

# Improved end-member characterization of modern organic matter pools in the Ohrid Basin (Albania, Macedonia) and evaluation of new palaeoenvironmental proxies

J. Holtvoeth<sup>1,2</sup>, D. Rushworth<sup>1</sup>, H. Copsey<sup>2</sup>, A. Imeri<sup>3</sup>, M. Cara<sup>3</sup>, H. Vogel<sup>4</sup>, T. Wagner<sup>5</sup>, and G. A. Wolff<sup>1</sup>

[1]{Department of Earth, Ocean and Ecological Sciences, School of Environmental Sciences, University of Liverpool, Liverpool, UK}

[2]{School of Chemistry, Organic Geochemistry Unit, University of Bristol, Bristol, UK}

[3]{Faculty of Agriculture and Environment, Agricultural University of Tirana, Tirana, Albania}

[4]{Institute of Geological Sciences & Oeschger Centre for Climate Change Research, University of Bern, Bern, Switzerland}

[5]{School of Civil Engineering & Geosciences, University of Newcastle, Newcastle-upon-Tyne, UK}

Correspondence to: J. Holtvoeth (j.holtvoeth@bristol.ac.uk)

## Abstract

We present elemental, lipid biomarker and compound-specific isotope ( $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ ) data for soils and leaf litter collected in the catchment of Lake Ohrid (Albania, Macedonia), as well as macrophytes, particulate organic matter and sediments from the lake itself. Lake Ohrid provides an outstanding archive of continental environmental change of at least 1.2M years and the purpose of our study is to ground truth organic geochemical proxies that we developed in order to study past changes in the terrestrial biome. We show that soils dominate the lipid signal of the lake sediments rather than the vegetation or aquatic biomass. There is a strong imprint of suberin monomers on the composition of total lipid extracts and chain-length distributions of *n*-alkanoic acids, *n*-alcohols,  $\omega$ -hydroxy acids and  $\alpha,\omega$ -dicarboxylic acids. Our end-member survey identifies that ratios of mid-chain length suberin-derived to long-chain length cuticular-derived alkyl compounds as well as their average chain length distributions can be used as new molecular proxies of organic matter sources to the lake. We tested these for the 8.2 ka event, a

1 pronounced and widespread Holocene climate fluctuation. In SE Europe climate became drier  
2 and cooler in response to the event, as is clearly recognizable in the carbonate and organic  
3 carbon records of Lake Ohrid sediments. Our new proxies indicate biome modification in  
4 response to hydrological changes, identifying two phases of increased soil OM supply, first  
5 from soils with moderately degraded OM and then from more degraded soils. Our study  
6 demonstrates that geochemical fingerprinting of terrestrial OM should focus on the main lipid  
7 sources, rather than the living biomass. Both can exhibit climate-controlled variability, but are  
8 generally not identical.

9

## 10 **1 Introduction**

11 Climate-controlled changes in hydrology are reflected in the development of the terrestrial  
12 biome, which determines the amount and the quality of organic matter (OM) supplied to  
13 environmental archives (Meyers and Lallier-Vergès, 1999). Currently, reconstructions of biome  
14 dynamics are largely based on pollen data, focussing on the development of the vegetation  
15 (Birks and Birks, 2006). The dynamics of the soil organic carbon pool, however, is poorly  
16 constrained, mainly because palynologists cannot easily discriminate between direct supply of  
17 pollen and intermediate storage of pollen in soils. Similarly, paleoclimatologists using  
18 established organic geochemical proxies such as the organic carbon to nitrogen ratio (C/N) or  
19 bulk organic carbon isotopes ( $\delta^{13}\text{C}_{\text{org}}$ ) to identify changing proportions of OM from aquatic and  
20 terrestrial sources struggle to differentiate between the individual elements of the terrigenous  
21 OM pool. Particularly problematic is the variable composition of terrestrial OM resulting from  
22 changing proportions of soil and plant litter and the degree of biological degradation that is  
23 closely related to the moisture regime. Furthermore, potential end-member materials are often  
24 not properly defined and proxies are transferred between supposedly comparable settings  
25 untested, reducing their actual applicability and potentially leading to erroneous interpretations.

26 We focus on Lake Ohrid in the Western Balkans (Fig. 1), which provides an outstanding archive  
27 of continental environmental change dating back at least 1.2M years (Wagner et al., 2014). In  
28 2013, almost 600 m of sediment core was recovered during a drilling campaign for the  
29 International Continental Drilling Program (ICDP). Initial analyses of core catcher material and  
30 logging data reveal a continuous limnic sequence, showing pronounced cyclicality that clearly  
31 relates to eccentricity and obliquity cycles (Wagner et al., 2014; Baumgarten et al., 2015). This  
32 exceptional continental sediment record thus appears very promising for high-resolution  
33 palaeoenvironmental reconstructions of orbitally controlled Northern Hemisphere climate

1 fluctuations. Earlier studies of pilot cores suggest a close relationship between the North  
2 Atlantic climate of the past 130,000 years and the sedimentation of inorganic as well as organic  
3 matter in the lake (Vogel et al., 2010; Holtvoeth et al., 2010). Whichever proxy is used to  
4 reconstruct changes in biogeochemical fluxes towards a sedimentary archive, the sources of  
5 each component have to be known in order to correctly interpret proxy variability. For distal  
6 marine sediment archives, mixing associated with long-distance transport results in averaged  
7 end-member characteristics for marine and terrestrially-sourced matter that are reasonably well  
8 defined. Terrestrial ecosystems, by contrast, show far greater complexity and diversity, with a  
9 great variety of local factors and processes that modify specific end-members. The local  
10 vegetation type, geology, geomorphology or hydrology may all reveal peculiarities that  
11 potentially interfere with the regional or global climate signal that paleoclimatologists are keen  
12 to extract from terrestrial archives.

13 As part of a longer term study of the Ohrid sedimentary record, we aim to improve the end-  
14 member definition of organic matter (OM) sources, and test new geochemical proxies across  
15 the 8.2 ka climate event. We provide lipid biomarker distributions and elemental compositions  
16 of modern materials from the Lake Ohrid Basin, including terrestrial plant litter, soils,  
17 macrophytes and lake water particles, all of which may influence the geochemical signature of  
18 the OM deposited in the lake.

19 A great advantage of the Lake Ohrid climate archive is its small, mountainous catchment (Fig.  
20 1). Up to 55% of water inflow derives from karst springs fed by seepage from the higher altitude  
21 Lake Prespa. Although these waters do not deliver any sediment they do supply small amounts  
22 of nutrients to Lake Ohrid, e.g., 7% of the total phosphorous load (Matzinger et al., 2006), as  
23 well as calcium ions allowing carbonate precipitation. As there are no major rivers entering the  
24 Ohrid Basin, the supply of allochthonous sediment to the lake depends almost entirely on  
25 surface run-off from the surrounding mountain ranges in the form of small streams and gullies.  
26 The Triassic limestones forming the eastern, southeastern and northwestern slopes of the Ohrid  
27 Basin are highly permeable so that much of the precipitation is taken up by karstic systems  
28 before reaching the lake. Thus, the quantity and composition of terrestrial OM supplied to the  
29 lake will reflect hydrologically-controlled changes in vegetation and soil stocks of the  
30 immediate surroundings, while productivity in the lake reflects run-off-controlled nutrient  
31 supply. The steep morphology and the geological conditions in the catchment force a rapid  
32 response of the vegetation cover to precipitation decrease and are responsible for relatively low  
33 soil stability. Primary productivity in Lake Ohrid is low due to the low nutrient levels (e.g.,  
34 total phosphorous:  $0.15 \pm 0.026 \text{ mmol m}^{-3}$ , Matzinger et al., 2007). Short distances, rapid

1 response and little dilution through aquatic productivity are excellent preconditions for the  
2 reconstructions of biome dynamics using sedimentary OM composition.

3 While the use of biological markers as proxies for environmental change is well established,  
4 we present the first data set of this kind for the Ohrid Basin to improve the interpretation of  
5 sedimentary OM compositional changes. We incorporate observations made during the study  
6 of sediments from pilot cores taken prior to the 2013 ICDP drilling. Our catchment data then  
7 allows us to identify the main OM sources to the Lake Ohrid sediments.

8 We also determined the carbon and hydrogen stable isotope composition for *n*-alkanoic acids  
9 of modern terrestrial materials and macrophytes ( $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ ). Similar data from algal biomass  
10 could not be established and signals largely overlapped, therefore isotope-based proxies could  
11 not be developed. However, as the data produced may be of interest to scientists working on  
12 comparable subjects, it are reported and discussed in a supplementary section (see Supplement  
13 2).

## 14 **2 Sampling, materials and methods**

### 15 **2.1 Sample collection**

16 Sampling was carried out along the southern and southeastern shores of Lake Ohrid (Fig. 1).  
17 We did not sample each component of the ecosystem, but instead focused on the major OM  
18 sources. Leaf litter and underlying topsoils, i.e. O2 and A horizons, respectively, were collected  
19 in June 2012 and 2013 on the Macedonian side of the lake at three sites: under light forest  
20 vegetation dominated by small oaks in the vicinity of the Saint Naum karst springs (SN, Fig.  
21 1), from a similar site about 2 km north of Trpejca (TP) and in a high-altitude beech forest of  
22 the Galicica National Park (GN). In September 2014, two species of grass were sampled, one  
23 of which belongs to the evergreen genus *Festuca* and typically grows in shady locations on  
24 rocky substrates (GN-G). The generally most abundant but unidentified species was collected  
25 from the high-altitude grasslands at the top of Galicica Pass (GP, Fig. 1). Furthermore, we  
26 sampled a prominent type of subsoil (B horizon), a deep red Chromic Luvisol (Terra Rossa)  
27 that is frequently exposed in gullies or along road cuttings and typically sits above the Mesozoic  
28 carbonates. Two samples were collected from the same spot at a near vertical roadside soil  
29 profile (DG, Fig. 1) in June 2013 (DG-SS) and September 2014 (DG-SS 2). Both subsoil  
30 samples were taken from about 50 cm below the soil surface and about 30 cm below the A-  
31 horizon. A noteworthy difference is that sample DG-SS included exposed surface material

1 while DG-SS 2 was taken from after removing 10-15 cm of soil from the surface. In contrast to  
2 the topsoils, these samples do not visibly contain significant amounts of fine root material.

3 At two littoral sites, near the DG site and ~2 km north of Pogradec, we collected samples of  
4 reed (*Phragmites* spp.). Submerged macrophytes were collected from a small vessel along two  
5 transects from 4-16 m water depth off the town of Pogradec and the village of Tushemisht  
6 (Albania; Fig. 1) using a small Van Veen bottom sampler (Hydro-Bios, Kiel, Germany). From  
7 shallow to deep water, we collected benthic algae (*Cladophora* spp.), one species of pondweed  
8 (*Potamogeton perfoliatus*) and 3 species of charophytes (*Characea tomentosa*, *C. gymnophilia*,  
9 *C. contraria*).

10 Lake water samples were collected from two sites (DEEP and Co1202; Fig. 1) by deployment  
11 of Niskin Bottles (20 L). The water samples were filtered immediately after collection in the  
12 laboratory of the Hydrobiological Institute in Ohrid using an electrical pump and pre-  
13 combusted glass fiber filters with a nominal pore size of 0.7  $\mu\text{m}$ . The filters were stored frozen  
14 (-20°C) until freeze-drying in the laboratory at Cologne University.

15 11 sediment samples were taken from core Lz1120 situated close to the southeastern shores of  
16 Lake Ohrid (Fig. 1). The detailed site description and age model are provided by Wagner et al.  
17 (2009). Accordingly, our samples cover the time span from 8.65 to 8.05 ka (8647-8049  $\pm$  130  
18 cal. years BP), sampled with a time resolution of 60 years. The sediment core sections were  
19 stored cold in the repository of the Institute of Geology and Mineralogy at Cologne University  
20 (Germany) prior to sub-sampling. Samples were freeze-dried before shipment and stored frozen  
21 (-20°C) prior to analysis. All terrestrial samples and macrophytes were stored cold in the field  
22 (4 °C) and then dried (70°C, 48 h).

## 23 **2.2 Elemental analysis**

24 Total carbon (TC) and total nitrogen (TN) contents were measured in duplicate (values were <  
25 10% of the mean) using a CE Instruments NC 2500 elemental analyzer. Total organic carbon  
26 (TOC) was determined after acid vapor (HCl) digestion of the carbonate fraction (Yamamuro  
27 and Kayanne, 1995). Carbonate contents (assuming all carbonate was as CaCO<sub>3</sub>) were then  
28 calculated from the difference between TC and TOC measurements using the equation  $\text{CaCO}_3$   
29 = (weight% TC – weight% TOC) \*  $M_{\text{CaCO}_3}/M_{\text{C}}$  (M = molar/atomic mass; CaCO<sub>3</sub> = 100, C =  
30 12). Contents of total organic carbon (TOC) and carbonate (CaCO<sub>3</sub>) are given as percentages  
31 of dry weight. The ratio of total organic carbon to total nitrogen (TOC/TN) is given as the molar  
32 ratio.

## 1 **2.3 Lipid analysis**

2 For extraction of the lipid fraction, about 0.7 g of plant matter and 2 g of soil and sediment were  
3 homogenized and sonicated (3 x 15 min) in a mixture of dichloromethane (DCM) and methanol  
4 (9:1, v:v; 10 mL). About 0.2 g of the grass samples and 5 g of the duplicate subsoil sample  
5 (DG-SS 2) were extracted by microwave-assisted solvent extraction (10 min at 70°C) using the  
6 same solvent mixture. The total lipid extract (TLE) was concentrated and any water removed  
7 by passing it through a Pasteur pipette column packed with anhydrous sodium sulphate. Acid-  
8 catalysed transmethylation of *n*-alkanoic acids was achieved by adding a solution of acetyl  
9 chloride in methanol (1:30, v:v; 1 mL; 0°C) and then warming the samples (45°C, 12 h). This  
10 methylates the carboxyl group of free *n*-alkanoic acids and also breaks the ester bonds in bio-  
11 polyesters such as suberin and in cuticular wax esters, thus, releasing the (previously bound)  
12 alkyl monomers as methyl esters. The methylated TLEs were dried under nitrogen and re-  
13 dissolved in DCM and passed through a column loaded with potassium carbonate to remove  
14 excess acetic acid. Finally, N,O-bis-(trimethylsilyl)-trifluoroacetamide with 1%  
15 trimethylchlorosilane was added to the TLE and warmed (65°C, 30 min) to derivatise  
16 compounds with hydroxy groups (e.g., *n*-alcohols, sterols).

17 Water filtrates were extracted using a Dionex Accelerated Solvent Extraction system (ASE),  
18 with DCM and methanol (9:1, v:v) as solvent. Aliquots of the resulting total lipid extracts were  
19 separated into aliphatic, aromatic and hetero-compound fractions by column chromatography  
20 using deactivated SiO<sub>2</sub> (mesh size 60) and elution with hexane, DCM:hexane (2:1, v:v) and  
21 methanol, respectively. The polar fractions of the water filtrates were transmethylated and  
22 derivatised following the same protocol described above.

23 Aliquots of the transmethylated and derivatised extracts of samples collected in 2012/2013 were  
24 injected onto a Trace 2000 Series gas chromatograph (GC) fitted with an on-column injector  
25 and a fused high-temperature silica column (60 m × 0.25 mm i.d.; film: (5 % phenyl-)  
26 methylpolysiloxane; DB5-HT equivalent; J&W) with helium as the carrier gas (*ca.* 1.6 mL min<sup>-1</sup>  
27 <sup>1</sup>). A retention gap of deactivated fused silica (1 m × 0.32 mm i.d.) was used at the front of the  
28 column. The oven was programmed from 60°C to 170°C at 6°C min<sup>-1</sup> after 1 minute, then to  
29 315°C at 2.5°C min<sup>-1</sup> and held for 10 minutes. The GC column was fed directly into the EI  
30 source of a Thermoquest Finnigan TSQ7000 mass spectrometer (ionisation potential 70 eV;  
31 source temperature 315°C; trap current 300 μA), operated in Full Data Acquisition mode, (50  
32 - 600 Thompsons cycled every s). TLEs of the samples collected in 2014 (grasses and DG  
33 subsoil duplicate) were treated as above but analysed using a Trace 1300 Series GC fitted with

1 a different capillary column (fused silica; 50 m × 0.32 mm i.d.; film: dimethylpolysiloxane;  
2 Rtx-1, DB-1 equivalent; Thames Restek) and a programmed temperature vaporising (PTV)  
3 injector in splitless mode. The carrier gas was helium (2 mL min<sup>-1</sup>). The GC was connected to  
4 a Thermo Fisher Scientific ISQ mass spectrometer in EI mode (ionisation potential 70 eV,  
5 source temperature 320°C). Data were processed using Xcalibur software. Compounds were  
6 identified either by comparison of their mass spectra and relative retention indices with those  
7 available in the literature and/or by comparison with authentic standards. Quantitative data were  
8 calculated by comparison of peak area of the internal standard, 5 $\alpha$ (H)-cholestane (spiked onto  
9 the samples before extraction), with those of the compounds of interest, using the total ion  
10 current chromatogram. The relative response factors of the analytes were determined  
11 individually for 36 representative compounds using authentic standards. For analytes for which  
12 authentic standards were not available, the response factors for similar compounds of the same  
13 class and/or similar structure were used. Response factors of standards were not determined for  
14 the grass samples (GN-G, GP-G) and the duplicate sample from site DG (DG-SS 2) and were  
15 assumed to be 1, hence data for those samples are semi-quantitative. Reproducibility of similar  
16 lipid analyses was determined to be <  $\pm 15$  % (Kiriakoulakis et al., 2000). Data quality was  
17 checked regularly with procedural blanks for each extracted sample batch and organic  
18 contamination was subtracted from the sample values, although it typically was insignificant  
19 ( $\ll 1$  % of the sample values). Total lipids were calculated as the sum of all identified  
20 compounds from the total ion chromatograms (TIC).

## 21 **2.4 Statistical methods**

22 Statistical analyses (Multi-dimensional scaling, MDS; Analysis of Similarity, ANOSIM;  
23 Similarity Percentages, SIMPER) were conducted on lipid biomarkers, their concentrations  
24 being normalised to percentage of total identified lipids (%<sub>lipid</sub>). The analyses were carried out  
25 separately for soils, leaf litters, macrophytes, lake particulate organic matter (POM) and  
26 sediment samples from core Lz1120. 78 variables were chosen; these were saturated and  
27 branched fatty acids, hydroxy acids, alcohols, *n*-alkanes and amyrins. Where there were zero  
28 values, a minimum detection limit was chosen of one half of the lowest recorded concentration,  
29 namely 0.001 %<sub>lipid</sub> (Yunker et al., 2005). Data were fourth root transformed and screened to  
30 confirm a normal distribution using a Draftsman's plot for all variables prior to analyses, which  
31 were carried out using PRIMER software (Primer-E Ltd., UK). All quantitative data can be  
32 found in the Supplementary Material.

## 1 **3 Results and Discussion**

### 2 **3.1 Elemental analysis**

#### 3 **3.1.1 Leaf litter and soils**

4 Leaf litter samples from the near-shore low-altitude forests (sites SN and TP) have high TOC  
5 contents of  $43 \pm 2$  % and TOC/TN ratios of  $\sim 27 \pm 2$  (Table 1). TOC contents of the Leptosol  
6 A-horizons at SN and TP range from 7.5 to 11 %, while their TOC/TN ratios vary little around  
7 17 (Table 1). The TOC/TN ratio of the high-altitude Leptosol (GN) is slightly lower, with a  
8 value of  $15.3 \pm 0.8$ . For all Leptosols, carbonate contents are low and vary between 5 and 8 %.  
9 The surface-exposed material from the B-horizon of the Chromic Luvisol (Terra Rossa; DG-  
10 SS) has low TOC and carbonate contents of 0.9 % and  $< 0.5$  %, respectively, within reported  
11 ranges for this soil type in the Mediterranean (e.g., Costantini et al., 2013: TOC 0.5 %,  $\text{CaCO}_3$ :  
12 0.5 %,  $n = 48$ ). The second sample (DG-SS 2) shows higher TOC and carbonate contents of  $1.5$   
13  $\pm 0.1$  % and  $2.3 \pm 1.5$  %, respectively. The TOC/TN ratios of the Terra Rossa samples are  
14 almost identical, and have an average value of  $11.6 \pm 0.7$ , which is also significantly lower than  
15 the Leptosols (Table 1).

#### 16 **3.1.2 Macrophytes**

17 The macrophyte samples can be separated into two types of plant that (a) do precipitate  
18 carbonate, i.e., *Characeae* spp., and (b) do not, i.e., *Cladophora* spp., *Potamogeton* spp.,  
19 *Cladophora* spp., *Potamogeton* spp. and the leaves of *Phragmites* spp.. Plants from group (b)  
20 have similar TOC contents ( $42 \pm 2$  %); TOC/TN ratios are also similar ( $13.5 \pm 0.3$ ; Table 2).  
21 The two specimens of *Characeae* (*Chara tomentosa*, *Chara gymnophylla*) have high amounts  
22 of carbonate ( $66 \pm 7$  %), diluting the TOC contents to  $10 \pm 0.3$  %. The TOC/TN ratios are  
23 higher than those of the other macrophytes:  $17.8 \pm 1.2$  %. High carbonate contents of  $>70$  %  
24 are common for charophytes in freshwater systems (see Apolinarska et al., 2011 and references  
25 therein). Królikowska (1997) reports calcium contents of  $234 \text{ mg g}^{-1}$  and  $246 \text{ mg g}^{-1}$  dry weight  
26 for *Chara tomentosa* and *Chara contraria* from a shallow Polish lake, or carbonate contents of  
27  $59 \pm 4$  % and  $61 \pm 2$  %, respectively, assuming that all calcium is present as carbonate, close to  
28 our value for *Chara tomentosa* (61.5 %).

#### 29 **3.1.3 Sediments**

30 Carbonate contents of the Lz1120 sediments range from 20 to 56 % and TOC from 1.5 to 2.3  
31 % (Fig. 2). The carbonate record of Lake Ohrid appears to be controlled by temperature and



1 terrestrial run-off, with precipitation and production of largely endogenic carbonate occurring  
2 mostly during the summer (Wagner et al., 2008; Vogel et al., 2010) and relying on the supply  
3 of calcium ions and nutrients from the catchment. Accordingly, minima in sedimentary  
4 carbonate represent periods of drier and cooler climate. Between 8.8 and 7.9 ka, the Lz1120  
5 carbonate record features two pronounced minima at around 8.3 and 8.15 ka that appear to  
6 correspond to phases of the 8.2 ka event (Fig. 2) as documented in sediment records from the  
7 North Atlantic (MD99-2251) and Greenland ice cores (GISP 2; Ellison et al., 2006). TOC  
8 decreases from 2.3 to a minimum of 1.5 % at *ca.* 8.3 ka and remains below 2 % thereafter.  
9 Carbonate and organic carbon contents appear to co-vary apart from in the three youngest  
10 samples between 8.05 and 7.9 ka and the sample separating the two carbonate minima at 8.23  
11 ka. In the remaining samples, TOC and carbonate correlate closely ( $R^2 = 0.86$ ). TOC/TN ratios  
12 range from 7 to 10.7, with values  $> 10$  between 8.9 and 8.3 ka (average:  $10.5 \pm 0.2$ ) and  $< 10$   
13 thereafter (average:  $8.1 \pm 0.7$ ). Higher TOC/TN values generally coincide with higher organic  
14 carbon contents ( $R^2 = 0.91$ ). When TOC is plotted against TN, however, the samples are clearly  
15 separated into two groups (Fig. 2, insert): samples from before and after 8.3 ka show similar  
16 concentrations of TN but significantly different concentrations of TOC, with lower TOC  
17 relative to TN after 8.3 ka. Before 8.3 ka, there is a strong linear relationship between TOC and  
18 TN ( $R^2 = 0.94$ ), with a y-axis intercept of 0.057 % TN for 0 % TOC, suggesting that around a  
19 quarter of the nitrogen is inorganic. The amount of organic nitrogen ( $N_{org}$ ) that results from this  
20 assumption leads to an average TOC/ $N_{org}$  ratio of 13.6 for the samples before 8.3 ka. After 8.3  
21 ka, the correlation between TOC and TN disappears, implying that higher nitrogen  
22 concentrations do not generally result from the supply of nitrogen-rich OM, but from variable  
23 amounts of inorganic nitrogen ( $N_{in}$ ) such as ammonium ( $NH_4^+$ ). The implications for the  
24 interpretation of the TOC/TN record of Lz1120 are discussed below (section 4.1 Assessing  
25 aquatic and terrestrial OM supply).

## 26 **3.2 Lipid Biomarkers**

### 27 **3.2.1 Leaf litter, topsoils, grasses**

#### 28 **3.2.1.1 Biomarker content and composition**

29 Leaf litter under both types of vegetation, low-altitude oak-dominated forest (sites SN, TP) and  
30 high-altitude beech forest (GN) contain very similar total amounts of lipids: *ca.*  $458 \pm 63 \mu\text{g g}^{-1}$   
31 dry weight ( $n = 4$ ). Lipid contents of the topsoils are approximately 6x lower, with an average  
32 of  $82 \pm 37 \mu\text{g g}^{-1}$  dry weight ( $n = 6$ ) for the Leptosols. Grasses revealed the by far highest

1 amounts of lipids, containing about  $1.4 \pm 0.2 \text{ mg g}^{-1}$ . Both high- and low-altitude leaf litter do  
2 not contain visible contributions from grass but are dominated by leaf fragments of the main  
3 tree species, i.e. small oaks (SN, TP) and beeches (GN). Leaf litter can be regarded as a main  
4 contributor to soil organic matter and the compositional changes occurring during leaf litter  
5 incorporation into the topsoil are described in the following.

6 There are compositional differences in the major lipid compound classes, between leaf litter  
7 and corresponding topsoils and between high-altitude and low-altitude sites (see Figure 3 and  
8 Table 2). Lipids of the near-shore oak-dominated leaf litter (sites SN, TP) are dominated by  
9 saturated FAs that account for  $37 \pm 2 \%$ lipids, while the other major compound classes, i.e. mono-  
10 and poly-unsaturated fatty acids (MUFAs, PUFAs) and sterols, vary between 13 and 18 %lipids.  
11 PUFAs and MUFAs are present in similar proportions,  $9.3 \pm 1.4$  and  $7.1 \pm 0.3 \%$ lipids,  
12 respectively. In high-altitude beech-dominated litter (GN), FAs make up only 23 %lipids and the  
13 proportions of MUFAs and PUFAs are considerably higher, 21 and 17 %lipids, respectively.  
14 Sterols make up 24 %lipids, with  $\beta$ -sitosterol (24 $\beta$ -ethylcholest-5-en-3 $\beta$ -ol; 17 %lipids) and  $\beta$ -  
15 sitostenone (24 $\beta$ -ethylcholest-4-en-3-one; 5 %lipids) together accounting for 96 % of the total  
16 sterols.  $\beta$ -Sitosterol is also the dominant sterol in low-lying oak-dominated litter (SN, TP;  $10.7$   
17  $\pm 0.3 \%$ lipids). In SN leaf litter, the pentacyclic triterpenoid taraxasterol (urs-20(30)-en-3 $\beta$ -ol) is  
18 also abundant ( $11.3 \pm 1.1 \%$ lipids, on average). It also occurs in leaf litter at site TP (7.1 %lipids)  
19 but is absent in the litter and soil samples at GN. Details on the potential source of this  
20 compound are provided in Supplement 2.

21 The proportions of the major compound classes in the topsoils differ from those observed in  
22 the overlying leaf litter. While the relative amounts of FAs remain similar ( $35 \pm 4.7 \%$ lipids), the  
23 MUFAs increase in relative abundance from leaf litter to soil from ca. 7 to 20 %lipids at TP and  
24 SN and from 12 to 25 %lipids at GN with *n*-C<sub>18:1</sub>(*cis*-9) being the dominant MUFA. Small  
25 amounts of *n*-C<sub>20:1</sub>(*cis*-9) MUFA might derive from collembolans (springtails), small  
26 detritivorous and microbivorous hexapods feeding on nematodes as well as fungi in leaf litter  
27 (Ruess et al., 2005). PUFAs, on the other hand, decrease from leaf litter to soil in relative  
28 abundance at all sites, most notably at GN, to between 5 and 6 %lipids. The dominant PUFA in  
29 all leaf litter samples is C<sub>18:2</sub>. Leaf litter at sites TP and GN contained C<sub>18:2</sub> only, albeit in higher  
30 amounts than at site SN with 8 %lipids (TP) and 17 %lipids (GN) vs. 8 %lipids (SN). C<sub>18:3</sub> occurs  
31 only at site SN where it accounts for 2 % lipids. For C<sub>18:2</sub>, this pattern is also preserved in the  
32 corresponding soils. The proportion of PUFA is also higher in the soil sample affected by white  
33 rot (TP-F), which is the only soil sample where we detected a small amount of C<sub>18:3</sub> and, notably



1 eicosapentaenoic acid (EPA; C<sub>20:5</sub>). The latter probably has a fungal source as some soil fungi  
2 such as *Mortierella alpina* or plant pathogens such as *Pythium ultimum* are known to  
3 biosynthesize EPA (Shimizu et al., 1988; Ghandi and Weete, 1991).

4 The higher amounts of *n*-C<sub>18:1</sub>(*cis*-9) MUFA in the soils (58 % of all unsaturated FAs at TP and  
5 SN, up to 71 % at GN) are likely to derive from root suberin in addition to cutin. Besides *n*-C<sub>16</sub>  
6 FA and *n*-C<sub>18</sub> FA, the *n*-C<sub>18:1</sub> MUFA is one of the main monomers synthesized *de novo* to form  
7 both cutin and suberin (Mertz and Brutnell, 2014).

8 The relative amount of steroids also decreases from leaf litter to soil. At GN, their relative  
9 abundance nearly halves compared to the leaf litter (24 to 13 %<sub>lipids</sub>). At TP and SN the decrease  
10 is marginal, from 14 to 11 %<sub>lipids</sub>. The main sterol in both leaf litter and topsoils is  $\beta$ -sitosterol  
11 accounting for 60 to 80 % of the total sterols.

12 A notable difference in the total lipid composition between leaf litter and underlying soils is the  
13 significantly higher proportion of *n*-alcohols in the latter. *n*-Alcohols account for  $3.1 \pm 0.3$  %  
14 <sub>lipids</sub> in leaf litter at SN and TP and 6.3 %<sub>lipids</sub> at GN. However, their proportions in the soils are  
15 4 and 2.5-times higher, respectively.

16 Minor compound classes present in leaf litter and soils include hydroxy acids, *n*-alkanes and  
17 branched FA. Hydroxy acids (OH-FA) are more abundant in leaf litter at SN ( $3.9 \pm 1.0$  %<sub>lipids</sub>)  
18 than TP (2.1 %<sub>lipids</sub>) and GN (0.9 %<sub>lipids</sub>). At SN, relative concentrations of  $\alpha$ -hydroxy acids ( $\alpha$ -  
19 OH-FA) are *ca.* 10x higher than  $\omega$ -hydroxy acids ( $\omega$ -OH-FA absent in TP sample), while leaf  
20 litter at GN is dominated by  $\omega$ -OH-FA. However, soils from all sites show similar relative  
21 amounts of  $\omega$ - and  $\alpha$ -OH-FAs (5 – 8 %<sub>lipids</sub>). The substantial increase in the amounts of  $\omega$ -OH-  
22 FAs from litter to soil by an order of magnitude and the chain-length range from C<sub>16</sub> to C<sub>26</sub> (site  
23 GN: C<sub>16</sub> - C<sub>28</sub>) suggest their source is suberin, a protective biopolymer mainly found in the  
24 periderm of plant root and stem tissues, but also in bundle sheaths of grasses (Pollard et al.,  
25 2008; Molina et al., 2006). *n*-Alkanes account for 5 - 7 %<sub>lipids</sub> in the leaf litter samples, but  
26 only 3 %<sub>lipids</sub> in the topsoil samples at all three sites. The proportions of branched FAs increase  
27 by an order of magnitude from 0.2 to 2.7 %<sub>lipids</sub> and 0.1 to 1.7 %<sub>lipids</sub> at SN and GN, respectively;  
28 they are absent in leaf litter at TP. There is a difference in the composition of branched FAs  
29 between litter and topsoil samples. While the litter samples mainly contain *iso*- and *anteiso*-  
30 branched C<sub>15</sub> FA as well as *iso*-C<sub>16</sub> FA, the topsoil samples additionally contain *iso*- and  
31 *anteiso*-C<sub>17</sub> FA as well as 10-methyl hexadecanoic acid. Finally,  $\alpha,\omega$ -dicarboxylic acids ( $\alpha,\omega$ -  
32 DiFAs) occur almost exclusively in the soil samples, the exception being one leaf litter sample  
33 from site SN where  $\alpha,\omega$ -DiFAs were detected in small amounts (0.3 %<sub>lipids</sub>). In all other leaf

1 litter samples,  $\alpha,\omega$ -DiFAs were not detectable. By contrast,  $\alpha,\omega$ -DiFAs accounted for 1 %<sub>lipids</sub>  
2 (SN) and 0.7 %<sub>lipids</sub> (TP) in the low-altitude soils and for 0.4 %<sub>lipids</sub> in the high-altitude sample  
3 (GN). As for the  $\omega$ -OH-FAs, their occurrence with a chain length range of C<sub>16</sub> - C<sub>26</sub> strongly  
4 suggest that the main source of the  $\alpha,\omega$ -DiFAs is suberin.

5 Compared to leaf litter and topsoils, TLEs of grasses show a far less complex composition, with  
6 the alkyl compounds being dominated by only a few individual biomarkers (Fig. 3b). Apart  
7 from representing single species samples, a further reason for this may be that the grasses were  
8 not sampled as litter from the ground but as upright, although dry, late-season plant matter. This  
9 means that degradation was at the earliest stage and contribution of lipids from degrading  
10 organisms likely minimal, which is also indicated by the absence of branched FAs. The grasses  
11 show significantly higher amounts of PUFAs ( $29 \pm 2$  %<sub>lipids</sub>) and a far lower proportion of  
12 MUFAs ( $3 \pm 1$  %<sub>lipids</sub>) than leaf litter and topsoils. The dominant PUFA and, in fact, the most  
13 abundant individual compound is C<sub>18:3</sub>(*cis*-9), which accounts for 20% of the total lipids. The  
14 proportions of short-chain FAs, *n*-alkanes and *n*-alcohols are also elevated, which is due to  
15 three compounds: C<sub>16</sub> FA, the C<sub>31</sub> *n*-alkane and C<sub>26</sub> *n*-alcohol. Together with C<sub>18:3</sub>(*cis*-9) PUFA  
16 and C<sub>18:2</sub>(*cis*-9) PUFA (8 %<sub>lipids</sub>) these compounds account for about 70 % of the total lipids in  
17 the grass samples. Long-chain FAs account for 6 %<sub>lipids</sub>, while the main sterol, sitosterol,  
18 contributes 4 %<sub>lipids</sub> and  $\beta$ -amyirin 5 %<sub>lipids</sub>. The amounts of  $\alpha$ - and  $\omega$ -OH-FAs are very low: 0.3  
19 %<sub>lipids</sub> and 0.2 %<sub>lipids</sub>, respectively, comparable to leaf litter samples. Saturated C<sub>24</sub> and mono-  
20 unsaturated C<sub>24:1</sub>  $\alpha$ -OH-FA are the only  $\alpha$ -OH-FA detected. In contrast to the leaf litter  
21 samples, the  $\omega$ -OH-FAs in the grasses are dominated by C<sub>24</sub> and C<sub>22</sub>  $\omega$ -OH-FA (~80 % of total  
22  $\omega$ -OH-FAs), with C<sub>16</sub>  $\omega$ -OH-FA accounting for the rest.

### 23 **3.2.1.2 Carbon number distributions of *n*-alkyl compounds**

24 Chain length distributions of *n*-alkyl compounds (saturated *n*-FA, *n*-alcohols,  $\alpha$ -/ $\omega$ -OH-FA and  
25 *n*-alkanes) show subtle differences between high-altitude beech-dominated (GN) and near-  
26 shore low-lying oak-dominated leaf litters (TP, SN), and between litter and underlying soils,  
27 which could nevertheless be useful in tracing the provenance of OM in the lake sediments. The  
28 main *n*-alkanoic acid in both leaf litter and topsoils is C<sub>16</sub> FA. At TP and SN, the leaf-litter  
29 samples have a trimodal chain-length distribution, with long-chain and mid-chain length C<sub>30</sub>  
30 FA and C<sub>22</sub> FA, respectively, being the other modes (Fig. 3b). This trimodal distribution is more  
31 pronounced at GN, with C<sub>22</sub> FA being the mid-chain mode, while the C<sub>28</sub> FA is the long-chain  
32 mode. The proportion of mid-chain compounds, in particular, the amounts of C<sub>22</sub>- and C<sub>24</sub>-FA

1 increase significantly in the low-altitude topsoils (TP, SN), relative to the overlying leaf litters  
2 (Figs. 3, 4a). Accordingly, the average chain-length (ACL; Eq. 1) between C<sub>22</sub> and C<sub>26</sub>-FA  
3 shifts from 24.2 to 23.6 between litter and topsoil.

$$4 \text{ ACL} = (22 \cdot C_{22} + 24 \cdot C_{24} + 26 \cdot C_{26}) / (C_{22} + C_{24} + C_{26}). \quad (1)$$

5 At GN, by contrast, ACL increases from leaf litter to soil from 23.1 to 23.9, suggesting a relative  
6 decrease of the mid-chain compounds.

7 The *n*-alcohols show pronounced bimodal distributions of mid- and long-chain compounds in  
8 leaf litter and topsoils. In GN leaf litter C<sub>20</sub> and C<sub>28</sub> *n*-alcohols (OH) dominate, while the GN  
9 topsoil peaks at C<sub>22</sub> and C<sub>28</sub> OH. In leaf litter at TP and SN C<sub>24</sub> OH and C<sub>30</sub> OH are the dominant  
10 mid- and long-chain modes, respectively. The bimodal character in both litter and topsoils  
11 results from the *n*-alcohols deriving from two major sources: (a) cuticular wax esters, with chain  
12 lengths of C<sub>26</sub> and C<sub>28</sub> or, in some cases, C<sub>30</sub> and C<sub>32</sub> (e.g., Samuels et al., 2008 and references  
13 therein) and (b) suberin, with C<sub>22</sub> and C<sub>24</sub> OHs being the main monomers (Molina et al., 2006).  
14 The source of C<sub>20</sub> OH in high-altitude leaf litter remains uncertain. Note that, although suberin  
15 is a major bio-polyester in root material, it is not exclusively present in roots but also forms part  
16 of other tissue types and is therefore present in leaf litter. In contrast to leaf litter and soils, the  
17 *n*-alcohols distribution in the grasses is unimodal and dominated by C<sub>26</sub> OH, accounting for 85  
18 % of the *n*-alcohols.

19 Comparison of the relative abundances of individual FAs and OHs in the two samples from site  
20 TP provides clues as towards the effect of white rot on chain-length distribution (Fig. 4b, c).  
21 Fungal biomass contribution and breakdown of plant material appears to increase the proportion  
22 of short-chain C<sub>16</sub> and C<sub>18</sub> FA and of C<sub>30</sub> FA (Fig. 4b) as well as the proportions of C<sub>30</sub> and C<sub>32</sub>  
23 OH, relative to mid- and short-chain OHs (Fig. 4c).

24 Chain lengths of  $\omega$ -hydroxy acids in all leaf litter samples range from 16 to 22 but are dominated  
25 by short-chain C<sub>16</sub>  $\omega$ -OH-FA (Fig. 3). This distribution is typical for cutin monomers (Matzke  
26 and Riederer, 1991), the lipid polymer being part of the protective hydrophobic layer of plant  
27 cell walls (Pollard et al., 2008). By contrast, the dominance of C<sub>24</sub> and C<sub>22</sub>  $\omega$ -OH-FAs in the  
28 grasses point towards suberin incorporated in bundle sheaths as their main source (Mertz and  
29 Brutnell, 2014). In the topsoil samples,  $\omega$ -OH-FAs range from C<sub>16</sub> to C<sub>26</sub>, the dominant  
30 compounds being C<sub>22</sub>  $\omega$ -OH-FA and C<sub>24</sub>  $\omega$ -OH-FA, which together account for ~ 50 % of the  
31 total  $\omega$ -OH-FA in the TP and SN soils and 65 % of the total  $\omega$ -OH-FA in the GN topsoil, where  
32 the proportion of C<sub>24</sub>  $\omega$ -OH-FA is higher. C<sub>22</sub>  $\omega$ -OH-FA and C<sub>24</sub>  $\omega$ -OH-FA typically are the

1 main  $\omega$ -OH-FAs in suberin from *Quercus robur* L. and *Fagus sylvatica* L. (Matzke and  
2 Riederer, 1991) or *Quercus suber* (Graça and Santos, 2007) and a similar  $\omega$ -OH-FA distribution  
3 has been reported for an oak forest soil by Nierop et al. (2005).  $\alpha$ -Hydroxy acids range from  
4 C<sub>22</sub> to C<sub>24</sub> in GN litter and C<sub>15</sub> to C<sub>26</sub> in TP and SN litters, with C<sub>24</sub>  $\alpha$ -OH-FA being dominant,  
5 followed by C<sub>22</sub>  $\alpha$ -OH-FA; there are also high abundances of odd-numbered  $\alpha$ -OH-FAs, C<sub>23</sub>  
6 and C<sub>25</sub> in particular. The distribution of  $\alpha$ -OH-FAs is similar in the underlying soils.

7 *n*-Alkanes in the leaf litter samples (sites TP, SN, GN) range from *n*-C<sub>23</sub> to *n*-C<sub>31</sub> and reveal a  
8 clear compositional difference between high-altitude beech-dominated and low-lying oak-  
9 dominated leaf litters (Fig. 3): the main *n*-alkane at SN and TP is *n*-C<sub>29</sub> while litter at GN almost  
10 exclusively contains *n*-C<sub>27</sub>, accounting for 90 % of the total *n*-alkanes. Thus, the average chain  
11 length (*n*-C<sub>27</sub> to *n*-C<sub>31</sub>; ACL<sub>27-31</sub>) is 28.9 at TP and SN and 27.1 for the GN site. In the grasses,  
12 the *n*-alkanes range from *n*-C<sub>23</sub> to *n*-C<sub>33</sub>. The distribution is dominated the C<sub>31</sub> *n*-alkane, which  
13 accounts for 75 % of the total *n*-alkanes, while *n*-C<sub>29</sub> accounts for 15 %, giving an ACL<sub>27-33</sub>  
14 value of 30.8. In the soils, *n*-alkane chain lengths also range from C<sub>23</sub> to C<sub>33</sub>. The dominant *n*-  
15 alkane in all low-altitude topsoil samples is *n*-C<sub>29</sub>. Compared to the leaf litter samples, the ratio  
16 of *n*-C<sub>29</sub> relative to *n*-C<sub>31</sub> ( $C_{29}/C_{31} = n\text{-}C_{29}/(n\text{-}C_{29} + n\text{-}C_{31})$ ) decreases from 0.78 in leaf litter to  
17 0.63 in the corresponding topsoils at SN, TP. In the sample affected by white rot (TP-F) this  
18 ratio is even lower (0.56). In GN leaf litter *n*-C<sub>31</sub> was absent. Notably, *n*-alkanes in the  
19 underlying topsoil at site GN show a bimodal distribution, with *n*-C<sub>31</sub> and *n*-C<sub>27</sub> being the main  
20 *n*-alkanes and the C<sub>29</sub>/C<sub>31</sub> ratio being 0.36. Since grass is currently neither present at the  
21 sampling site nor a visible part of the leaf litter this suggests that it has contributed to the soil  
22 lipids in the past, i.e. before the establishment of the present mature beech canopy.

23 The  $\alpha,\omega$ -dicarboxylic acids ( $\alpha,\omega$ -DiFAs) in the topsoils reveal chain-length distributions that  
24 are similar to those of the  $\omega$ -hydroxy acids, i.e. bimodal, with peaks at C<sub>16</sub> and C<sub>22</sub>. Notably,  
25 the increased proportions of C<sub>24</sub> relative to C<sub>22</sub>  $\omega$ -OH-FA that is observed in the high-altitude  
26 soil when compared to the low-lying soils is reflected in the DiFA chain-length distribution  
27 confirming that both  $\omega$ -OH-FAs and  $\alpha,\omega$ -DiFAs most likely derive from suberin. The presence  
28 of  $\alpha,\omega$ -DiFAs in roots but not in leaves and stems of land plants, led Mendez-Millan et al.  
29 (2011) to conclude that  $\alpha,\omega$ -DiFAs represent subterranean biomass.

### 30 **3.2.2 Subsoil (Terra Rossa)**

31 As for TOC, the subsoil samples, DG-SS and DG-SS 2, contain the lowest amounts of lipids:  
32 1.3  $\mu\text{g g}^{-1}$  and 2.3  $\mu\text{g g}^{-1}$  dry weight, respectively, which is an order of magnitude lower than

1 the lipid content of the topsoils. Notably, there is a significant difference in lipid composition  
2 between the sample collected from the exposed surface of the soil profile (DG-SS) and the  
3 sample collected from slightly greater depth, for simplicity distinguished as ‘weathered’ and  
4 ‘un-weathered’ subsoil samples. The lipid composition of the un-weathered sample closely  
5 resembles those of the low-altitude topsoils, but appears to contain significantly lower amounts  
6 of sterols (3 %<sub>lipids</sub> vs. 11 %<sub>lipids</sub> in the topsoils). The proportion of  $\omega$ -OH-FAs (2 %<sub>lipids</sub>) is half  
7 compared to the topsoils while  $\alpha,\omega$ -DiFAs were not detected, which is consistent with the low  
8 visual abundance of root material. The amount of *n*-alcohols, on the other hand, is slightly  
9 enhanced, with 20 %<sub>lipids</sub> compared to 15 %<sub>lipids</sub> in the low-altitude topsoils. While these  
10 differences set the un-weathered subsoil sample slightly apart from the topsoils (also see Fig.  
11 5), some of the variation may arise from the different extraction method applied (microwave),  
12 the different analytical equipment (see methods) and, the semi-quantitative data determined for  
13 this sample.

14 The lipid composition of the weathered sample contrasts with the un-weathered subsoil sample  
15 and the topsoils. Almost half of the lipids (48 %) are *n*-alcohols, followed by unsaturated FAs  
16 (20 %<sub>lipids</sub>), saturated FAs (15 %<sub>lipids</sub>) and steroids (11 %), with  $\beta$ -sitosterol being the dominant  
17 sterol (9 %<sub>lipids</sub>). Minor components include *n*-alkanes (3.5 %<sub>lipids</sub>), OH-FAs (1.4 %<sub>lipids</sub>) and  
18 branched FAs (0.6 %<sub>lipids</sub>). As in the un-weathered subsoil sample,  $\alpha,\omega$ -DiFAs were absent.  
19 Notably, almost all compounds with carboxylic groups (i.e. fatty acids) appear depleted relative  
20 to *n*-alcohols, *n*-alkanes and sterols when compared to the un-weathered subsoil sample and the  
21 topsoils (Fig. 3), with the exception of PUFAs. Short-chain FAs and MUFAs appear less  
22 severely depleted than mid-chain and long-chain saturated FAs. The fact that the amount of  
23 unsaturated FAs in the weathered subsoil sample is higher than the amount of saturated FAs  
24 suggests that a large proportion of the unsaturated FAs in this sample is produced *in situ*, with  
25 the dominant *n*-C<sub>18:1</sub>(*cis*-9) MUFA (45 % of unsaturated FA) most likely deriving from micro-  
26 organisms.

27 The dominant *n*-alcohol in the un-weathered Terra Rossa sample is C<sub>24</sub> OH. In the  
28 weathered/degraded Terra Rossa sample, the *n*-alcohols show a bimodal chain-length  
29 distribution peaking at 28 and 24, with C<sub>28</sub> OH accounting for 25 % and C<sub>24</sub> OH for 19 % of all  
30 *n*-alcohols. The saturated *n*-alkanoic acids are dominated by short-chain (C<sub>16</sub>, C<sub>18</sub>) and mid-  
31 chain (C<sub>24</sub>, C<sub>22</sub>) FAs, with C<sub>16</sub> FA in the degraded Terra Rossa sample showing the highest  
32 relative amounts compared to the longer chain *n*-alkanoic acids in all other soil samples (36  
33 %<sub>FA</sub> vs. 20 %<sub>FA</sub>). As for the unsaturated compounds, this may reflect *in-situ* contributions from

1 micro-organisms. The chain-length distributions of  $\omega$ -OH-FAs and *n*-alkanes in both Terra  
2 Rossa samples appear very similar. As in the topsoils, the dominant  $\omega$ -OH-FA is the C<sub>22</sub>  
3 compound, suggesting suberin as their main source. The only  $\alpha$ -OH-FA is the C<sub>24</sub> homologue.  
4 Chain lengths of the *n*-alkanes range from 23 to 33, with *n*-C<sub>31</sub> and *n*-C<sub>29</sub> being dominant  
5 compounds and being present in almost equal amounts (C<sub>29</sub>/C<sub>31</sub> = 0.49) accounting for 68 % of  
6 the total *n*-alkanes. This suggests higher *n*-alkane input from grasses (*n*-C<sub>31</sub>) compared to the  
7 low-altitude topsoils under oak-dominated vegetation (*n*-C<sub>29</sub>).

8 The differences between the un-weathered and the weathered/degraded Terra Rossa sample  
9 may be indicative of the compositional changes soil lipids may undergo when the soil dries out  
10 frequently and becomes aerated. *n*-Alcohols appear selectively preserved relative to *n*-alkanoic  
11 acids to an even greater extent than during the degradation process in the transition from plant  
12 litter to soil organic matter. As leaf litter and weathered Terra Rossa have highest and lowest  
13 concentrations of total organic carbon and total lipids, these may represent the end-members of  
14 continuous plant lipid transformation determined for this initial survey of lipid biomarker  
15 sources in the SE Ohrid Basin. However, the projection towards the weathered/degraded stage  
16 is solely based on one pair of samples and potential controls such as soil moisture, oxygen  
17 availability, pH or temperature are unknown.

### 18 **3.2.3 Macrophytes**


19 Submerged macrophytes contain between 0.2 and 1.1 mg g<sup>-1</sup> dry wt. of lipids, which is in the  
20 same range as the leaf litter. Highest concentrations are found in *Cladophora* spp. (1.07 ± 0.33  
21 mg g<sup>-1</sup>). *Potamogeton* spp. and *Characeae* spp. contain lower amounts (0.22 ± 0.13 mg g<sup>-1</sup> and  
22 0.53 ± 0.34 mg g<sup>-1</sup>, respectively), which is likely due to a higher proportion of supportive tissue  
23 in the latter and, in case of *Characeae* spp., carbonate incrustations. Sub-aquatic and sub-aerial  
24 parts of *Phragmites* spp. were analysed separately; lipid concentrations in the root and stem  
25 (sub-aquatic) are in a similar range to the submerged macrophytes (0.44 and 0.34 mg g<sup>-1</sup>,  
26 respectively). The highest lipid concentrations were in the (sub-aerial) *Phragmites* spp. leaves  
27 (1.7 ± 0.3 mg g<sup>-1</sup>).

28 The macrophyte lipids are dominated by unsaturated and saturated fatty acids that, together,  
29 account from 64 %<sub>lipids</sub> (*Potamogeton*) to 95 %<sub>lipids</sub> (*Cladophora*). Unsaturated FAs dominate,  
30 with slightly higher proportions in *Phragmites* sp. leaves (59 %<sub>lipids</sub>) and *Characeae* spp. (57  
31 %<sub>lipids</sub>) and lower proportions in *Potamogeton* spp. (41 %<sub>lipids</sub>) and the *Phragmites* sp. roots (48  
32 %<sub>lipids</sub>). The C<sub>18:2</sub> PUFA is the dominant unsaturated FA in the *Phragmites* sp. stem (21 %<sub>lipids</sub>)



1 and roots (27 %<sub>lipids</sub>), while in the leaves it is the C<sub>18:3</sub> PUFA (48 %<sub>lipids</sub>). The latter also  
2 dominates in *Potamogeton* sp. (23 %<sub>lipids</sub>). C<sub>16:1</sub> (*cis*-9) MUFA is most abundant in *Cladophora*  
3 sp. (29 %<sub>lipids</sub>), which also contains the highest amounts of C<sub>18:1</sub> (*cis*-9) MUFA (8 %<sub>lipids</sub>). C<sub>16:1</sub>  
4 (*cis*-9) MUFA was also present in traces in *Phragmites* stems (13 %<sub>lipids</sub>) but absent in roots  
5 and leaves.

6 Saturated *n*-alkanoic acids have highest relative abundance in *Cladophora* spp. (45 %<sub>lipids</sub>) and  
7 lowest concentrations in *Phragmites* sp. leaves (15 %<sub>lipids</sub>). They are dominated by C<sub>16</sub> FA,  
8 which accounts for about three quarters of all saturated FA, with lower values only in the  
9 *Phragmites* stem and root (67 and 55 % of saturated FA, respectively), where there are slightly  
10 elevated amounts of C<sub>20</sub> FA and C<sub>24</sub> FA. The remaining compounds are mainly sterols,  
11 accounting for 3.6 - 12 % of the total lipids and with highest amounts in *Potamogeton* spp. (33  
12 %<sub>lipids</sub>) and elevated proportions in the *Phragmites* sp. root and stem (12 and 11 %<sub>lipids</sub>,  
13 respectively). The main sterol present in all samples is  $\beta$ -sitosterol followed by stigmasterol  
14 (absent in *Cladophora* sp.). Small amounts of cholesterol (0.1 – 2.3 %<sub>lipids</sub>) were detected in all  
15 samples except for the *Phragmites* sp. stem.

16 *n*-Alcohol concentrations are generally very low (< 1.5 %<sub>lipids</sub>), with the exception of the  
17 *Phragmites* leaves where long-chain C<sub>28</sub> OH and C<sub>30</sub> OH account for 19 % of the total lipids  
18 and likely to derive from cuticular waxes [Samuels et al., 2008]. In most samples, hydroxy acids   
19 are either absent (*Characeae* spp.) or detected in small amounts of less than 0.4 %<sub>lipids</sub>  
20 (*Cladophora* spp., *Potamogeton* spp., *Phragmites* spp. leaves). Higher amounts were detected  
21 in the *Phragmites* stem and root: 1.4 and 3.5 %<sub>lipids</sub>, respectively. Thus, *Phragmites* spp. roots,  
22 in particular, are a potential macrophytic source for sedimentary  $\alpha$ - and  $\omega$ -OH-FA. As in leaf  
23 litter, the amount of  $\alpha$ -OH-FA is higher than the amount of  $\omega$ -OH-FA in all samples where  
24 OH-FAs are present. The  $\alpha$ -OH-FA chain-length distribution is bimodal in all *Phragmites* spp.  
25 tissues, with  $\alpha$ -C<sub>24</sub> OH-FA dominant followed by the  $\alpha$ -C<sub>20</sub> OH-FA. In *Potamogeton* spp. and  
26 *Cladophora* spp.  $\alpha$ -C<sub>24</sub> OH-FA dominates, followed by  $\alpha$ -C<sub>22</sub> OH-FA, and only *Potamogeton*  
27 spp. contained a small amount of  $\alpha$ -C<sub>16</sub> OH-FA. The main  $\omega$ -OH-FA is the  $\omega$ -C<sub>24</sub> OH-FA  
28 compound, which is present in *Potamogeton* spp. (traces) and the *Phragmites* spp. stem and  
29 root samples (0.2 and 0.9 %<sub>lipids</sub>, respectively). The latter also contain  $\omega$ -C<sub>22</sub> OH-FA,  $\omega$ -C<sub>26</sub>  
30 OH-FA and  $\omega$ -C<sub>20</sub> OH-FA. Although  $\omega$ -C<sub>16</sub> OH-FA was not detected, this distribution suggests  
31 that the  $\omega$ -OH-FA in *Phragmites* spp. also derive from suberin, which is a known biopolymer  
32 in *Phragmites australis* (Soukup et al., 2007).

33 *n*-Alkanes were either absent (*Characeae*, *Phragmites* root and stem) or found in very low

1 concentrations, e.g., 0.25 %<sub>lipids</sub> in *Phragmites* sp. leaves (dominated by *n*-C<sub>29</sub>, ACL<sub>23-31</sub> = 28.3)  
2 and 1 %<sub>lipids</sub> in *Potamogeton* spp. (dominated by *n*-C<sub>25</sub>, ACL<sub>23-31</sub> = 25.6). The differences in  
3 chain-length distributions between *Potamogeton* spp. and the leaves of *Phragmites* sp. are  
4 consistent with observations made by Ficken et al. (2000) on the *n*-alkane composition of  
5 macrophytes from lakes on Mt. Kenya. There, *n*-alkanes produced by submerged macrophytes  
6 were dominated by mid-chain compounds (C<sub>23</sub>, C<sub>25</sub>), whereas *n*-alkanes produced by emergent  
7 macrophytes were dominated by long-chain compounds (> C<sub>29</sub>) as in land plants. Ficken et al.  
8 (2000) suggested a proxy (eq. 2) to determine varying contributions from submerged and  
9 emergent macrophytes defined as:

$$10 \quad P_{\text{aq}} = (n\text{-C}_{23} + n\text{-C}_{25}) / (n\text{-C}_{23} + n\text{-C}_{25} + n\text{-C}_{29} + n\text{-C}_{31}), \quad 2)$$

11 with values between 0.1 and 0.4 suggested to correspond to emergent macrophytes and values  
12 between 0.4 and 1 to submerged macrophytes. Accordingly, the  $P_{\text{aq}}$  value of 0.78 for  
13 *Potamogeton perfoliatus* in Lake Ohrid matches the values reported by Ficken et al. (2000) for  
14 *Potamogeton thumbergii* (0.73 and 0.92) while the value for *Phragmites* leaves (0.24) does fall  
15 in the suggested range for emergent macrophytes.

### 16 **3.2.4 Lake water particulate organic matter (POM)**

17 The total lipid composition of the lake particulate organic matter (POM) was not determined,  
18 as extracts had previously been separated into non-polar and polar fractions. Here we report the  
19 results from analyses of the polar components that include fatty acids, alcohols and steroids of  
20 the water filtrates taken at Co1202 and the DEEP site. At both sites, these were dominated by  
21 short-chain FAs (C<sub>16</sub> FA and C<sub>18</sub> FA), accounting for 26 % of polar lipids (%<sub>polar lipids</sub>), C<sub>18</sub>  
22 MUFAs (21 %<sub>polar lipids</sub>) and some sterols (20 %<sub>polar lipids</sub>), mainly cholesterol (8 %<sub>polar lipids</sub>),  
23 sitosterol (6 %<sub>polar lipids</sub>) and stigmasterol (5 %<sub>polar lipids</sub>). Minor polar compounds were C<sub>18:2</sub>  
24 PUFA (7 %<sub>polar lipids</sub>), *n*-alcohols (4 %<sub>polar lipids</sub>), mid- and long-chain *n*-FAs (3 %<sub>polar lipids</sub>),  
25 branched FAs (2 %<sub>polar lipids</sub>) and OH-FAs (1.3 %<sub>polar lipids</sub>). The proportion of C<sub>18:2</sub> PUFA  
26 increased with water depth at site Co1202, from 4 to 12 %<sub>polar lipids</sub> suggesting that most recently  
27 produced labile OM had sunk to depth at the time of sampling. Long-chain *n*-alkanoic acids  
28 and *n*-alcohols were detected in trace amounts, only. Notably, the *n*-alcohol distribution in the  
29 water filtrates suggests a bacterial source (Fig. 3b). Yang et al. (2014) observed a very similar  
30 *n*-alcohol distribution in *Erythrobacter* sp. isolated from the South China Sea. Albaigés et al.  
31 (1984) noted that C<sub>18</sub> OH and C<sub>22</sub> OH dominated the free and bound *n*-alcohol fraction in  
32 lacustrine sediments from Spain and assigned them to bacterial inputs.

### 1 3.2.5 Sediments

2 Total lipid contents of sediments from core Lz1120 range from 7.6 to 79  $\mu\text{g g}^{-1}$ , with an average  
3 of 32  $\mu\text{g g}^{-1}$ . Notably, the amounts of lipids normalized to TOC correlate closely to the lipid  
4 concentrations of the dry samples ( $R^2 = 0.92$ ), suggesting that, for example, a drop in TOC  
5 corresponds to a drop in the lipid concentration of the OM supplied.

6 With regard to the major lipid compound classes, there are clear compositional differences  
7 between the samples with low and high carbonate contents, i.e. between the samples dated to  
8 8.29, 8.17 and 8.11 ka, representing cool, dry episodes associated with the 8.2 ka event, and  
9 those from the rest of the investigated time slice. In particular, the proportion of *n*-alkanoic  
10 acids is significantly lower in the samples from 8.17 and 8.11 ka compared to the high-  
11 carbonate samples (16 %<sub>lipids</sub> vs. 40 %<sub>lipids</sub>, on average), while the proportions of sterols and *n*-  
12 alcohols are higher (24 %<sub>lipids</sub> vs. 15 %<sub>lipids</sub> and 37 %<sub>lipids</sub> vs. 21 %<sub>lipids</sub>, respectively). In the  
13 sample from 8.29 ka, both *n*-alkanoic acids (27 %<sub>lipids</sub>) and *n*-alcohols (18 %<sub>lipids</sub>) are present in  
14 lower amounts but similar proportion relative to each other as in the high-carbonate samples  
15 (see ‘Supplementary Data’).

16 The concentrations of OH-FAs range from 1 to 6.7 %<sub>lipids</sub>, with averages of 3.3 and 4.6 %<sub>lipids</sub>  
17 for low- and high-carbonate samples, respectively. These values are similar to those of the  
18 topsoil (average: 5.7 %<sub>lipids</sub>) and leaf litter samples (average: 2.5 %<sub>lipids</sub>), but significantly higher  
19 than in the macrophytes, with the exception of *Phragmites* stems (1.4 %<sub>lipids</sub>) and roots (3.5  
20 %<sub>lipids</sub>). As in the soils, but unlike the leaf litters and macrophytes, the majority of the OH-FA  
21 in the sediments are  $\omega$ -OH-FA, except at 8.17 ka where  $\alpha$ -OH-FAs dominate (2.1 vs. 1.1  
22 %<sub>lipids</sub>). Branched FAs account for 0.9 %<sub>lipids</sub> on average in the high-carbonate samples. Of the  
23 low-carbonate samples, the sample at 8.11 ka shows an exceptionally high value of 2.5 %<sub>lipids</sub>  
24 while the sample at 8.29 ka contains the lowest amount of branched FAs of all sediment samples  
25 (0.2 %<sub>lipids</sub>). *n*-Alkane concentrations are more than twice as high in the three low-carbonate  
26 samples than in the high-carbonate samples (3.3 %<sub>lipids</sub> vs. 1.5 %<sub>lipids</sub>).

27 In contrast to macrophytes, leaf litter and soils, the early Holocene sediment samples contain  
28 almost no unsaturated fatty acids. The only detectable MUFA is C<sub>18:1</sub>(*cis*-9) accounting for  
29 about 0.1 to 0.5 %<sub>lipids</sub>, if present. PUFAs are not preserved in the sediments. On the other hand,  
30 the C<sub>21</sub> - C<sub>33</sub> methyl ketones were identified only in the sediments (0.1 to 1.1 %<sub>lipids</sub>; with the  
31 exception of C<sub>27</sub> in SN leaf litter).

32 Other compounds account for 17 % of the total lipids in both high- and low-carbonate samples.

1 While most of these compounds are minor components ( $<2\%$  lipids), long-chain diols, lanosterol  
2 and  $17\beta(H),21\beta(H)$ -bis-homohopanoic acid, on average, account for 3, 4 and 8 %lipids,  
3 respectively (Table 2). Like many of the minor compounds they were absent in the terrestrial  
4 samples and imply production in the water column or sediment. Further details and references  
5 as towards potential sources are provided Supplement 2. The distributions of *n*-alkanoic acids  
6 reveal significant differences in chain length between samples with high and low carbonate  
7 contents. The input of long-chain *n*-alkanoic acids from terrestrial plant waxes appears strongly  
8 reduced relative to short-chain  $C_{16}$ -FA and mid-chain  $C_{22}$ - and  $C_{24}$ -FAs during periods of  
9 reduced or diluted carbonate sedimentation. By contrast, the chain-length distribution of *n*-  
10 alcohols shows rather subtle differences that, in fact, result from the depletion of long-chain *n*-  
11 alcohols in one sample, only (8.29 ka, Fig. 5). *n*-Alkanes show a unimodal distribution peaking  
12 at *n*- $C_{29}$  and do not show a significant difference between high- and low-carbonate samples. *n*-  
13  $C_{31}$  is slightly more abundant relative to *n*- $C_{29}$ . The ratio of these two compounds ( $C_{29}/C_{31}$ )  
14 changes marginally, with values of 0.53 and 0.56 for low- and high-carbonate samples,  
15 respectively. These values match the value of the soil sample influenced by white rot (0.56) but  
16 would also result from a hypothetical 3:4 mix of low-altitude and high-altitude woodland  
17 topsoils, for example. The methyl ketone distribution is unimodal peaking at  $C_{27}$ . Methyl  
18 ketones appear to correlate with *n*-alkanes with regard to concentrations ( $R^2 = 0.73$ ) and chain-  
19 length distribution ( $R^2 = 0.94$ ). Microbial oxidation of *n*-alkanes has widely been suggested to  
20 explain the presence of methyl ketones in soils and sediments (e.g., van Bergen et al., 1998;  
21 Cranwell et al., 1987; Albaigés et al., 1984) and their increase in relative concentration with  
22 sediment depth (Cranwell et al., 1987). We did not find methyl ketones to be a lipid fraction in  
23 the soil samples and so we assume methyl ketones to be *in situ* products of microbial *n*-alkane  
24 breakdown in the lake sediments.

25

#### 26 **4 Implications for proxy records**

27 The principle behind paleoenvironmental reconstructions using organic-geochemical proxies is  
28 that OM fluxes from aquatic and terrestrial sources within the catchment of a given sedimentary  
29 archive change in response to changes in regional or global climate. The main purpose of this  
30 study is to characterize the organic geochemical composition of potential sources of OM buried  
31 in sediments of Lake Ohrid in order to improve the interpretation of organic geochemical proxy  
32 data in reconstructions of hydrology-controlled environmental change in the Ohrid Basin. Our  
33 initial data set provides significant insight towards an improved interpretation of changes in

1 elemental (TOC/TN) and biomarker composition.

## 2 **4.1 Assessing aquatic and terrestrial OM supply**

### 3 **4.1.1 Elemental composition of aquatic and terrestrial OM**

4 TOC/TN ratios of bulk sediment are often used for assessment of aquatic and terrestrial  
5 contributions, with two values frequently applied in simple 2-endmember models: the Redfield  
6 Ratio of 6.6 for the aquatic/algal end member and the value of 20 for the terrestrial end-member  
7 (see, e.g., Meyers, 1994). As such, this approach appears of limited value in case of the Lz1120  
8 sediments since there is a range of terrestrial OM of different levels of degradation present in  
9 the Ohrid Basin, reaching from plant litter to degraded (or “weathered”) soil. This degradation  
10 gradient coincides with a substantial range of TOC/TN signatures, while plant matter/litter and  
11 soils are mobilized from a great variety of topographical settings (mountain slopes to flood  
12 plains) at different rates under specific circumstances. Furthermore, macrophytes have  
13 intermediate TOC/TN ratios of  $15 \pm 2.4$  and produce a significant amount of biomass in the  
14 littoral, intercepting nutrient supply from terrestrial run-off and directly tapping into the  
15 sedimentary nutrient pool. For example, *Potamogeton perfoliatus* L. produces a biomass of  
16  $2,747 \times 10^6 \text{ g y}^{-1}$  (Talevska, 2006), while the stock of charophytes in Lake Ohrid is estimated  
17 at  $10,500 \times 10^6 \text{ g}$  (Trajanovska et al., 2012). Lake Ohrid is oligotrophic and the biomass from  
18 the epilimnion may well be the smallest source of sedimentary OM. A maximum phytoplankton  
19 biomass of  $131 \text{ mg m}^{-3}$  in spring 2001 (Petrova et al., 2008) leads us to estimate a peak  
20 phytoplankton biomass in the upper 50 m of the water column of  $2,300 \times 10^6 \text{ g}$  for the spring  
21 season (calculated for lake surface area of  $358 \text{ km}^2$ ), which is comparable to the annual  
22 production of *Potamogeton perfoliatus* L. and a quarter of the standing stock of charophytes.  
23 This OM nevertheless represents high-value food for the lake’s heterotrophic consumers and is  
24 likely largely remineralised in the water column.

25 Straightforward interpretation of the Lz1120 TOC/TN record based on the conventional two  
26 end members therefore is not possible, but the variability of the TOC/TN record does provide  
27 valuable information on changes in OM sources in the given paleoenvironmental context.  
28 Before 8.3 ka, the estimated TOC/N<sub>org</sub> ratio is 13.6 (see section 3.1.3). This is higher than the  
29 value of Terra Rossa ( $11.6 \pm 0.8$ ), within the range of macrophytes ( $15 \pm 2.4$ ), and may result  
30 from any mix of these with leaf litter, topsoils and a small amount of algal material. Still, the  
31 majority of the nitrogen appears to be organic and to vary with organic carbon supply. After  
32 8.3 ka, by contrast, TOC/TN ratios are very low, with a value of 7 at 8.05 ka close to the

1 Redfield ratio, suggesting that the majority of the sedimentary OM is of algal origin. This,  
2 however, is not supported by the lipid biomarkers, which are dominated by terrestrially-derived  
3 compounds throughout this period. An alternative explanation for the very low TOC/TN ratios  
4 in the Lz1120 sediments after 8.3 ka other than algal matter would be elevated amounts of  
5 inorganic nitrogen ( $N_{in}$ ), for which there is some evidence (see also section 3.1.3). Within the  
6 terrestrial biome, the highest proportions of  $N_{in}$  are likely to be found in the clay fraction of  
7 mineral soils. This is due to the fact that ammonium ( $NH_4^+$ ), in particular, can firmly bind to  
8 clay minerals and strongly influence the soil TN pool (e.g., Elmacı et al., 2002; Kothawala and  
9 Moore, 2009; Nieder et al., 2011). The low TOC/TN value for Terra Rossa is likely due to  
10 enhanced  $NH_4^+$  levels. Soils developing from former lake sediment during lake level low stands  
11 are also likely to be charged with  $NH_4^+$ , in particular. It has frequently been reported that the  
12 fine fraction of mineral soils contains substantially higher amounts of ammonium than the  
13 coarser soil fractions (see Nieder et al., 2011 and references therein). Size fractionation during  
14 transport of eroded mineral soil may therefore result in the deposition of clay-rich material with  
15 TOC/TN ratios that are lower than those determined for the bulk soils *in situ*. Hence, the low  
16 TOC/TN ratios in Lz1120 sediments after 8.3 ka probably result from enhanced supply of clay-  
17 bound ammonium following mineral soil erosion rather than algal OM.

#### 18 **4.1.2 Aquatic and terrestrial biomarkers**

19 Nearly all of the biomarkers preserved in the sediments can be ascribed to terrestrial OM, or  
20 have a non-specific source. The exception is dinosterol (from dinoflagellates), but this is a very  
21 minor component of the total lipid extracts (see Table 2, Supplement 2) and we consider it not  
22 to be a robust quantitative proxy for lake-derived OM.

23 The amount of lipids relative to the total OM of individual sources is likely to vary with  
24 environmental conditions. Thus, without knowledge on how representative individual  
25 biomarkers are for the size of the OM pool they derive from under specific hydrological  
26 conditions, assessments of relative contributions remain speculative unless they are backed up  
27 by other proxy data (e.g., elemental data, petrology, bulk isotopes).

#### 28 **4.2 Identifying the changing composition of terrestrial OM using lipid** 29 **biomarkers**

30 As evident from the pie diagrams in Figure 3, topsoils contain significantly higher amounts of  
31 *n*-alcohols (OHs) relative to *n*-alkanoic acids (FAs) than leaf litter. The weathered/degraded  
32 subsoil sample contains even higher proportions of OHs, accounting for almost half of the total

1 lipids, and very low amounts of saturated FAs. Thus, the ratio of sedimentary *n*-alkanoic acids  
2 over *n*-alcohols (FA/OH) is indicative of shifts in the relative amounts of moderately to strongly  
3 degraded OM from the major terrestrial sources, with higher ratios indicating higher  
4 proportions of plant litter-type OM relative to soil-type OM and lower ratios indicating  
5 enhanced contributions of stronger degraded OM from young topsoils and/or degraded subsoils.  
6 Since a significant amount of short-chain FAs, *n*-C<sub>16</sub> FA in particular, may also derive from  
7 aquatic sources we consider the ratio of long-chain FA over long-chain OH of certain terrestrial  
8 origin (terr FA/OH) to be a more reliable measure. Notably, both ratios, FA/OH and terr  
9 FA/OH, correlate well in the sediment samples ( $R^2 = 0.99$  for all samples,  $R^2 = 0.95$  for high-  
10 carbonate samples) suggesting that aquatic sourced short-chain FAs are of minor importance.

11 The chain-length distributions in Figure 3 also reveal another important difference between leaf  
12 litter and soils. In the near-shore oak-dominated setting, the proportion of mid-chain FAs, C<sub>22</sub>  
13 and C<sub>24</sub> FA in particular, is higher relative to long-chain FAs ( $\geq$  C<sub>26</sub>) in the topsoil and in Terra  
14 Rossa than in the corresponding leaf litter. The ratio of sedimentary mid-chain C<sub>22</sub> and C<sub>24</sub> FAs  
15 over long-chain C<sub>26</sub>, C<sub>28</sub> and C<sub>30</sub> FAs should therefore be sensitive to soil OM supply from the  
16 near-shore setting. Similar increases in the proportion of mid-chain FAs in soils relative to litter  
17 have also been observed in topsoils of a high-altitude beech forest in Central France (Marseille  
18 et al., 1999) and of an evergreen oak forest in Central Spain (Almendros et al., 1996). In a forest  
19 topsoil from the surroundings of Lake Suwa in Japan (Matsuda and Koyama, 1977), which  
20 represents a setting very similar to the Ohrid Basin (mid-latitude, high-altitude intra-  
21 mountainous lake basin with moist forest biome), FA distributions in the topsoils are very  
22 similar to that at site SN ( $R^2 = 0.90$ ), providing evidence that, generally, similar natural  
23 processes operate under similar environmental conditions. The chain-length distributions of the  
24 *n*-alcohols also reveal differences between the terrestrial sources (Fig. 3), which are most  
25 pronounced between high-altitude and low-altitude sources when expressed, for example, as  
26 the average chain length (ACL) of C<sub>24</sub> to C<sub>28</sub> OHs (ACL<sub>24-28</sub>). The amounts of *n*-alcohols are  
27 significantly higher in soils than in leaf litter. Changes in ACL<sub>24-28</sub> in the sediments therefore  
28 potentially indicate variable contributions of soil OM from high-altitude beech-dominated or  
29 low-altitude oak-dominated habitats. *n*-Alkane distributions, finally, allow for a clear  
30 distinction between C<sub>27</sub>-dominated and C<sub>29</sub>-dominated leaf litter from the high and low-altitude  
31 forests, respectively. In the soils, the input of C<sub>31</sub> *n*-alkane significantly increases (Fig. 3) and  
32 either reflects over-proportional incorporation of lipids from the grassy undergrowth or from  
33 an earlier, more open type of vegetation with higher proportions of grass. In the sediments, the  
34 amounts of *n*-C<sub>27</sub> alkane are always significantly lower than the *n*-C<sub>29</sub> alkane, suggesting that

1 contribution from the high-altitude habitat is minor. In fact, the *n*-alkane distribution of a  
2 hypothetical 1:1 mix of *n*-alkanes from low-altitude topsoil and weathered Terra Rossa  
3 correlates very well with the average sedimentary *n*-alkane distribution ( $R^2 = 0.95$ ). Similarly,  
4 the high-proportion of the *n*-C<sub>31</sub> alkane indicates that direct inputs of leaf litter from beech or  
5 oak are minor relative to soil OM. Two observations suggest that the popular application of  
6 specific *n*-alkane ratios (*n*-C<sub>27</sub> or *n*-C<sub>29</sub> relative to *n*-C<sub>31</sub>) as proxies for the development of the  
7 vegetation (mainly for trees *vs.* grass/shrubs; e.g., Schwark et al., 2002, Zhang et al., 2006;  
8 Leider et al., 2013) is compromised in the Ohrid Basin: a) the bimodal *n*-alkane distribution in  
9 high-altitude topsoil and b) the similarity of the distributions in soils and sediments that  
10 distinctly differ from the pronounced unimodal leaf litter distributions. The ambiguity of  
11 proxies based on these ratios in the Ohrid Basin is highlighted by the fact that, for example, the  
12 C<sub>29</sub>/C<sub>31</sub> *n*-alkane ratio in both high-carbonate samples (warmer, more humid climate) and low-  
13 carbonate samples (cooler, more arid climate) are very similar, 0.56 and 0.53, respectively, even  
14 though the terrestrial biome certainly changed significantly through the pronounced climatic  
15 fluctuation of the 8.2 ka event.

16 MDS shows separation of sample types, namely soils, leaf litter, grasses, macrophytes, lake  
17 POM and sediments, which 1-way ANOSIM shows to be significantly different. The “weakest”  
18 differences are between subsoil and grass ( $R = 0.25$ ,  $P = 33.3\%$ ) and leaf litter and grass ( $R =$   
19  $0.5$ ,  $P = 20\%$ ), they not being significantly different. This is also evident in the MDS plot  
20 (Figure 5), which shows that the soils are close in Euclidian space to the sediments and implies  
21 that the composition of the sediments is dominated by soil-derived lipids. If the analysis is  
22 restricted to the lake sediments only, then again, there is a significant difference between the  
23 low carbonate and other sediments ( $R=0.734$ ,  $P=0.6\%$ ). This is particularly clear for the  
24 sediments that are interpreted to reflect the 8.2 ka event (Fig. 2 and Fig 6), which are on the  
25 other hand, much closer in MDS space to the single sample of degraded Terra Rossa soil. This  
26 finding would support the notion that soils eroded around 8.11 and 8.17 ka contained more  
27 strongly degraded OM. However, this assumption is based on the findings from a single sample,  
28 only.

### 29 **4.3 Application of new proxies across the 8.2 ka climate event**

30 Figure 6 compares the elemental records across the 8.2 ka event to biomarker proxies that are  
31 based on our geochemical fingerprinting of modern OM sources. The carbonate record shows  
32 two pronounced minima in response to changes in temperature and hydrology (Wagner et al.,  
33 2008) that likely reflect the Northern Hemisphere climate development controlled by two major





1 phases of freshwater release into the North Atlantic (Lajeunesse and St-Onge, 2008; Roy et al.,  
2 2011). In the following, we refer to the timing of these minima as Phase 1 and Phase 2 according  
3 to their chronological succession. While the carbonate record is closely linked to climate  
4 forcing, the proxies that incorporate an organic component, i.e. TOC, TOC/TN, the  
5 concentration of total lipids and the biomarker ratios, are controlled by the response of the  
6 biome and associated changes in OM fluxes to the lake sediments.

7 Between 8.7 and 8.35 ka, TOC and TOC/TN values are relatively high as is the terr FA/OH  
8 ratio. These data are consistent with moderately degraded OM from high- and low-altitude  
9 topsoils (terr FA/OH 0.7 and 0.9, respectively) and little degraded leaf litter (terr FA/OH 0.5  
10 and 0.7, respectively) being the main sources of sedimentary OM between 8.7 and 8.35 ka,  
11 while the contribution from weathered (degraded) Terra Rossa-type soil (terr FA/OH 0.1) is  
12 minimal. Notably, the concentration of the total lipids steadily decreases from 8.7 ka onwards,  
13 indicating a decline in the supply of lipid-rich material and a gradual change in the make-up of  
14 the terrestrial biome. A similar trend can be seen in the ACL<sub>22-26</sub> FA between 8.7 and 8.5 ka,  
15 but not in ACL<sub>24-28</sub> OH indicating an early change in the lipid sources. This subtle shift can also  
16 be discerned in the TOC/TN ratios. While the ultimate driver behind these early trends is  
17 certainly a slowly changing regional climate, more immediate forcing factors may have  
18 included:

19

- 20 i. A slowly decreasing lake level, exposing the flood plains to the south of site Lz1120  
21 and reducing the flux of lipid-rich terrestrial OM to the lake.
- 22 ii. A change in the soil moisture budget leading to increased degradation of lipids.
- 23 iii. A slow decline in terrestrial productivity.

24

25 Due to its tectonic origin, the Ohrid Basin is characterized by a pronounced terraced  
26 morphology, particularly at its northern and southern ends where today's agricultural plains  
27 were once marsh land and/or lake floor. As a result, lake level change can introduce a threshold-  
28 type control of biome characteristics and, thus, cause abrupt changes in material fluxes within  
29 the catchment of site Lz1120.

30 A major change occurred shortly after 8.35 ka, with the onset of Phase 1, when values for  
31 carbonate, TOC and TOC/TN dropped sharply, indicating a substantial change in the deposition  
32 of OM quantity and composition. While the carbonate record suggests a short "recovery"  
33 followed by another, slightly prolonged but less pronounced minimum during Phase 2, the

1 records of TOC and TOC/TN reveal that the non-carbonate sediment delivered during Phases  
2 1 and 2 contained bulk OM of significantly different quantity and composition. During Phase  
3 1, an increased supply of non-carbonate material apparently contributed little OM and diluted  
4 both carbonate and TOC while the sedimentary OM shows low TOC/TN ratios. During Phase  
5 2, by contrast, TOC/TN ratios were higher and TOC was either less diluted and/or the non-  
6 carbonate material supplied to the lake contained higher amounts of OM. This difference in  
7 OM composition between Phases 1 and 2 is confirmed by the composition of the total lipid  
8 extracts that generally contain high proportions of both long-chain terrestrial *n*-alkanoic acids  
9 and *n*-alcohols, but show a substantial drop in the terr FA/OH ratio in Phase 2, only. Conversely,  
10 the average chain length of *n*-alcohols (ACL<sub>24-28</sub> OH) does not show any significant change  
11 except for a minimum in Phase 1. The ratio of largely suberin-derived mid-chain over plant  
12 wax-derived long-chain *n*-alkanoic acids (m-FA/l-FA), on the other hand, shows maxima in  
13 both phases. In order to explain these patterns, variable contributions from terrestrial OM pools  
14 containing little, moderately and strongly degraded OM from the low- and high-altitude habitats  
15 need to be considered. For Phase 1, higher values of m-FA/l-FA and a lower ACL<sub>22-26</sub> FA and  
16 ACL<sub>24-28</sub> OH are consistent with the lipid fingerprint of a low-altitude topsoil. However, the  
17 shifts in the elemental data require substantial supply of a material with very low amounts of  
18 carbonate and TOC, lower TOC/TN ratios and lowest lipid concentrations. An enhanced supply  
19 of un-weathered Terra Rossa-type soil would explain this pattern. An alternative OM source  
20 with low TOC/TN ratios and TOC values could be former lake sediment. This would have been  
21 deposited in the shallow areas to the south and southwest of Lz1120 at lake level high-stands  
22 and would have been spiked with suberin-derived mid-chain FA and OH during a slowly falling  
23 lake level and the development of marshy vegetation while the high groundwater table would  
24 have dissolved the carbonate. In fact, the lipid composition of the sample from 8.29 ka (Phase  
25 1), despite showing different values for the m-FA/l-FA ratio and ACL<sub>22-26</sub> FA, overall does not  
26 significantly differ from the total lipid composition of samples with high carbonate contents  
27 (Fig. 5). Silty agricultural soils of brown-grey color that appear most likely to have developed  
28 from former lake sediment can be found, for example, in the fields to the south of Pogradec,  
29 with variable amounts of fine sand incorporated into the clayey silt matrix deriving from former  
30 beach lines. The shift in the geochemical records in Phase 1 may thus be explained by relatively  
31 enhanced contribution from (un-weathered) Terra Rossa-type subsoils and erosion of material  
32 from the exposed plains, combined with some contribution from surrounding low-altitude and  
33 moderately degraded topsoils. This also implies that the lake level must have dropped to  
34 roughly the modern level or below at the time. Unfortunately, we do not have the geochemical

1 fingerprint for the marshy element of the biome to verify this hypothesis. By contrast, while an  
2 even more pronounced peak in the m-FA/l-FA ratio and a minimum in ACL<sub>22-26</sub> FA again  
3 suggest increased supply of suberin-derived lipids, the significant drop in the terrigenous  
4 FA/OH ratio observed in Phase 2 clearly suggests substantial contributions from soils with a  
5 lipid fingerprint comparable to weathered (or degraded) Terra Rossa. MDS analysis confirms  
6 the assumption of significant contribution from degraded Terra Rossa-type soil as the samples  
7 from 8.17 and 8.11 ka plot closest to the weathered Terra Rossa sample (Fig. 5).

8 Degraded soils as the main source of sedimentary lipids and little contribution from topsoils or  
9 of terrestrial vegetation suggests that the latter were both diminished, leaving the deeper soil  
10 layers vulnerable to erosion. A depleted topsoil pool and, possibly, a vegetation cover that had  
11 not yet fully recovered from a period of at least seasonally increased aridity would also explain  
12 why the samples from 8.23 ka (separating Phases 1 and 2) and from 8.05 ka show TOC values  
13 that appear low relative to their carbonate contents, with these two proxies otherwise correlating  
14 closely ( $R^2 = 0.88$  between 8.8 and 8.0 ka, without samples from 8.05 and 8.23 ka). By contrast,  
15 carbonate supply from primary production and carbonate precipitation would have increased  
16 rapidly under warmer and more humid conditions and with increased nutrient supply.

17 Finally, the composition of *n*-alkanes in Figure 6 apparently follows the concentration of the  
18 total lipids, with higher proportions of grass-derived *n*-C<sub>31</sub> alkane relative to the tree-derived *n*-  
19 C<sub>27</sub> and *n*-C<sub>29</sub> alkanes coinciding with lower concentrations of sedimentary lipids. The supply  
20 of Terra Rossa-type soil may contribute to this pattern, as it contains very low amounts of lipids  
21 and significantly higher proportions of the C<sub>31</sub> alkane. Considering the fact that the  
22 incorporation of lipids into the subsoil is likely lagging the development of the overlying topsoil  
23 and vegetation makes the application of *n*-alkane distributions for vegetation reconstructions in  
24 the Ohrid Basin challenging. By contrast, biomarker proxies based on chain-length distributions  
25 and proportions of the major lipid compound classes, FA and OH, appear to follow a pattern  
26 that is consistent with the climatic development and likely biome and lake level changes.

27 Since Lake Ohrid appears to have been an oligotrophic ecosystem for most of its history, it is  
28 not surprising that soil OM is the major source of the sedimentary lipids. Our lipid-based  
29 proxies suggest that soil OM supply was relatively enhanced during the 8.2 ka event in response  
30 to a dryer and cooler climate and the associated recession of terrestrial vegetation. This  
31 contradicts the conclusions of Lacey et al. (2014) who assume a reduction in primary  
32 productivity and an even more profound reduction in the contribution of soil-derived OM for  
33 the period between 8.5 and 8.0 ka, based on their interpretation of proxy data from bulk organic

1 carbon isotope measurements and Rock-Eval pyrolysis of sediments from the western part of  
2 Lake Ohrid. The authors postulate lower soil-OM contributions on the basis of heavier bulk  
3 carbon isotopes and higher values of the oxygen index (OI) from Rock-Eval pyrolysis.  
4 However, soil OM is frequently characterized by the same pattern, i.e. bulk carbon isotope  
5 ratios that are heavier by 1 – 3 ‰ compared to the original vegetation (Lichtfouse et al., 1995;  
6 Ehleringer et al., 2000 and references therein) and have higher OI values (Disnar et al., 2003).  
7 This results from initial OM degradation in the soils rather than in the water column as assumed  
8 by Lacey et al. (2014). We therefore suggest that the data reported by Lacey et al. (2014)  
9 actually supports our conclusion of relatively increased amounts of soil-derived OM in the Lake  
10 Ohrid sediments between about 8.5 and 8.0 ka.

11

## 12 **5 Summary and conclusions**

13 Lake Ohrid is an outstanding sedimentary archive of SE European continental climate change,  
14 recording environmental changes in the Ohrid Basin continuously for at least 1.2 million years.  
15 OM buried in Lake Ohrid sediments can deliver information on the response of the ecosystem  
16 to climatically controlled changes in hydrology. In order to optimize the reconstruction of  
17 environmental changes we carried out the first organic geochemical survey of potential sources  
18 of sedimentary OM in the Ohrid Basin.

19 We determined the organic geochemical fingerprints of leaf litter, topsoil, un-weathered and  
20 weathered subsoil, macrophytes and filtrates of suspended and slow-sinking particles from the  
21 Ohrid water column. Comparison with the TLE composition of Early Holocene sediments from  
22 site Lz1120 in the southeast of Lake Ohrid reveals that little of the sedimentary lipids appear to  
23 derive from aquatic OM sources such as macrophytes or phytoplankton. Labile compounds  
24 including MUFAs and PUFAs, accounting for a third of the polar lipids in water filtrates and  
25 up to half of the total lipids in macrophytes, are not preserved in the sediments, while the  
26 dominant saturated fatty acid in these samples, the *n*-hexadecanoic acid, is not source specific.  
27 By contrast, the majority of the sedimentary lipids are from land sources. Apart from long-  
28 chain (> C<sub>24</sub>) *n*-alkanoic acids (FAs) and *n*-alcohols (OHs) that derive from cuticular waxes,  
29 mid-chain C<sub>22</sub> and C<sub>24</sub> FAs, ω-hydroxy FAs (ω-OH-FAs) and OHs most likely are from suberin,  
30 a bio-polyester forming protective tissue mainly in roots but also barks and bundle sheaths of  
31 grasses. The C<sub>22</sub> and C<sub>24</sub> ω-OH-FAs appear to be particularly reliable indicators for soil-derived  
32 OM as they were present in all topsoil samples but largely absent in leaf litter, grasses,  
33 macrophytes and water filtrates. With proportions of ω-OH-FA showing an almost identical

1 range in soils and sediments of about  $4 \pm 2$  %<sub>lipids</sub> on average, each, we conclude that the  
2 majority of the extracted lipids originates from soil OM with considerable inputs from root  
3 material. This is supported by the generally high amounts of suberin-derived mid-chain FA and  
4 OH. Terra Rossa, the most abundant type of subsoil in the East and South of the Ohrid Basin,  
5 contains little organic carbon compared to the topsoils (1 – 2 % vs. 8 - 11 %) and, accordingly,  
6 only small amounts of lipids. Notably, the terr FA/OH ratio is significantly lower in the  
7 weathered Terra Rossa, making the ratio of these compound classes a useful indicator for soil  
8 degradation and erosion. The lipid composition of the sediments is closest to the composition  
9 of topsoils and, in some cases, likely represents a mixture of lipids from moderately and  
10 strongly degraded soils.

11 We tested a set of new proxies based on our improved end-member characterizations for a  
12 section of sediment core Lz1120 that includes the prominent 8.2 ka event, a 400- to 500-year  
13 episode of cold and dry climate in the North Atlantic realm affecting much of the Northern  
14 Hemisphere, including SE Europe. Both the carbonate record and the biomarker proxies  
15 highlight two distinct phases of the 8.2 ka event affecting the ecosystems of the Ohrid Basin.  
16 Changing proportions of suberin-derived mid-chain compounds relative to cuticular long-chain  
17 compounds as expressed in ratios (m-FA/l-FA) or average chain lengths (ACL<sub>C22-26</sub> FA,  
18 ACL<sub>C24-28</sub> OH) combined with the proportion of terrestrial *n*-alkanoic acids over *n*-alcohols (terr  
19 FA/OH) indicating contributions from degraded soils also reveal significant differences  
20 between the two phases with regard to lipid fluxes from the major OM sources. Accordingly,  
21 Phase 1 (~ 8.3 ka) is characterized by relatively enhanced contribution of lipids from  
22 moderately degraded (top)soils while Phase 2 (8.2 - 8.05 ka) shows clear evidence for a  
23 substantial proportion of lipids derived from degraded soil, suggesting that the moderately  
24 degraded soil OM pool had already been depleted in Phase 1, or that soils were less protected  
25 by the modified vegetation cover. Notably, the biomarker proxies show a consistent trend of  
26 biome modification starting early from 8.65 ka onwards that is consistent with a similar trend  
27 in carbonate, TOC and TOC/TN and occurs well before the substantial change at the onset of  
28 Phase 1. These trends result from a continuous change in the hydrology of the Ohrid Basin and  
29 underline the sensitivity of the biomarker approach.

30 Overall, it appears that soil-derived lipids have the greatest potential to be preserved in the  
31 sedimentary archive of the Ohrid Basin. However, the extent as to which terrigenous and  
32 aquatic biomarkers are representative of the terrestrial and aquatic OM pools still is a matter of  
33 uncertainty. Multi-proxy approaches combining lipid analyses with, for example, bulk carbon  
34 and hydrogen stable isotope data, pyrolysis GC-MS analyses or Rock-Eval analyses of both

1 OM sources and sediments may help resolve this issue. Further improvements of biomarker-  
2 based reconstructions of the terrestrial environment may be achieved by completing the  
3 geochemical characterization of elements of the Ohrid Basin's habitats that are currently  
4 missing including, e.g., marshland soils and soils developing on the ultrabasic rocks along the  
5 western shores. However, our first survey of major OM sources in the Ohrid Basin demonstrates  
6 that organic-geochemical fingerprinting and the development of proxies adjusted to the local  
7 OM inventory can lead to an improved understanding of terrestrial biome dynamics and OM  
8 fluxes towards the lake sediments, in particular, when short-distance OM transport in a  
9 heterogeneous and variable catchment significantly increase complexity. When applied to the  
10 sedimentary record of a well-known climate fluctuation, the 8.2 ka event, the adjusted proxies  
11 demonstrably improve the interpretation of bulk-proxy data such as TOC and TOC/TN.

1 **Appendix A: Abbreviations used for lipid biomarkers in the main text and**  
2 **supplements and their IUPAC names**

3

4 ***n-Alkanes***

5 These are abbreviated by their carbon number, for example:

6 *n*-C<sub>27</sub>                      *n*-heptadocosane

7

8 ***Fatty acids***

9 *n*-Alkanoic acids are abbreviated by their carbon number, for example:

10 C<sub>16</sub> FA                      hexadecanoic acid

11 C<sub>18</sub> FA                      octadecanoic acid

12

13 Monounsaturated fatty acids (MUFAs) are abbreviated using IUPAC recommended  
14 numerical symbols, for example:

15 C<sub>16:1</sub> (*cis*-9)              *cis*-9-hexadecenoic acid

16 C<sub>18:1</sub> (*cis*-9)              *cis*-9-octadecenoic acid

17 C<sub>20:1</sub> (*cis*-9)              *cis*-9-docasenoic acid

18

19 Polyunsaturated acids (PUFAs) are abbreviated in the same way, but where double bond  
20 positions are unsure, no designated position is given, for example:

21 C<sub>18:2</sub>                      octadecanadienoic acid

22

23 Branched fatty acids are abbreviated as follows:

24 *iso*-C<sub>15</sub>                      13-methyltetradecanoic acid

25 *anteiso*-C<sub>15</sub>                12-methyltetradecanoic acid

26 *iso*-C<sub>16</sub>                      14-methylpentadecanoic acid

27 *iso*-C<sub>17</sub>                      15-methylhexadecanoic acid

1 *anteiso*-C<sub>17</sub> 14-methylhexadecanoic acid

2

3 Hydroxy fatty acids, either  $\alpha$ - or  $\omega$ - are abbreviated as follows:

4 C<sub>22</sub>  $\alpha$ -OH-FA 2-hydroxydocosanoic acid

5 C<sub>22</sub>  $\omega$ -OH-FA 22-hydroxydocosanoic acid

6

7 Diacids are denoted  $\alpha,\omega$ -DiFAs, for example:

8 C<sub>16</sub>  $\alpha,\omega$ -DiFA hexadecadioic acid

9

### 10 ***Fatty alcohols***

11 *n*-Alcohols are abbreviated by their carbon number, for example:

12

13 C<sub>16</sub> OH 1-hexadecanol

14 C<sub>18</sub> OH 1-octadecanol

15

16 Branched alcohols are abbreviated as follows:

17 *iso*-C<sub>15</sub> OH 13-methyltetradecan-1-ol

18 *anteiso*-C<sub>15</sub> OH 12-methyltetradecan-1-ol

19

20 Long chain diols are abbreviated as follows:

21 C<sub>30</sub> 1,15-diol 1,15-triacontadiol

22 C<sub>32</sub> 1,15-diol 1,15-dotriacontadiol

23

### 24 ***Long-chain ketones***

25 Long-chain hydroxy-ketones are abbreviated as follows:

26 1,15( $\omega$ 16)C<sub>30</sub> keto-ol 1-hydroxy-tetradecan-15-one



1		
2	<b>Terpenes</b>	
3	<i>Isoprenoids</i>	
4	$\alpha$ -tocopherol	2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol
5		
6	<i>Sterols</i>	
7	$\beta$ -sitosterol	24 $\beta$ -ethylcholest-5-en-3 $\beta$ -ol
8	$\beta$ -sitostenone	24 $\beta$ -ethylcholest-4-en-3 $\beta$ -one
9	stigmasterol	24 $\beta$ -ethylcholesta-5,22 <i>E</i> -dien-3 $\beta$ -ol
10	stigmasteranol	24-ethyl-5 $\alpha$ (H)-cholestan-3 $\beta$ -ol
11	cholesterol	cholest-5-en-3 $\beta$ -ol
12	cholestanol	5 $\alpha$ (H)-cholestan-3 $\beta$ -ol
13	coprostanol	5 $\beta$ (H)-cholestan-3 $\alpha$ -ol
14	epicoprostanol	5 $\beta$ (H)-cholestan-3 $\beta$ -ol
15	epicholestanol	5 $\alpha$ (H)-cholestan-3 $\alpha$ -ol
16	brassicasterol	24 $\beta$ -methylcholesta-5,22 <i>E</i> -dien-3 $\beta$ -ol
17	campesterol	24 $\beta$ -methylcholest-5-en-3 $\beta$ -ol
18	ergosterol	ergosta-5,7,22-trien-3 $\beta$ -ol
19	dinosterol	4 $\alpha$ ,23,24-trimethylcholest-22 <i>E</i> -dien-3 $\beta$ -ol
20	dinostanol	4 $\alpha$ ,23,24-trimethylcholestan-3 $\beta$ -ol
21	lanosterol	5 $\alpha$ -lanosta-8,24-en-3 $\beta$ -ol
22		
23	<i>Triterpenoids</i>	
24	taraxasterol	5 $\alpha$ -taraxast-20(30)-en-3 $\beta$ -ol
25	tetrahymanol	gammaceran-3 $\beta$ -ol
26	$\beta$ -amyirin	olean-12-en-3 $\beta$ -ol

1

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8

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4



1 Table 1a. Total organic carbon contents (TOC), organic carbon to total nitrogen ratio (TOC/TN)  
 2 and carbonate (CaCO<sub>3</sub>) contents of soils and leaf litter in the southeastern Ohrid Basin (TS =  
 3 topsoil, TS/F = topsoil with white rot, SS = subsoil, LL = leaf litter). For leaf litter, total nitrogen  
 4 equals organic nitrogen.

5

sample ID	location; coordinates	soil type; vegetation	TOC (%)	TOC/TN (atomic)	CaCO <sub>3</sub> (%)
SN-TS	above St. Naum springs; 40°54'43.78" N	Leptosol/Rendzina; small oak & beech, shrubs	11.0 ± 0.8	17.5 ± 0.1	7.7 ± 3.0
SN-LL	20°44'35.18" E, 708 m.a.s.l.		41.1 ± 0.6	28.8 ± 0.4	-
TP-TS	near Trpejca; 40°58'45.46" N	Leptosol/Rendzina; small oak & beech, shrubs	7.5 ± 0.2	17.3 ± 0.0	5.4 ± 0.8
TP-LL	20°47'46.62" E, 808 m.a.s.l.		44.2 ± 0.3	25.5 ± 0.2	-
TP-TS/F		with white rot	10.1 ± 0.7	17.4 ± 0.3	5.4 ± 4.9
GN-TS	Galicica National Park; 40°57'59.64" N	Leptosol/Rendzina; mature beech forest	8.9 ± 0.3	15.3 ± 0.8	7.4 ± 1.6
GN-LL	20°48'52.10" E, 1380 m.a.s.l.		n.d.	n.d.	-
DG-SS	near border (dogana); 40°54'29.47" N	Chromic Luvisol/Terra Rossa (B-horizon); shrubs	1.0 ± 0.1	11.4 ± 0.8	<0.5
DG-SS 2	20°43'48.04" E, 708 m.a.s.l.		1.5 ± 0.1	11.8 ± 0.6	2.3 ± 1.5

6

7 Table 1b. Total organic carbon contents (TOC), organic carbon to organic nitrogen ratios  
 8 (TOC/N<sub>org</sub>) and carbonate contents (CaCO<sub>3</sub>) of major Lake Ohrid macrophytes. Reed leaves  
 9 (RL) were sampled near the Albanian-Macedonian border post (DG = Dogana). Submerged  
 10 macrophytes were collected off Tushemisht (TU) and Pogradec (PG).

11

sample ID	species	water depth (m)	TOC (%)	TOC/N <sub>org</sub> (atomic)	CaCO <sub>3</sub> (%)
DG-RL	<i>Phragmites</i> leaves	0.5	44.2	13.3	-
TU-3	<i>Cladophora</i>	3	40.3	13.8	-
TU-4	<i>Potamogeton perfoliatus</i>	4	41.0	14.7	-
TU-6	<i>Potamogeton perfoliatus</i>	6	40.7	12.3	-
TU-12	<i>Chara tomentosa</i>	12	9.9	18.7	61.5
PG-14-II	<i>Chara gymnohylla</i>	14	10.4	17.0	71.2

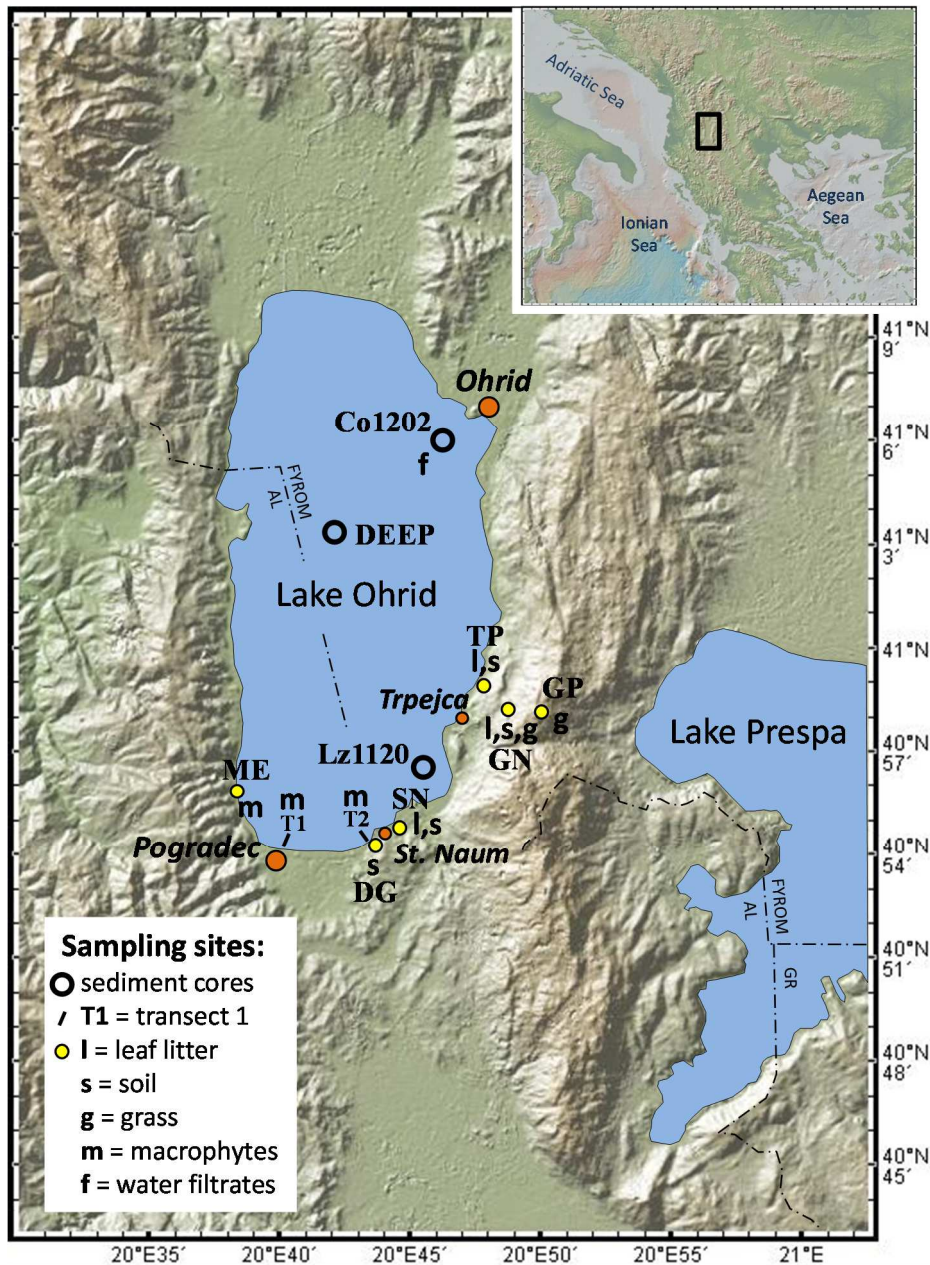
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13

1 Table 2. Composition of the total lipid extracts (TLEs) and amounts of compound classes, sub-  
2 categories and individual lipids of soils, sediments, leaf litter, macrophytes and water filtrates  
3 from the Lake Ohrid Basin. Values are given as percentages of the total lipids (%<sub>lipids</sub>); SN = St  
4 Naum, TP = Trpejca, GN = Galicica National Park, DG = Dogana; ME = Memëlisht; ll = leaf  
5 litter, ts = topsoil, ss = subsoil, hc = high-carbonate sediment, lc = low-carbonate sediment,  
6 clad. = *Cladophora*, potam. = *Potamogeton*, chara. = *Characeae* spp., phrag-r, -s, -l =  
7 *Phragmites* spp. roots, stem, leaves. Grey shading indicates the main compound in each  
8 category, boxes indicate the two most abundant compounds where the amount of the second-  
9 most abundant compound is less than 20 % below the amount of the most abundant compound.  
10 \* Indicates only polar fractions were analysed. For the full list of compounds see  
11 'supplementary data'.

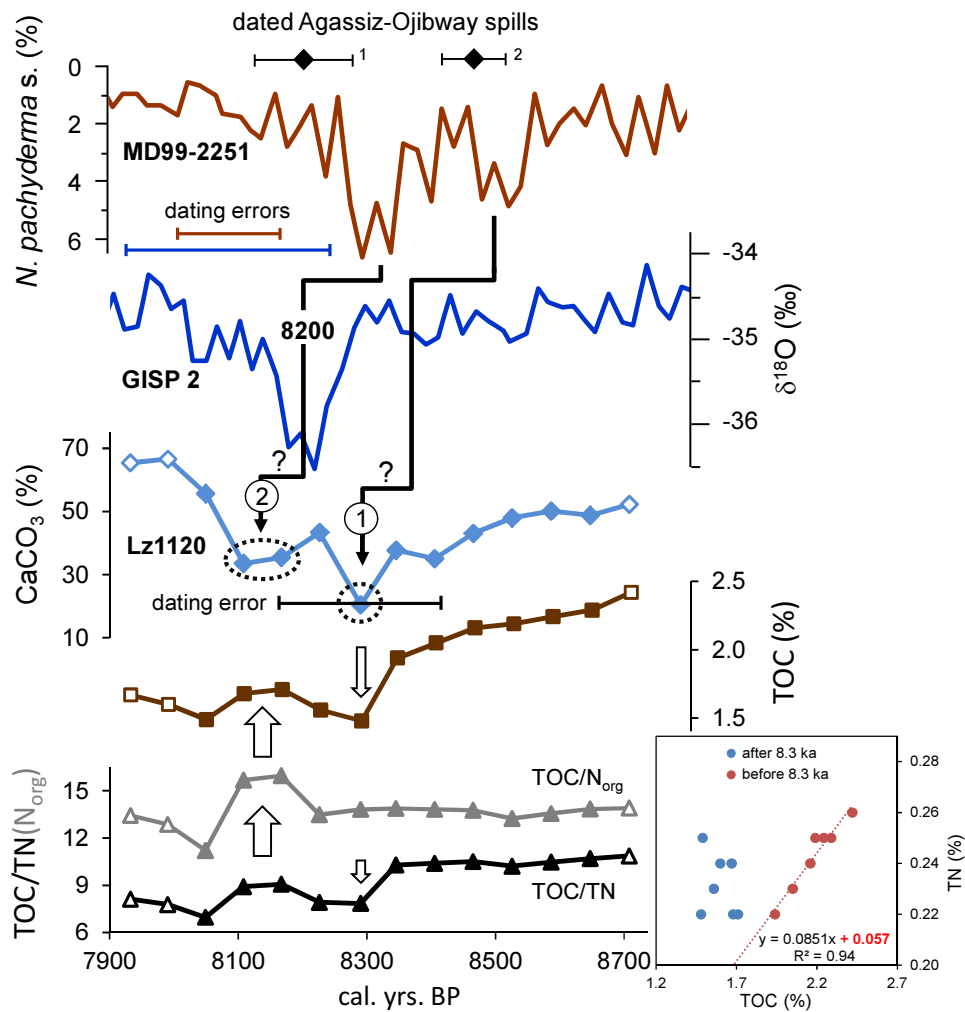
site	SN	TP	GN	SN	TP	TP/F	GN	DG	DG	GN	GP	Lz1120	TU/PG	PG	TU/PG	DG	DG	DG/	
sample type/species	II	II	II	ts	ts	ts	ts	ss	ss II	g	g	hc	lc	clad.	potam.	chara.	phrag-r	phrag-s	phrag
<i>number of samples</i>	2	1	1	2	2	2	2	1	1	1	1	8	3	2	2	3	1	1	2
total lipids (µg/g dry weight)	643	471	653	63	97	89	79	1.3	2.3	1599	1298	40	13	1068	224	533	443	345	172
<b>lipid fractions (%)</b>																			
<i>n</i> -alkanoic acids (FA)	35.0	38.3	22.5	34.9	35.9	39.6	29.0	14.7	37.8	30.4	27.3	39.6	19.6	44.8	22.7	32.7	31.8	35.8	15
hydroxy acids (OH-FA)	3.9	2.1	0.9	5.6	7.9	5.3	5.4	1.4	4.2	0.3	0.2	4.6	3.3	0.2	0.3	-	3.5	1.4	0.3
branched alkanolic acids	0.2	-	0.1	2.6	1.5	1.4	1.5	0.6	2.3	-	-	0.9	1.2	0.6	0.04	1.7	0.4	0.1	-
mono-unsaturated alkenoic acids	6.9	7.5	20.6	19.4	22.1	17.1	25.4	14.7	21.9	2.0	3.3	0.2	0.2	40.6	6.0	20.7	4.7	15.5	1.9
poly-unsaturated alkenoic acids	9.8	8.2	17.3	3.4	5.4	5.6	5.4	5.4	4.7	27.6	29.7	-	-	9.5	35.3	36.1	43.6	34.6	57
<i>n</i> -alcohols (OH)	3.2	3.1	6.3	15.3	13.6	15.8	16.6	47.6	20.2	20.2	16.3	21.1	37.0	0.8	1.5	0.1	0.6	1.4	19
<i>n</i> -alkanes	4.8	6.7	5.4	3.4	2.2	1.7	3.1	3.5	5.1	10.7	8.5	1.5	3.3	0.05	1.0	-	-	-	0.3
methyl ketones	0.04	-	-	-	-	-	-	-	-	-	-	0.4	0.9	-	-	-	-	-	-
sterols	13.1	15.7	23.7	13.2	9.7	11.0	12.8	11.0	3.4	3.1	6.2	14.7	24.2	3.6	32.9	8.7	12.3	10.6	4.9
others	23.1	18.5	3.2	2.1	1.7	2.5	0.9	1.2	-	5.5	8.2	17.0	16.6	-	0.3	0.1	3.2	0.7	0.3
<b><i>n</i>-alkanoic acids</b>																			
Σ C <sub>14</sub> - C <sub>19</sub> FA (short-chain)	15.5	17.1	13.8	10.5	9.9	13.2	6.1	7.1	12.3	18.2	22.4	7.3	7.2	44.3	19.2	31.7	19.4	28.1	12
Σ C <sub>20</sub> - C <sub>25</sub> FA (mid-chain)	6.4	10.0	5.7	11.3	13.3	11.4	9.5	5.7	14.4	2.4	2.2	13.7	7.3	0.5	2.8	1.0	10.0	5.6	1.1
Σ C <sub>26</sub> - C <sub>34</sub> FA (long-chain)	13.2	11.2	2.9	13.1	12.7	15.0	13.4	1.8	11.1	9.8	2.7	18.6	5.1	0.1	0.8	0.01	2.3	2.0	1.3
<b>hydroxy acids</b>																			
Σ ω-hydroxy acids	0.3	0.1	0.6	4.4	6.1	4.0	4.7	1.1	1.9	0.2	0.1	4.4	2.6	-	0.02	-	1.6	0.4	-
Σ α-hydroxy acids	3.5	2.0	0.2	1.2	1.7	1.3	0.7	0.3	2.3	0.1	0.1	0.3	0.7	0.2	0.3	-	2.0	1.0	0.3
<b>branched alkanolic acids</b>																			
C <sub>14</sub> - C <sub>16</sub> branched FA	0.2	-	0.1	1.7	0.8	0.9	1.0	0.6	0.5	-	-	0.9	1.1	0.4	-	0.7	-	0.1	-
C <sub>17</sub> - C <sub>19</sub> branched FA	-	-	-	0.9	0.7	0.5	0.5	-	1.7	-	-	0.01	0.1	0.1	-	1.0	0.4	-	-
C <sub>20</sub> - C <sub>25</sub> branched FA	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-
<b>mono-unsaturated alkenoic acids</b>																			
C <sub>16:1</sub> FA	0.7	0.9	1.0	4.4	5.2	8.4	2.7	4.0	2.1	-	-	-	-	29.3	2.7	11.8	-	13.1	1.1
C <sub>18:1</sub> FA	6.1	6.3	19.4	14.5	16.6	8.5	22.5	10.2	19.7	1.9	3.3	0.2	0.5	11.3	2.7	7.1	0.5	0.7	0.3
C <sub>19:1</sub> - C <sub>24:1</sub> FA	0.1	0.3	0.2	0.5	0.2	0.2	0.2	0.5	0.1	0.2	-	-	-	0.02	0.6	1.8	4.1	1.2	0.0
<b>poly-unsaturated alkenoic acids</b>																			
C <sub>18:2</sub> FA	7.9	8.2	17.3	3.4	5.4	5.3	5.4	5.4	4.7	8.1	7.7	-	-	2.5	11.2	8.8	27.0	21.1	10
C <sub>18:3</sub> FA	2.0	-	-	0.02	0.1	0.3	0.02	-	-	19.4	21.9	-	-	3.7	22.9	11.3	16.4	12.7	47
	T E R R E S T R I A L										S E D I M.				A Q U A T I C				

site	SN	TP	GN	SN	TP	TP/F	GN	DG	DG	GN	GP	Lz1120	TU/PG	PG	TU/PG	DG	DG	DG/	
sample type/species	ll	ll	ll	ts	ts	ts	ts	ss	ss ll	g	g	hc	lc	clad.	potam.	chara.	phrag-r	phrag-s	phrag
<b>poly-unsaturated alkenoic acids</b>																			
C <sub>20:5</sub> FA (EPA)	-	-	-	-	-	0.1	-	-	-	-	-	-	-	1.5	0.6	5.5	-	0.7	-
C <sub>22:6</sub> FA (DHA)	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.2	2.2	-	-	-
<b>n-alcohols</b>																			
Σ C <sub>12</sub> - C <sub>19</sub> OH (short-chain)	0.2	0.1	1.4	0.8	0.7	0.5	0.6	0.6	0.9	0.1	-	1.1	1.3	0.2	0.1	0.1	0.1	-	0.1
Σ C <sub>20</sub> - C <sub>25</sub> OH (mid-chain)	1.8	1.5	3.8	7.5	6.8	6.3	5.3	16.6	15.9	17.1	15.2	7.6	11.1	0.1	0.8	0.01	0.3	0.4	0.3
Σ C <sub>26</sub> - C <sub>34</sub> OH (long-chain)	1.2	1.4	1.1	7.0	6.1	9.0	10.7	30.4	3.3	2.9	1.0	12.4	18.1	0.4	0.6	-	0.2	1.1	18.1
<b>n-alkanes</b>																			
Σ C <sub>19</sub> - C <sub>20</sub> (short-chain)	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-
Σ C <sub>21</sub> - C <sub>26</sub> (mid-chain)	0.5	0.3	0.3	0.2	0.1	0.1	0.1	0.3	1.1	0.6	0.1	0.2	0.4	-	0.4	-	-	-	0.0
Σ C <sub>27</sub> - C <sub>33</sub> (long-chain)	4.3	6.4	5.1	3.2	2.1	1.6	2.9	3.2	4.0	10.1	8.5	1.3	3.0	0.05	0.3	-	-	0.3	-
<b>sterols</b>																			
cholesterol	-	0.1	-	0.5	0.5	0.2	0.9	0.6	-	-	-	1.5	1.9	0.6	0.4	2.3	0.1	-	0.1
cholestanol	-	-	-	-	-	-	-	-	-	-	-	0.8	2.2	-	-	-	-	-	-
ergosterol	0.2	-	0.4	0.1	0.2	0.1	0.1	-	-	-	-	-	-	-	-	-	-	-	-
stigmasterol	0.03	0.2	0.3	0.4	0.6	0.6	0.3	-	0.6	-	-	0.3	0.5	-	8.1	0.1	1.6	1.7	0.1
sitosterol	10.5	11.0	16.6	9.5	5.9	7.8	9.4	9.1	2.9	2.8	5.1	1.7	3.0	2.8	15.3	3.4	10.3	8.6	3.1
stigmasterol	0.3	0.6	0.4	0.7	0.5	0.5	0.8	0.9	-	-	0.5	3.3	6.2	-	-	-	0.3	0.3	-
dinosterol	-	-	-	-	-	-	-	-	-	-	-	1.2	1.1	-	-	-	-	-	-
dinostanol	-	-	-	-	-	-	-	-	-	-	-	1.4	1.8	-	-	-	-	-	-
β-sitostenone	1.6	2.9	5.1	1.0	0.9	0.6	0.8	0.3	-	-	-	-	-	-	-	-	-	-	-
lanosterol	-	-	-	-	-	-	-	-	-	-	-	3.3	5.9	-	-	-	-	-	-
<b>others</b>																			
dicarboxylic acids (DiFA)	0.2	-	-	1.0	0.5	1.0	0.4	-	-	-	-	-	-	-	-	-	0.2	-	-
α-tocopherol	2.6	3.6	3.2	0.7	0.4	0.3	0.3	-	-	0.4	0.2	0.7	0.4	-	0.2	0.1	0.1	0.1	0.1
α+β-amyrin	1.2	1.8	-	0.7	0.8	0.7	0.1	1.2	-	5.1	8.0	0.7	1.2	-	-	-	-	-	-
tetrahymanol	-	-	-	-	-	-	-	-	-	-	-	0.7	1.5	-	-	-	-	-	-
1,15(ω16)C <sub>30</sub> diol	-	-	-	-	-	-	-	-	-	-	-	2.0	2.6	-	-	-	-	-	-
1,15(ω16)C <sub>30</sub> keto-ol	-	-	-	-	-	-	-	-	-	-	-	1.0	1.3	-	-	-	-	-	-
1,15(ω18)C <sub>32</sub> diol	-	-	-	-	-	-	-	-	-	-	-	0.8	0.8	-	-	-	-	-	-
17β(H),21β(H)-bishomohopanoic acid	-	-	-	-	-	-	-	-	-	-	-	9.3	6.1	-	-	-	-	-	-
	<b>T E R R E S T R I A L</b>										<b>S E D I M.</b>				<b>A Q U A T I C</b>				

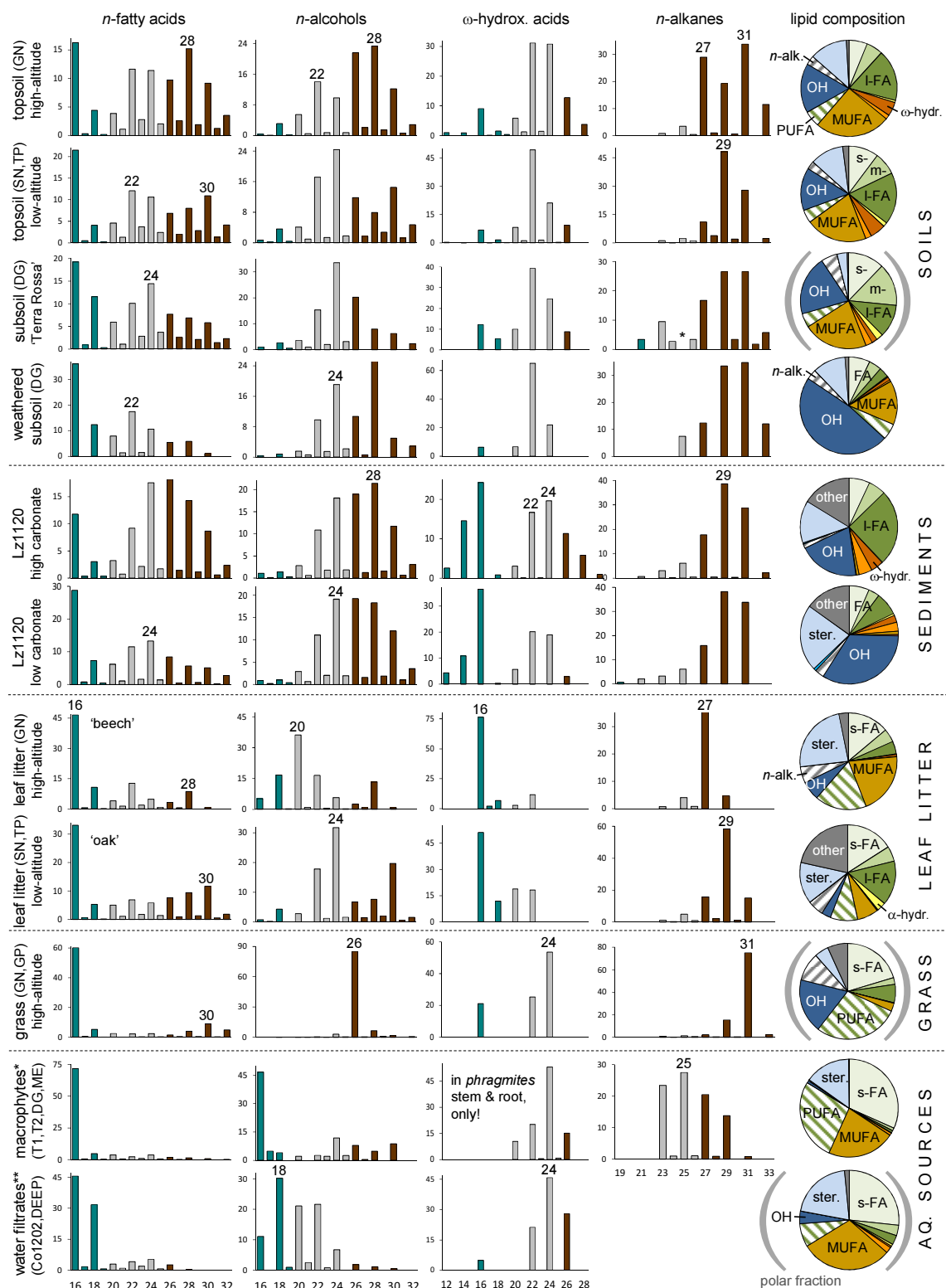


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Figure 1. Locations of sediment core Lz1120 and sampling sites for modern materials in the Ohrid Basin. Lake Prespa contributes 28% of the inflow of meteoric water through karst systems. Inset: Location of Lake Ohrid in the Balkans region of South Eastern Europe.



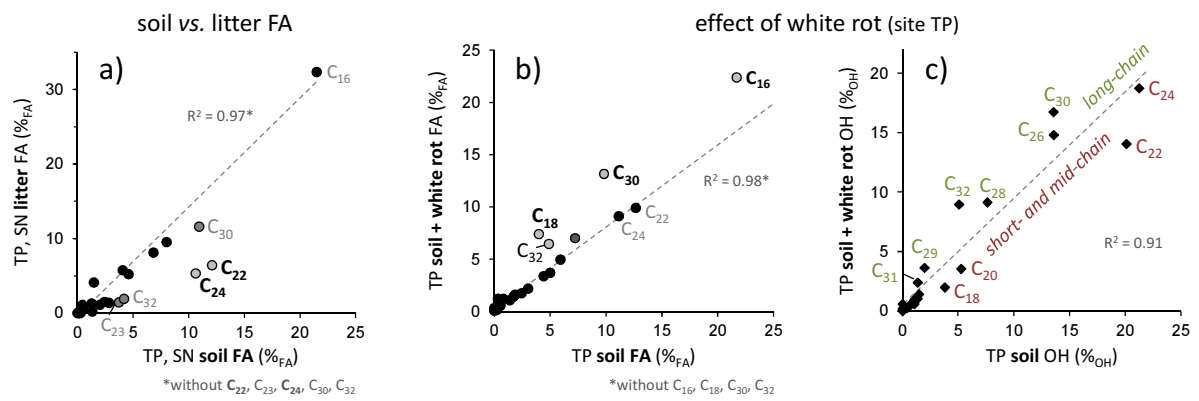
1  
 2 Figure 2. Records of carbonate ( $\text{CaCO}_3$ ), total organic carbon (TOC) and the organic carbon to  
 3 total nitrogen ratio (TOC/TN) of core Lz1120 compared to proxy data from the North Atlantic.  
 4 Phases of increased percentages of *N. pachyderma* s. in MD99-2251 indicate at least two  
 5 southward shifts of colder surface waters (Ellison et al., 2006) while the excursion in the  $\delta^{18}\text{O}$   
 6 record of Greenland ice cores (GISP), which first defined the 8.2 ka event, indicates a drop in  
 7 atmospheric temperature over the Greenland Ice Sheet (Grootes et al., 1993). The dashed circles  
 8 mark those samples that are discussed as low-carbonate samples in carbonate minima 1 and 2  
 9 and are possibly corresponding to the climatic deteriorations occurring over the course of the  
 10 8.2 ka event caused by at least two catastrophic freshwater spills into the North Atlantic (<sup>1</sup>Roy  
 11 et al., 2011; <sup>2</sup>Lajeunesse and St-Onge, 2008). Closed symbols mark samples analysed for their  
 12 lipid biomarker composition. White arrows highlight different fluxes of organic carbon and  
 13 total nitrogen during the carbonate minima. Insert: TOC vs. TN for samples from before (red)  
 14 and after (blue) 8.3 ka.  
 15



1  
 2 Figure 3. Chain-length distributions of *n*-alkyl compounds (bar diagrams) in high- and low-  
 3 altitude topsoil, subsoil (un-weathered and weathered Terra Rossa), early Holocene sediments,  
 4 in high- and low-altitude leaf litter, high-altitude grasses, macrophytes and water filtrates and  
 5 the composition of GC-amendable lipids (pie diagrams). Chain-length distribution y-axis

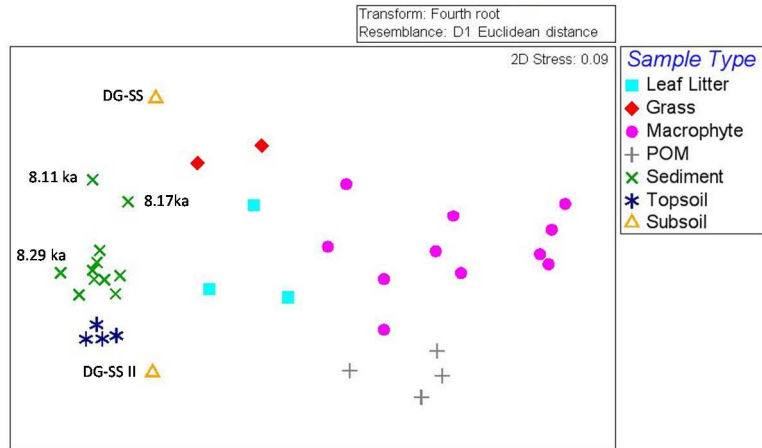
1 values are percentages of the total amount of each compound class, i.e. %<sub>FA</sub>, %<sub>OH</sub> *etc.*; s-, m-,  
2 l-FA = short-, mid- and long-chain FA; MUFA = mono-unsaturated FA; PUFA = poly-  
3 unsaturated FA; ster. = sterols; hydr. = hydroxy acids; br. FA = branched FA; asterix (\*): co-  
4 elution of *n*-C<sub>25</sub> with contaminant; lipid composition for un-weathered Terra Rossa is likely  
5 less accurate as response factors for main compounds were not determined. Note the distinct  
6 shift towards C<sub>22</sub> and C<sub>24</sub> in chain-length distributions of FAs and towards C<sub>24</sub> in chain-length  
7 distributions of OHs from high to low carbonate sediment samples. C<sub>12</sub> and C<sub>14</sub> ω-hydroxy  
8 acids in the sediments appear to derive from an *in-situ* source. Macrophyte data derives from  
9 submerged species, except for the ω-hydroxy acids, which exclusively occur in (emergent)  
10 *Phragmites* sp.. Accuracy of the lipid composition of the grasses is limited since response  
11 factors could not be determined for the main compound classes. Water filtrate data does not  
12 include non-polar compounds (e.g., *n*-alkanes) as these were separated from the polar TLE  
13 fractions prior to further sample processing.



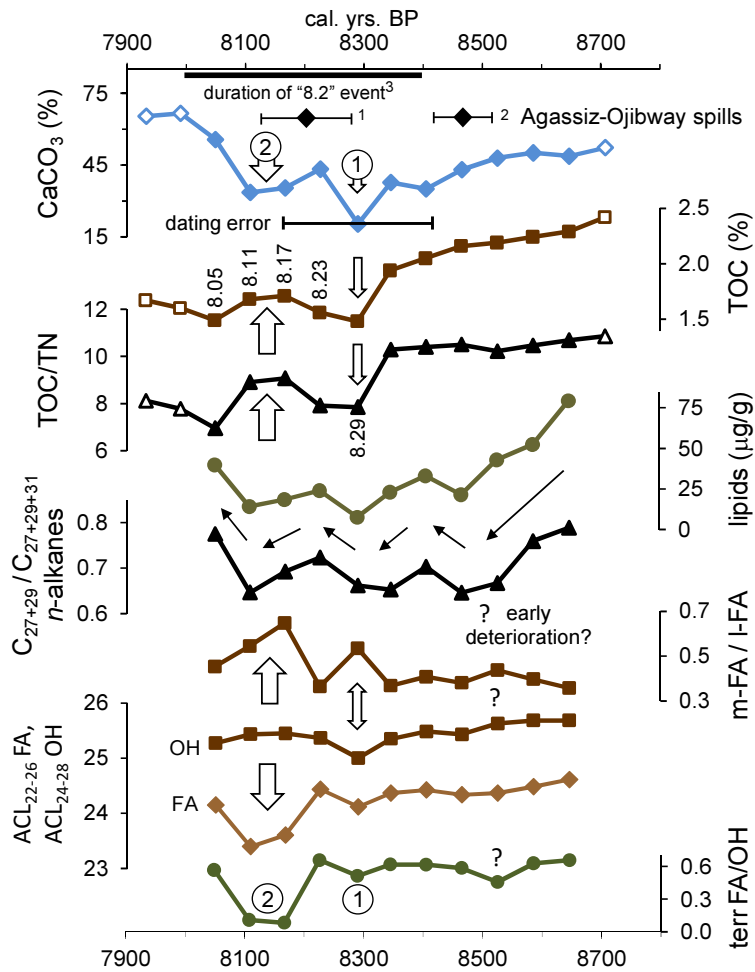


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3 Figure 4. Correlations of compound class-specific relative abundances of a) *n*-alkanoic acids in  
 4 soils and leaf litter of the low-altitude, oak-dominated sites (TP, SN), b) *n*-alkanoic acids and  
 5 c) *n*-alcohols in the two soil samples from site TP, one of which is affected by white rot. The  
 6 patterns illustrate enrichment of mid-chain FA (C<sub>22</sub>, C<sub>24</sub>) from leaf litter to soil and of long-chain  
 7 *n*-alcohols (C<sub>26-32</sub>) as well as long-chain FA (C<sub>30</sub>, C<sub>32</sub>) and short-chain FA (C<sub>16</sub>, C<sub>18</sub>) due to visibly  
 8 enhanced fungal degradation (white rot) within the soil at site TP. Chain-length shifts in the  
 9 latter suggest either selective degradation or biosynthesis of specific compounds by fungi.



1  
2 Figure 5. MDS plot showing separation of sample types in Euclidean space. For details see  
3 methodology section 2.4. Low-carbon sediment samples are labelled with their age (ka). DG-  
4 SS and DG-SS II refer to the weathered and un-weathered subsoil samples (Terra Rossa) from  
5 site DG.



1  
 2 Figure 6. Records from elemental ( $\text{CaCO}_3$ , TOC, TOC/TN) and lipid biomarker analyses  
 3 (closed symbols) of sediment core Lz1120 over the course of the 8.2 ka climate deterioration.  
 4 White arrows indicate contrasting response of elemental and biomarker proxies in response to  
 5 the reduced carbonate supply, i.e. in carbonate minima. Black diamonds indicate Agassiz-  
 6 Ojibway freshwater spillages dated by <sup>1</sup>Roy et al. (2011) and <sup>2</sup>Lajeunesse and St-Onge (2008),  
 7 <sup>3</sup>duration of 8.2 climate deterioration (Barber et al., 1999). While *n*-alkane composition ( $\text{C}_{27}$   
 8 and  $\text{C}_{29}$  relative to  $\text{C}_{31}$ ) appears to co-vary with the total concentration of lipids in the sediment  
 9 (indicated by the black arrows), proxies based on *n*-alkanoic acid and *n*-alcohol distribution  
 10 appear to correspond to the environmental changes indicated by the variable supply of  
 11 carbonate and non-carbonate material.