Response to the reviewers' comments

We would like to thank all reviewers for their efforts; their comments are greatly appreciated and carefully considered. They led us to gain new insights and we have adjusted the manuscript accordingly. In the following, we first summarise our replies to comments common to all reviewers and in the second section, we reply to the specific comments of each reviewer.

A) Response to general issues:

All reviewers mention the small amount of samples investigated in our pilot study as a limiting factor. However, they also kindly acknowledge that we are not intending to actually apply biomarker tools for any reconstructions of environmental change. Instead, it has been the purpose of our study to identify major and minor lipid compounds of Lake Ohrid sediments, discuss their sources and to assess their potential applications in future high-resolution reconstructions of environmental change using the Lake Ohrid sedimentary archive. Such an inventory will allow the design of focussed scientific approaches and research funding requests. Despite the number of our samples being small we believe that we have delivered on this point. Although the *dynamics* of mechanisms controlling the supply of certain organic matter compounds cannot be identified using a small set of isolated samples from various climate stages, the observation of significant compositional differences between these samples nevertheless does allow concluding for the existence of such mechanisms. Hence, the potential of certain compounds as tools to reconstruct environmental dynamics can be assessed, particularly so in multi-proxy approaches when specific biomarkers are put into a reasonable context through their relations to other compounds or proxies (see also response to specific comment 1 by reviewer 1)

The reviewers were intrigued by the finding of coprostanol, the dominant sterol in human faeces, in an early Holocene sample. A small quantity of this biomarker was certainly identified in the sample, based on comparison of its retention time and mass spectrum with an authentic standard. However, the reviewers are concerned whether we could actually conclude for the presence of a human population, in particular, since other sources of coprostanol are known and archaeological evidence for human settlements from this early stage of the Neolithic transition is still missing in the area around Lake Ohrid. We now emphasise in our manuscript that, although the presence of a settled human population clearly is a possibility, the coprostanol found in the pre-8.2 ka sample may as well derive from animals such as wild pigs, although the coprostanol content of their faeces is much lower. An alternative source could also be domesticated animals (pigs, cattle, sheep goats) as their presence is well known from the archaeological record of the Western Balkans (Bailley, 2000). The fact that coprostanol has *not* been detected in younger samples apart from the surface samples as pointed out be reviewer 3 does not imply that humans and/or their domesticated animals have not been around. An important factor probably is where exactly around the lake humans lived and kept their animals, i.e. right along the shores or further inland. Furthermore, coprostanol might have been diluted towards levels below its detection limit when the aquatic productivity and terrestrial input

were higher as suggested, for example, by the carbonate and TOC records for the time following the 8.2 ka event until about 2.5 ka.

Reviewers 2 and 3 requested the presentation of compound distributions for the complete set of samples and including the *n*-alkanes. We now have extended the corresponding figures presenting the full data accordingly.

***B) Response to specific comments*:**

Reviewer #1 (R. Jaffe)

1) The reviewer is right with his suggestion that subtle differences in the relative amounts of minor lipid compounds might reflect natural variability. As acknowledged by the reviewer, major compositional differences such as the striking differences observed in the proportions of terrestrial *n*-alkanols and fatty acids appear as robust features of environmental change, in particular as they follow identical patterns at both sites. Such consistent patterns, however, are not necessarily observed for minor compounds when investigated as standalone proxies for specific organic matter types. To make matters more complicated, all of our samples apart from two (Holocene samples 399 and 483) represent very different stages of the environmental development in the Ohrid Basin as well as organic matter degradation in the sediments: there is only one sample from before, during and after the 8.2 ka event from each of the two sites, plus one glacial sample and one sample representing relatively un-degraded organic matter from site Lz1120. We therefore cannot determine the natural variability of a single minor compound directly. However, in many cases we are able to assess whether the amount of a certain minor compound is varying randomly within natural ranges or not from its relationship to other compounds. For example, the amounts of β-amyrin vary on a low level in a narrow range (0.2 - 0.9 $\%_{lipids}$). Nevertheless they correlate well with the (higher) amounts of lanosterol (1.8 - 5.3 $\%_{lipids}$). Since the two compounds are most likely to share a common, terrestrial source we may assume that the variability of β-amyrin is in fact systematic. Identifying the factors controlling the variability of the common source, however, is a task that goes beyond the purpose of our study, i.e. identifying biomarkers or combinations of biomarkers that are likely to provide a future tool for reconstructing environmental changes in high-resolution time series. The variability of a minor compound that does not show any correlation or co-variation with any other compound is most likely random, indeed, and the biomarker can be regarded of little use, accordingly.
   In this context, the reviewer asks about the statistical relevance of the observed differences in the amounts of lipids relative to the total organic carbon content (%TOC) between the two investigated sites. These amounts differ between the Holocene samples from each site by a factor of ~2. Although this factor is not particularly high and the number of samples small we are confident that this observation is relevant, in particular, since it mirrors the differences in other proxies between the sites such as the TOC contents. We agree that assumptions based on

such a small number of samples are generally risky. We therefore draw our cautious conclusions from multiple observations that, when combined, nevertheless illustrate the great potential of lipid investigations of Lake Ohrid sediments. This aspect is kindly acknowledged by reviewers 2 and 3.

2) Good point. Organic matter degradation is now considered in the text as an explanation for the different amounts of extractable lipids between the sites.

3) see point 1.

4) It is not surprising that bacterial biomarkers from different sources such as hopanoic acid supposedly deriving mainly from soils and epicholestanol deriving from bacteria living in the water column do not correlate. However, we do actually observe some correlations between bacterial markers from identical or potentially associated sources and have now added a short discussion of this issue to the manuscript.

5) We are very grateful for the hint towards the potential contribution of C_{22} *n*-alkanol from epiphytes. In fact, epiphytes in Lake Ohrid have been studied in detail by Allen et al. (1981) who demonstrate high contributions from epiphytes to organic matter productivity in littoral areas of the lake. This specific source for the C_{22} *n*-alkanol is now considered in the manuscript.

6) The P_{aq} values of the samples are now listed in Table 3 (before in Table 2). As suggested by the reviewer, we have extended the discussion of the *n*-alkane distribution with regard to contributions from shallow lake areas.

7) We thank the reviewer for his approval of this specific section!

8) We have shortened and reworded this section. The different preservation potential of branched fatty acids and hopanoic acid mentioned by the reviewer is now included in the discussion of the correlations between the various bacterial markers (see point 4).

9) Thanks to the reviewer we have now identified and included tetrahymanol as a potential marker for an oxic/anoxic interface in our study. At Lz1120, this compound shows a good correlation with epicholestanol, thus delivers helpful complementary information. Further details are now discussed in the manuscript. Adding this compound to the total of extracted lipids resulted in small changes in the data tables and figures which have been carried out accordingly.

10) See response to general comments above.

Reviewer #2 (P.A. Meyers)

Following the suggestion of the reviewer we have now calculated two more proxies based on chain-length distributions of fatty acids, TAR_{FA} (according to Bourbonniere and

Meyers, 1996) and CPI_H (following Matsuda and Koyama, 1977), and discuss these in the manuscript.

Two issues referring to the interpretation of lanosterol and coprostanol have been brought up by the reviewer. The reasoning for why we think lanosterol is of predominantly fungal origin has now been made clearer in the manuscript. Regarding the finding of coprostanol in an early Holocene sample see the general response above.

The editorial corrections suggested by the reviewer have been carried out. We did not replace "emergent" by "emersed" in connection with the P_{aq} value, though. We prefer continuing the terminology used by the authors who introduced this proxy (Ficken et al., 2000).

Reviewer #3 (anonymous)

The reviewer rightly asks why we think the amount of C_{22} *n*-alkanol reflects relatively increased algal productivity in the glacial sample but is not observed in the samples representing the 8.2 ka event at both sites although these supposedly represent similar climate conditions. Thanks to the comments of reviewer 1 we now know that there are, in fact, two possible sources of this compound both of which are present in the Lake Ohrid ecosystem: eustigmatophytes (freshwater micro-algae) and epiphytic algae associated to macrophytes in the littoral areas. Two samples show unusually high proportions of C_{22} *n*-alkanol: the surface sample and the glacial sample pointed out by the reviewer. In case of the surface sample it is very likely that recent high anthropogenic nutrient input has triggered enhanced algal growth including eustigmatophytes. Phytoplankton do not produce high amounts of C_{22} *n*-alkanol, but they do biosynthesize C_{16} fatty acid which is present in high amounts in the samples as well. In case of the glacial sample, however, increased proportions of *n*-alkanes with P_{aq} values >0.1 suggest a relatively higher contribution from macrophytes. We now believe that the elevated amount C_{22} *n*-alkanol observed in this sample is likely to derive from epiphytes associated to the macrophytes and not from algal productivity in the open lake as assumed earlier.

In this context, the reviewer wonders why the amount of *n*-alkanes is increased during the glacial and the 8.2 ka event. Actually, the amount of *n*-alkanes is not significantly increased during the 8.2 ka event at site Lz1120 but only in the glacial sample. We suggest that the proportion of macrophyte-derived *n*-alkanes is relatively increased during the glacial due to the overall reduced productivity on land as well as in the open waters of Lake Ohrid in response to a smaller soil carbon and nutrient pool and low nutrient supply from surface run-off leaving the littoral zones as a relatively more productive element of the ecosystem. This carbon and nutrient pool, however, had formed during the early Holocene and probably still contributed to an overall higher productivity in the lake as well as on land during the 8.2 ka event resulting in relatively lower contribution from the littoral zones relative to the glacial.

The proportion of *n*-alkanes is slightly higher, though, in the sample representing the 8.2 ka event at site Co1202. However, the relative amounts of compounds summarised as "others" such as triterpenoids and sterols increase by about the same factor (1.5 - 2). We therefore assume that the proportional increase of *n*-alkanes in this case results from the dramatic decrease in the amounts of fatty acids.

All comments on technical issues have been gratefully considered and the discrepancies between the main text and the reference list have been sorted out.

References (see also manuscript):

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