

Interactive comment on “The Holocene sedimentary record of cyanobacterial glycolipids in the Baltic Sea: Evaluation of their application as tracers of past nitrogen fixation” by Martina Sollai et al.

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Received and published: 14 September 2017

We thank referee #1 for taking the time to read and comment on our manuscript. We do appreciate the positive assessment of our work. Here we respond to the various issues raised in the two concerns of the referee. - Concern #1: “My first concern relates to the preservation of HGs in sediments (as is briefly discussed by the authors in the text as well). How does HG decomposition vary in freshwater versus brackish water systems? In modern freshwater and brackish water systems, does HG composition show the same pattern as observed in the sediment core? Is it possible that HGs are

C1

better preserved in brackish waters, leading to their higher abundance as well as stability compared to in freshwater systems? If so, HGs in sediments are not only related to their inputs but also to their decay. As both processes are influenced by temperature, the presence of O₂ and possibly salinity, it is very difficult to conclude on “the potential of HGs as specific biomarker of heterocystous cyanobacteria in paleoenvironmental studies”. Instead, I would suggest considering whether there is a proxy or indicator that may be used to (even roughly) assess the preservation or degradation stage of HGs in sediments? In lines 27-35 (pg 9), it is mentioned that sea surface temperatures reconstructed using HGs were too high to be realistic and the causes were not clarified. To me, this seems like a hint that HG signatures in the sediments may be subject to diagenesis-related alterations and that different molecules have been influenced differentially. I think the authors need to clarify this possibility before making conclusions and in the abstract as well.”

This concern of the referee relates to two fundamental questions: what is the effect of changes in preservation conditions on 1) the concentration and 2) the distribution of HGs in sediments. Indeed the ability of HGs to be preserved in sediments represents a key premise to our work (as it is for every biomarker). We discuss potential break-down of HGs in sediments quite extensively (pages 10–11, lines 30–40 and 1–22) but this is mainly related to the breakdown in the anoxic sediments (i.e. the marked decline in the concentration of HGs in uppermost sediments). We have interpreted their decline in the sediments deposited during the freshwater phase as a much lower abundance of nitrogen-fixing cyanobacteria (which is supported by the increased $\delta^{15}\text{N}$ values) but it is true that we should have discussed more extensively the fact that the changing redox conditions in the surface sediments (i.e. from anoxic to oxic) will probably have affected the conditions for preservation of HGs. To compensate for this we normalized the HG concentrations on TOC but it is known that biomarkers are more readily degraded than TOC under oxic conditions. In the revised version of our manuscript we will discuss this topic more in depth. On the matter of how the HG decomposition varies in modern freshwater versus brackish water systems (i.e. effect of salinity), to the best

C2

of our knowledge, no studies are available. However, we think that other environmental parameters (e.g. oxygen exposure) are far more important. The second issue that was raised concerns the question whether perhaps partial degradation of HGs results in significant changes in the distribution of the HGs in such a way that differences in HG distribution, interpreted as arising from a difference in the composition of heterocystous cyanobacteria (e.g. as done in our study for the differences observed between the Ancylus Lake and brackish Baltic Sea), is in fact caused by differences in degradation of individual HGs. We don't feel that this is likely. First of all, all HGs are chemically quite similar and we don't expect large differences in oxic degradation rates. Secondly, in a number of systems a good match in the distribution of the HG of suspended water column and surface and (in some cases) deeper sediments is observed (i.e. Lake Challa, Bauersachs et al 2010; Lake Schreventeich, Bauersachs et al., 2015; equatorial Atlantic, Bale et al., 2017). Upon sedimentation, a substantial fraction of the HGs will be degraded, so these studies indicate no preferential degradation of specific HGs. Lastly, the HG distribution that we find in the surface sediments of the Baltic Sea are fully in line with the HG composition of the most important heterocystous cyanobacteria (see text of the manuscript). Consequently, we don't see this as a problem and have full confidence in using the HG distribution to infer potential sources but we will elaborate on this a little bit more in the revised version of our manuscript.

- Concern #2: "My second concern relates to the influence of multiple environmental variables on HG composition and distributions. As the authors mentioned (several times) in the text, HG variations may be related to temperature variations as well as salinity changes. I think that control experiments are needed to prove that HG shifts are related to cyanobacteria community changes only instead of being affected by physiochemical processes also."

We do agree with the suggestion that controlled experiments would help to further elucidate the influence of environmental factors such as temperature and/or salinity on the HG composition. As indicated by the referee, we already quote quite a number of

C3

studies that have examined the influence of temperature and these studies have even resulted in the potential application of HGs to reconstruct temperature. Studies on the effect of salinity on the HG composition of different heterocystous cyanobacteria have not been performed but are more difficult because of the restricted salinity range of heterocystous cyanobacteria. However, these kind of studies fall outside the scope of the present work that describes the HG composition of the Holocene sedimentary record of the Baltic Sea.

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Interactive comment on *Biogeosciences Discuss.*, <https://doi.org/10.5194/bg-2017-324>, 2017.

C4