	–	–	–	–	phytoplankton, bacteria, microalgae	Bobbie and White (1980), Kattner et al. (1983), Ahlgren et al. (1991)
n-alkanols											
ΣC_{12}–C_{19} OH (short-chain)	3.9	0.3	0.5	1.4	1.1	1.2	3.7	5.7	1.3	phytoplankton, bacteria	Meyers and Ishiwatari (1993),
ΣC_{20}–C_{23} OH (mid-chain)	6.2	7.1	4.3	6.3	3.6	14.1	4.6	10.3	5.2	microalgae, macrophytes?	Volkman et al. (1999), Ficken et
ΣC_{24}–C_{32} OH (long-chain)	8.1	24.1	18.3	37.7	23.7	28.4	17.6	40.4	21.9	higher plants	al. (2002)
n-alkanes											
ΣC_{12}–C_{19} (short-chain)	–	–	–	–	–	–	–	–	–	phytoplankton, bacteria	Meyers and Ishiwatari (1993), Ficken
Σ C_{20} - C_{23} (mid-chain)	1.79	0.24	0.54	0.25	0.08	1.16	0.23	–	0.18	macrophytes	et al. (2002)
ΣC_{24}–C_{32} (long-chain)	0.90	2.10	1.89	1.92	1.39	7.61	1.77	2.35	1.91	higher plants	
P_{aq}=(n-C_{23}+n-C_{25})/Σn-C_{23-31}	*0.38*	*0.13*	*0.14*	*0.18*	*0.18*	*0.11*	*0.30*	-	*0.21*		Ficken et al. (2000)
sterols											
coprostanol	0.76	–	–	–	0.12	–	–	–	–	human faeces	Sherwin et al. (1993), Bull et
epicoprostanol	0.79	–	–	–	–	–	–	–	–	faeces of higher mammals	al. (2000)
epicholestanol	0.65	0.25	0.71	0.35	0.22	–	–	0.12	–	phototrophic sulphur bacteria	Robinson et al. (1984),
cholesterol	6.03	2.10	3.04	1.82	1.64	1.09	0.60	1.87	0.58	non-specific; zooplankton,	Volkman et al. (1986), Goosens et
cholestanol	3.43	1.08	2.95	2.01	0.92	2.09	0.52	0.79	0.19	dinofagellates, diatoms	al. (1989), Thiel et al. (1997)
stigmasterol	2.25	0.62	0.93	0.33	0.29	–	–	–	–	higher plants	Hartmann (1998)
stigmastanol	3.48	2.59	2.14	5.07	2.83	3.42	1.06	1.62	0.86		
sitosterol	4.72	2.54	5.49	2.10	2.16	4.54	0.85	1.81	1.58	higher plants, also diatoms	Volkman et al. (1986, 2005)
dinosterol	–	–	2.01	1.02	1.40	–	0.38	0.38	0.19	dinoflagellates	Volkman et al. (1986)
dinostanol	1.20	–	3.10	1.44	1.41	–	1.30	2.10	0.98		
lanosterol	1.85	2.93	5.32	5.11	3.08	0.01	0.67	1.25	0.33	fungi, animals, dinoflagellates	Volkman (2005), Al-Mutlaq et al. (2008)
others											
β-amyrin	0.20	0.42	0.74	0.94	0.57	0.59	0.24	0.93	–	higher plants	Volkman et al. (2005)
17β(H),21β(H)-bishomohopanol	0.49	1.39	–	1.31	0.71	0.44	0.18	0.36	0.10	bacteria in soils and	Ries-Kautt and Albrecht (1989),
17β(H),21β(H)-bishomohopanoic acid	3.19	6.01	9.27	2.47	6.42	2.80	1.49	1.94	1.78	sediments	Innes et al. (1997)
Σ branched C_{15}, C_{16}, C_{17}, C_{22} alkanols	1.76	0.18	0.39	0.66	–	1.15	–	–	–	bacteria	Cranwell (1980), Thiel et al. (1997)

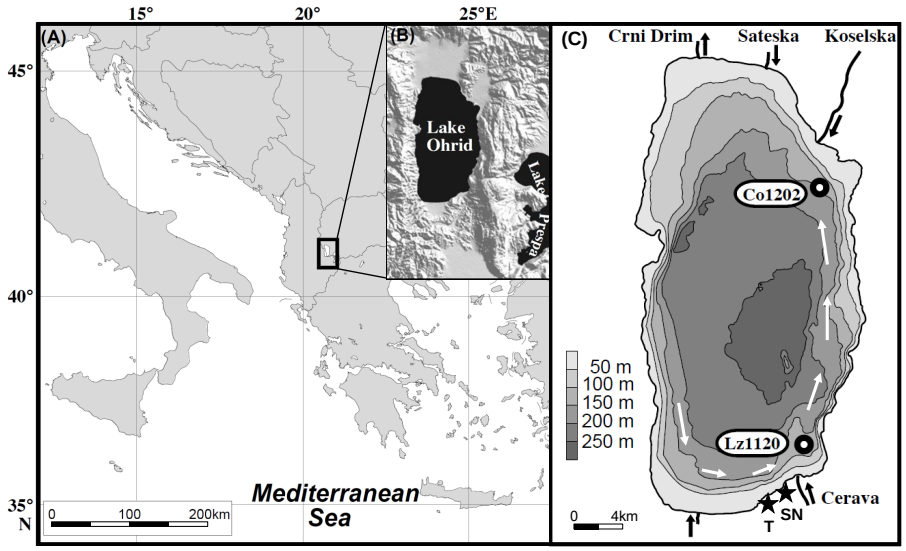

Fig. 1. Maps of SE Europe **(A)** and the Lake Ohrid Basin showing the topography and neighbouring Lake Prespa **(B)**, the bathymetry of Lake Ohrid and the positions of sites Lz1120 and Co1202 **(C)**. White arrows indicate the wind-induced surface currents in the catchments of the sites (according to Vogel et al., 2010). Asterisks mark the karst springs at Tushemisht (T) and Sveti Naum (SN). Note that Lz1120 is influenced by run-off from the low lands at the southern shores, the karst springs and the Cerava River while the catchment of Co1202 includes surface run-off from the steep eastern slopes, only.

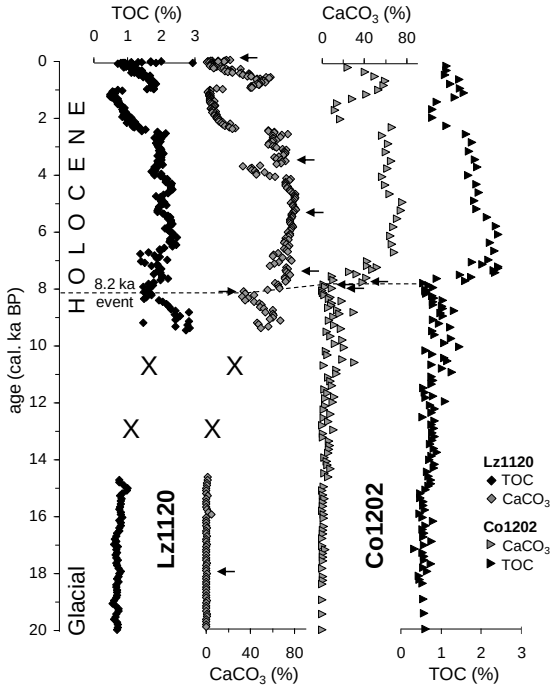

Fig. 2. Weight percentages of carbonate ($CaCO_3$) and total organic carbon (TOC) of Lz1120 and Co1202 (X = hiatus in sediments from site Lz1120; data from Wagner et al., 2009). Black arrows mark the samples selected for this study. Although the current stratigraphic model of site Co1202 suggests a lag of the early Holocene minima occurring in both proxies around 7.8 ka relative to similar minima observed around 8.2 ka at site Lz1120 we are confident that these are corresponding features and represent the prominent 8.2 ka cooling event.

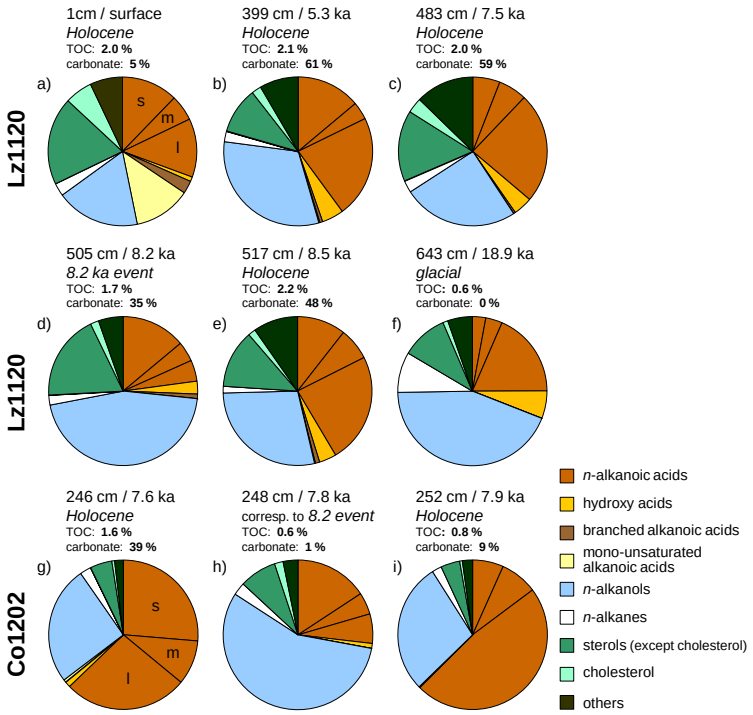

Fig. 3. Composition of total lipid extracts of samples from sites Lz1120 – **(a)–(f)** – and Co1202 – **(g)–(i)**. The segments within the *n*-alkanoic acid signature represent (clockwise) short- (s), mid- (m) and long-chain (l) *n*-alkanoic acids. Note the considerably higher proportions of *n*-alkanoic acids at site Co1202 and the increased amounts of *n*-alkanols in samples from the 8.2 ka event and the glacial. Labile unsaturated *n*-alkanoic acids are present in significant amounts in the surface sample, only.

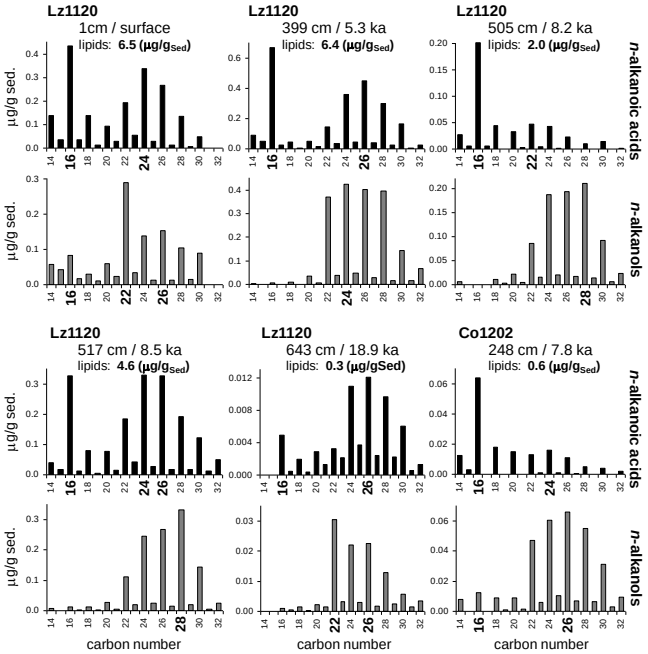

Fig. 4. Distributions of *n*-alkanoic acids (black bars) and *n*-alkanols (grey bars) in samples from Lz1120 and Co1202. Carbon numbers of some specific compounds (e.g. 16 and 24–28 for aquatic and terrestrial OM, respectively) are highlighted by larger numbers where dominant or indicating bimodal distributions. Samples from the 8.2 ka event are strongly depleted in terrestrial *n*-alkanoic acids relative to terrestrial *n*-alkanols suggesting increased proportions of soil organic matter enriched in *n*-alkanols. Considerably lower concentrations of lipids (see y-axis values) during the drier climate stages confirm overall lower OM input.