Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2017-324-RC3, 2017

Biogeosciences
Discussions

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.

Anonymous Referee #3

Received and published: 17 October 2017

The manuscript reports on the use of lipids specific to heterocystous cyanobacteria (heterocyst glycolipids) as (i) tracers for investigating past changes in the community of cyanobacterial blooms and (ii) paleo-proxy to trace back anoxic events in the Holocene Baltic Sea. Sediments sampled from a multicore and a gravity core collected in the Gotland Basin have been investigated for bulk geochemistry, nitrogen isotopes and the distribution and abundance of heterocyst glycolipids. While the use of heterocyst glycolipids as biomarkers to trace for cyanobacterial blooms in the Baltic Sea is principally interesting, I have some major concerns regarding the experimental setup, study de-

sign as well as data acquisition and interpretation that have to be addressed before I can recommend publication of the manuscript.

General comments

My first and most pressing concern is related to the reconstruction of the past Baltic Sea cyanobacterial community, which seems to be the major aims of the study. From reading the manuscript, I got the impression that only six C6 HGs were present in the Baltic Sea sediments and that those are indicative mainly for heterocystous cyanobacteria of the family Nostocaceae; in agreement with the major bloom-forming Baltic Sea cyanobacteria. However, while having a closer look at the method used for the detection of HGs (apparently the same method described by Bale et al. (2015; OG)), I could not fail to notice that the method specifically targets only these six C6 HG but it is neither able to detect HGs of longer chain length (e.g. C30 to C32 keto-ol, keto-diol, diol and triol HGs), which have been described from numerous heterocystous cyanobacteria previously (Gambacorta et al. 1998; Phytochemistry) nor HGs with deoxyhexose or pentose headgroup (attached to a C26 alkyl chain) as described by Wörmer et al. (2012; L&O). This essentially means that the authors limit themselves to a very narrow window of HGs and consequently members of the cyanobacterial community that can be detected with their method. Moreover, they limit themselves largely to the detection of Nostocaceae. So, my major concern is: do the presented HG profiles really reflect the complete cyanobacterial community or in fact only a small fraction of the community and is it then possible to draw any conclusion on the cyanobacterial community at all? It is very much likely that HGs with higher carbon chain length or other sugar head groups are also abundant and perhaps also more dominant than the six C6 HGs that were detected in the Baltic Sea sediments but we would never know because they are not included in the detection method. This might be in particular the case for the freshwater interval, for which major changes in the cyanobacterial community would be expected.

Moreover, it makes of course sense that the presented HG profiles agree with the major

bloom-forming genera if only those HGs are included in the detection method that are specific for cyanobacteria of the family Nostocaceae. In my opinion, the authors may have missed major changes in the cyanobacterial community due to the limited number of HGs that have been investigated. To obtain robust results and hence make reliable interpretations of cyanobacterial community changes over time, most if not all of the samples would need to be re-measured using a method that includes the full spectrum of HGs currently known from heterocystous cyanobacteria.

This identification of potential biological sources of HGs in the Baltic Sea sediments is similarly problematic. I got again the impression that the six C6 HGs shown in Table 2 and discussed in the text cover the full spectrum of HGs that are present in the listed heterocystous cyanobacteria. From reading the original literature, however, it seems that many of these species do not only contain the six C6 HGs but also other HGs in substantial abundances, in particular when they do not belong to the Nostocaceae. For example, according to the authors, Tolypothrix contains only C28 diol and keto-ol HGs but in fact it also contains significant quantities of C30 triol and keto-diol HGs that surprisingly have not been included in the table and again this component will be missed in the Baltic Sea sediments as it is not included in the detection method. Likewise, according to the authors the C28 triol HG should be the only HG present in Scytonema hofmanni. This by no means is the case if the original literature is consulted (Gambacorta et al. (1998; Phytochemistry)). In fact, this HG is not present in S. hofmanni at all. Instead, it only contains C30 triol and keto-diol HGs, both of which cannot be detected using the analytical protocol described in the present study. There are other examples, such as Aphanizomenon aphanizomenoides or Aphanizomenon ovalisporum, from which only incomplete HG profiles are described lacking e.g. C30 diol, triol, keto-ol and keto-diol HGs as well as HGs with deoxyhexose or pentose head group. I am wondering why only a selection of HGs is shown in the table and why this is biased towards Nostocaceae? In any case, the question remains: Can the cyanobacterial community reconstructed based on these incomplete records? I think not. Therefore, I strongly encourage the authors to carefully check the table and where

necessary to complete the full range of HGs. Otherwise, it is not possible to link HG profiles detected in the sediment record to the biological sources of HG and any attempt to reconstruct cyanobacterial community changes will be flawed.

Although I generally appreciate the authors' efforts to identify the sources of HGs in Baltic Sea sediments, I have my doubts that this is possible by comparing sedimentary HG profiles with HG distributions in cultured cyanobacteria. All cyanobacteria investigated for their HG content so far include either freshwater or marine representatives but brackish species (such as those from the Baltic Sea) have not been analyzed so far. Given that the environmental conditions in the Baltic Sea significantly differ from freshwater and marine environments, it is likely that Nodularia, Aphanizomenon or Anabaena species living in the brackish Baltic Sea will not necessarily show similar HG profiles as found in freshwater and marine cyanobacteria. Although it requires additional work, the authors may consider including modern Baltic Sea cyanobacteria in their study, so that HG distributions can be unequivocally linked to their biological sources and eventually be used to reconstruct changes in the cyanobacterial community in the Holocene Baltic Sea. This would significantly strengthen their conclusions.

While having a look at the HG structures, I am wondering if accelerated solvent extraction is the method of choice for extracting HGs from sediments? For my feeling, this particular extraction method is too harsh and may lead to the degradation of HGs. I assume that there is a reason why other studies dealing with HGs (such as Bale et al. 2015 (OG), 2016 (L&O); Schouten et al. 2013 (Phytochemistry); Bauersachs et al. 2009 (Phytochemistry); 2015 (Biogeosciences)) have used the more gentle Bligh and Dyer extraction method? Can the authors proof beyond doubt that the extraction method did not flaw the generated HG profiles and that these profiles are indeed representatives for the sedimentary signal? While reading some of the original literature, I noticed that a comparison between ASE and Bligh & Dyer extraction has been made previously (Bauersachs et al. 2010; PNAS). Yet, the comparison is only semi-quantitative and without quantification using a standard also less robust. From these

experiments it is also not clear whether ASE leads to selective degradation of keto-ol vs diol HGs or diol vs triol HGs. This is likely not an issue with Bligh and Dyer but with ASE it may indeed be problematic. This issue, however, it is not addressed in the manuscript. As it is now possible to quantify HGs (see Bale et al. 2016; OG), these experiments could easily be done and included in the manuscript.

The issue of how degradation may affect HGs is also only little addressed but essential to verify the robustness of these components as biomarkers for cyanobacterial HABs in the Baltic Sea and as paleo-proxies. As stressed by the other reviewers, the HG profile does not really match other profiles of cyanobacterial activity such as those based on cyanobacterial pigments reported by Bianchi et al. (2000; L&O) or Funkey et al. (2014; EST). Yet, the nitrogen isotope record for instance shows lowest values at the AL-LS transition and the lower part of the Littorina Sea phase, which may point to an increased loading of nitrogen derived from cyanobacterial N2 fixation. Therefore, it is surprising that this interval is not characterized by increased abundances of HGs. As indicated in the text, heterocystous cyanobacteria may not have formed blooms in the past Baltic Sea but this is in contrast to previous findings and certainly needs more attention in the manuscript. It could also very well be that HGs experienced some sort of degradation and are therefore not abundant in the lower part of the Littorina Sea phase anymore. Determining the degradation of HGs is certainly beyond the scope of the manuscript but it would be interesting to obtain additional proof for the presence/absence of cyanobacteria along the record. One such proxy is pigments but distributions of methyl branched alkanes (a well-established marker for cyanobacteria) are an alternative. If these independent proxies show similar profiles as the HGs, this would at least strengthen the authors' hypothesis that cyanobacterial HABs played only a minor role in past Baltic Sea.

The use of the temperature indices does not seem to add much to the manuscript and I am wondering if it is really needed. Temperatures reconstructed using the HDI26 or HDI28 are not described in the results section and only briefly touched on in the dis-

cussion section (p. 9, l. 20-35). I also find this discussion hard to follow. It is not really clear to me how they calculated the temperatures. It is indicated that proxy calibrations from cultures were used but the calibrations described in the 'materials and methods' section seem to be those established by Bauersachs et al. (2015; Biogeosciences) for a lake environment. This is confusing and it should be clarified which calibration has been applied before any robust discussion of temperature can actually be made. I also have my doubts that the culture or the lake calibrations are indeed applicable in the brackish Baltic Sea and that the 'somewhat unrealistic' and too high temperatures result from the lack of a calibration specifically established for the Baltic Sea. There are numerous other examples where specific calibrations have been generated for the Baltic Sea including e.g. the TEXL86.

The publication would greatly benefit from including a temperature record based on a well-established temperature proxy, such e.g. the TEXL86. As mentioned on several occasions in the manuscript, 16 °C seems to be a sort of threshold with temperatures >16 °C promoting bloom-formation. If established and plotted along with the HG data, it would allow identifying intervals during which past cyanobacterial HABS may have occurred in the Baltic Sea.

Specific Corrections

p. 1, l. 17-18. Please mention the different genera of bloom-forming heterocystous cyanobacteria.

p. 2, l. 7. Adams was certainly not the first one to describe the role of the heterocyst in the process of N2 fixation. A very nice overview on this topic is provided by Wolk (1982) and this author certainly deserves credit for his work. Please add the following reference to the manuscript:

Wolk, CP (1982). Heterocysts. In: Carr, N.G., Whitton, B.A. (Eds), The Biology of Cyanobacteria. Blackwell Scientific Publishers, Oxford, pp. 359-386.

p. 2, l. 7-9. The way the sentence is phrased, it seems that both the polysaccharide and the glycolipid layer are involved in regulating the diffusion of atmospheric gases to the heterocyst. Yet, the polysaccharide layer is considered to provide protection with regard to mechanical damages. Please rephrase to make clear that the glycolipid layer is the gas diffusion barrier.

p. 2, l. 15: I do not like the term 'free-living' too much. I think 'non-symbiotic' is more appropriate in this context.

p. 2, l. 31. A reference to studies addressing the nature of cyanobacterial HABs is missing here.

p. 3, l. 8. In addition, species of the genus Anabaena may also be important bloom-formers and they should be included here. They are mentioned as bloom-formers in the discussion section. So why not here as well?

p. 3, l. 33. The authors state to investigate 'past cyanobacterial communities' but in fact they limit themselves to a very narrow range of the cyanobacterial community as their method only allows the detection of six C6 heterocyst glycolipids. As expressed in detail above, I have major concerns that the past cyanobacterial community is expressed in full in the data set and additional measurements using the full range of known HGs are necessary to determine how and when the community of heterocystous cyanobacteria changed in the Baltic Sea.

p. 3, l. 35. Although it is an interesting idea, I do not really see the need and use of HGs as paleo-proxy to trace back anoxic events. There are other lithological and/or bulk-geochemical means that are better suited to investigate sediments for anoxic events. Also, do all anoxic events have to be characterized by the presence of HGs? I assume not as this depends on the nature of the bloom-forming cyanobacteria. Cyanobacterial HABs can also be caused by unicellular or filamentous non-heterocystous cyanobacteria and there is evidence that these cyanobacteria can be abundant in the Baltic Sea as well. Blooms of these types of cyanobacteria may also have occurred in the past

Baltic Sea, causing anoxia but no HGs would be produced and hence the anoxic event would not be visible in the HG downcore record.

p. 4, l. 12-13. Please check the description of the sampling resolution. How can samples from 0-377 cm be sampled as 1 cm slices and samples from 241-377 cm be collected simultaneously as 2 cm slices?

p. 4, l. 17-26. Some of the descriptions of how the bulk-geochemcial data have been obtained are not clear to me but they are essential to understand whether the data is robust or not. I find the description on how the TC, TIC and TOC content of the MUC sediments were obtained very confusing. Was the procedure identical to the measurement of the TOC content of the GC sediments? It is also described that stable carbon isotope values of organic matter were determined. Yet, no stable carbon isotope values are given in the manuscript? So, this does not have to be described here.

p. 5, l. 5. I am wondering why the reproducibility of HG measurements on the GC sediments is less robust?

p. 5, l. 8. What do the abbreviations HDI26 and HDI28 stand for? They should be explained. Also, some information on the temperature calibration should be provided. Have those been established for the Baltic Sea? Have they been tested in the Baltic Sea and are they applicable in this type of setting?

p. 5, l. 18. Please check the timing of the LIA again. I am fairly sure that this cold interval did not extend until the 1950s.

p. 5., l. 25-26: The phrasing suggests that only six C6 HGs could be detected in the Baltic sediments. Given the information in the 'materials and methods' section, however, these six C6 HGs were the only HGs for which the sediments were investigated. Again, this should be clearly expressed in the manuscript.

p. 6, l. 7-8. I am intrigued by the difference in HG abundance although the overlapping

sediment sequences should represent the same time interval. Can this be a result of different preservations at the different sampling sites?

p. 7, l. 25. HAB could indicate all kinds of harmful algal blooms and should be replaced by 'cyanobacterial HAB'

p. 7, l. 35. See above comment and replace 'harmful algal blooms' by 'cyanobacterial HABs'

p. 8, l. 31-32. Again, I do not think that this conclusion is a valid at this stage. The study is largely limited to HGs produced by cyanobacteria belonging to the Nostocaceae and of course then they will always appear as major bloom-former. The full suite of HGs should be analyzed to comprehensively reconstruct the past cyanobacterial community.

p. 9, l. 2-3. Do the authors have other evidence to proof this? For example, indications from bulk-geochemical data, such as increased sulphur content or biological markers specific for a more marine algae community? This would be important to determine whether changes in the HG distribution and thus cyanobacterial community are indeed caused by inflow of salt water or not.

p. 9, l. 27. In the 'materials and methods' section, SWT has been introduced as 'surface water temperature' and here it is referred to as 'sea water temperature'. Which term is correct? The latter implies that the proxy is applicable in marine systems? Is that the case?

p. 9, l. 28. Here it is indicated that the temperature equations are based on cultures. While checking the original literature, however, I noticed that the equations in the culture study by Bauersachs et al. (2014; OG) are different from the once reported here. It seems that the equations used are actually taken from Bauersachs et al. (2015; Biogeosciences) and refer to a lacustrine environment.

p. 11, l. 3. I find it very difficult to follow the authors here. Multiple times it is indicated

that periods of bottom water anoxia occurred and temperatures changed over the investigated sediment profile. It would be advantageous if the intervals characterized by bottom water anoxia would be clearly indicated in Figure 3. Also, is there a temperature reconstruction that can be shown together with the TOC and nitrogen isotope records? It is suggested that temperature changed at the AL-LS transition and throughout the LS but there is no evidence for this provided in the manuscript. It would be very helpful if such data would be shown.

p. 11, l. 9-10. Based on the declining abundance of HGs, it is concluded that cyanobacterial HABs may have been less common and intense in the past brackish Baltic Sea. Although this might be the case, I miss a more thorough discussion of the HG data in context with other studies that have reconstructed past cyanobacterial activity in the Baltic Sea and that contradict with the findings of this study, showing that cyanobacteria were apparently abundant at least during the initial LS phase. In fact, the decreasing stable nitrogen isotope values at the start of the Littorina Sea shown in this study suggest a higher contribution of diazotrophic biomass to the organic matter content even though HG do not increase in this time interval. This actually raises the question whether HGs experienced significant degradation or not. This is a very essential issue to discuss and investigate if any robust conclusion on the use of HGs to trace cyanobacterial HABs and communities over time shall be made. One way to address this issue would be to investigate for other biomarkers specific for cyanobacteria, such as methyl branched alkanes or pigments to determine if they show similar trends as HGs.

p.11, l. 21-22. It may not lead to a complete destruction of HGs but can the authors rule out any effect of selective degradation on HGs? For example that shorter chain HGs are more easily degraded than their longer chain homologues? If that is the case, does the sedimentary HG distribution allow for the reconstruction of past cyanobacterial communities?

Figure 3. I noticed that nitrogen isotope values only for the GC have been obtained.

This is unfortunate as no link between the intensity of N2 fixation and the abundance of HGs can be made. It would be interesting to compare whether nitrogen isotope values are low in the MUC surface sediments and coincide with high HG abundances. If that is the case, it could be estimated how much HGs should be present e.g. in the initial LS phase and compared with the measured HG abundance. There are certainly uncertainties with such a calculation but it may help to clarify whether HGs should have been present in deeper parts of the records or not.

Technical Corrections

l. 36. Change to: . . ...the fixation of N2

p. 3, l. 17-18. The authors should be more consistent in the choice of terms. On multiple instances in the manuscript different expressions for cyanobacterial blooms are used (e.g. 'cyanobacterial HAB', 'cyanobacterial bloom' or 'HAB'). The latter is particular confusing because it could mean any harmful algal bloom not specifically those caused by cyanobacteria. The authors should just stick to one abbreviation.

p. 3, l. 22. Change to: 'water column stratification'

p. 3, l. 32. Change to: 'anoxic events'

p. 3, l. 36-37. In the remainder of the text, 'total organic carbon' is used. Why not here as well?

p. 3, l. 37. Please change to 'nitrogen isotope record'

p. 3, l. 38: Change to 'specific biomarkers of'

p. 4, l. 3. Change to 'max. 248 m'

p. 4, l. 11. This should be '7200 cal. kyr BP', shouldn't it?

p. 4, l. 14. Delete 'of' in 'grounded before of further'

p. 5, l. 1. 'IPL' was not introduced before.

p. 6, l. 20-21. Change to 'C28 keto-diol HG'

p. 6, l. 27. Change to 'during the brackish phase'

p. 6, l. 31. Change to 'HG distribution'

p. 6, l. 32. Change to 'HG distribution'

p. 8, l. 24. Change to 'or occurred in traces'

p. 9, l. 37. Change to 'cyanobacterial HAB'

p. 12, l. 4. Change to 'summer cyanobacterial HABs'

Figure 3: The delta symbol is not displayed correctly.

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